EFFECTS OF DRINKING WATER BIODEGRADABILITY AND DISINFECTANT RESIDUAL ON BACTERIAL REGROWTH

by

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ABSTRACT

In this research an empirical biofilm accumulation model was developed which relates steady-state heterotrophic biofilm bacterial (HPC) numbers to chemical and physical system conditions in a drinking water distribution system. Biodegradable organic matter (BOM), which is a surrogate for the available organic carbon, disinfectant type and the concentration of the disinfectant are the chemical conditions. The investigated physical parameters are the shear stress or flow velocity, temperature, and the material of a pipe surface (substratum). Although biofilm detechment and the relationship between biofilm accumulation and suspended cell numbers are complicated phenomena, in general, reducing biofilm accumulation would be expected to reduce suspended cell numbers.

The model was developed utilizing data from experiments with both synthetic and real waters. These waters were fed to bench-scale annular reactors (AR), which represent a section of a distribution system. The feed water as well as a BOM cocktail, and either chlorine or chloramine disinfectants were dosed directly into the ARs. Shear conditions could be adjusted by the rotational speed of an inner drum in the AR. Liquid phase temperature was controlled using a recirculating temperature control unit. Biofilm was mechanically removed from flush-mounted polycarbonate or ductile iron coupons and quantified by HPCs. The quantitative variables were investigated at design levels which are typical in actual distribution systems.

Both experimental and modeling results clearly show the importance of the disinfectant. The increase of free chlorine residual from zero to 0.5 mg/L reduced HPC numbers by 3 to 4 orders of magnitude. When applied to an established biofilm, the efficacy of the disinfectant was somewhat lower. In a system with little or no disinfectants, the pipe material appears to affect the accumulation of biofilm, such that the corrosive ductile iron surface supports significantly higher net accumulation. Model output indicates a positive correlation between BOM levels and the steady-state HPC numbers. The rate of increase in HPC numbers, however, declines as BOM level increases.

Evidence suggests that the effect of shear on bacterial growth is a function of the BOM and disinfectant residual in a system. In the presence of a BOM supplement but in the absence of a disinfectant, HPCs were little affected by shear conditions. Shear appeared to be a significant factor for net accumulation only in the absence of both BOM and a disinfectant. This suggests a bioreaction limitation at higher BOM levels and mass transfer or diffusion limitation at lower nutritional conditions. It was speculated that in the presence of a disinfectant residual, higher flow velocities may lead to lower biofilm accumulation. The practical implication of this is that in the design of distribution system flow velocities, due consideration should be given to BOM and disinfectant conditions in the system. HPC numbers were less affected by temperature than by other factors such as disinfection residual and BOM level.

A user-friendly interface of the model was written in Visual Basic© programming language. The executable file of the interface is appended on two distribution (3 1/2") disks.

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LIST OF ABBREVIATIONS

AOB	Ammonia-oxidizing bacteria
AOC	Assimilable organic carbon
AR	Annular reactor
ATP	Adenosine triphophate
BAC	Biologically active carbon
BDOC	Biodegradable dissolved organic carbon
BOM	Biodegradable organic carbon
CFU	Colony forming unit
CSTR	Continuously stirred tank reactor
DBP	Disinfection by-product
DBPFP	Disinfection by-product formation potential
DO	Dissolved oxygen
EBCT	Empty bed contact time
EPS	Extracellular polymeric substance
GAC	Granular activated carbon
HDT	Hydraulic detention time
HPC	Heterotrophic plate count
MCL	Maximum contaminant level
MIC	Microbiologically influenced corrosion
PFR	Plug flow reactor
SDOC	Slowly degradable organic carbon
SRB	Sulfate reducing bacteria
TOC	Total organic carbon
WTP	Water treatment plant

LIST OF SYMBOLS

Cl_2	Gaseous chlorine
Cl^-	Chloride ion
$C_2H_3NaO_2$	Sodium acetate
KH_2PO_4	Potassium dihydrogen phosphate
K_2HPO_4	Potassium hydrogen phosphate
HOCl	Hypochlorous acid
H_3COO^-	Acetate
$NaNO_3$	Sodium nitrate
NaOCl	Sodium hypochlorite
NCl_3	Trichloramine (Nitrogen trichloride)
$NHCl_2$	Dichloramine
NH_4Cl	Ammonium chloride
NH_3	Ammonia
NH_2Cl	Monochloramine
OCl^-	Hypochlorite ion
K_S	Limiting nutrient concentration at which μ is half its maximum value
S_{min}	Minimum substrate concentration to achieve a steady-state biofilm
μ_{max}	Maximum growth rate achievable when $S>>K_S$
μ	Specific growth rate
$Df(\theta^*/y)$	Posterior probability
$Df(\theta^*)$	Prior probability
θ^*	True value of the parameter
ε	Experimental error
$\underline{F}$	Regression matrix
$\underline{U}$	Prior covariance matrix
$\underline{V}$	Posterior covariance matrix
$\lvert\underline{V}\rvert$	Generalized variance

CHAPTER 1: INTRODUCTION

Since the early 1980's an increasing number of studies have focused on water quality changes within potable water distribution systems. It has been recognized, initially in Western Europe, that the biochemical parameters of treated water are not necessarily constant as water moves through the distribution system resulting in a certain degree of water quality deterioration. One important aspect of this deterioration is the unwanted growth of bacteria. A high bacterial population in potable water can be associated with an increased possibility of waterborne disease (Payment *et al.*, 1997), taste and odor problems (Suffet *et al.*, 1996), corrosion (Geldrich, 1996) and the need to maintain a higher disinfectant residual (Rittmann and Huck, 1989).

Rittmann and Snoeyink (1984) have proposed the term 'biologically stable water' to describe water that does not promote the growth of microorganisms. This term has gained acceptance within the water industry. Conventional water treatment in North America has traditionally added a disinfectant (chlorine and/or chloramine) to achieve biological stability. An alternative approach is to reduce the concentration of the limiting bacterial substrate (usually organic carbon) prior to distribution. The latter process is receiving increased interest in North America as disinfectants typically react with organic material to form a range of potentially harmful by-products. In addition to (1) disinfectant residual, and (2) biodegradable organic matter (BOM) concentration, other factors which may influence biofilm accumulation include: (3) disinfectant type, (4) shear or flow velocity, (5) temperature, and (6) substratum or pipe material.

As heterotrophic bacteria are a significant portion of the microbial population of most distribution system biofilms, removing organic matter, specifically BOM, is conceptually a

superior approach to achieving biological stability. BOM is a generic term which includes both easily and slowly biodegradable organic carbon and may consist of humic and fulvic acids, polymeric carbohydrates, proteins and carboxylic acids (Rittmann and Huck, 1989).

Typically, in biologically unstable water, bacterial populations proliferate as a biofilm attached to the pipe wall. In a biofilm, immobilized cells are frequently embedded in an organic polymer matrix of microbial origin (Characklis and Marshall, 1990). In general, the population of planktonic, or suspended, cells in distribution systems is orders of magnitude less than the population of biofilm cells (Camper, 1996; van der Wende *et al.* 1989; Characklis, 1988). Since suspended cells are considered to be introduced to the liquid phase from the biofilm through the detachment process, the primary objective (minimizing the concentration of suspended cells) is best achieved by minimizing the number of biofilm cells.

For effective bacterial control it is necessary that the dosed bactericide be in contact with the target organism. Free chlorine reacts rapidly with electron donors of lower redox potential, such as corrosion products, and may be exhausted at an early stage of diffusing into cell clusters (LeChevallier *et al.* 1993). Monochloramine, on the other hand, being a weaker oxidizing agent, reacts more slowly with electron donors and is able to penetrate deeper into a biofilm matrix before being consumed (LeChevallier *et al.* 1990). Thus, if a long enough period is allowed for the diffusion-bioreaction process, attached bacteria would be controlled more effectively by monochloramine than free chlorine.

Recent evidence suggests that biofilm growth is closely associated with corrosion of pipe materials. LeChevallier *et al.* (1993) reported that low levels of corrosion could interfere with free chlorine disinfection, and higher corrosion rates affected monochloramine disinfection. Consequently, the corrosion potential of actual pipe material(s) influences biofilm regrowth and ultimately downstream water quality. In a recent full-scale drinking water distribution system study, Olson (1997) found that phosphate-based corrosion inhibitors could reduce the number of biofilm cells.

Flow velocity and shear at the biofilm-liquid interface are closely related parameters. If, in a system such as an annular reactor (AR) where flow velocity is independent of influent flowrate and therefore organic loading, biofilm accumulation is enhanced by an increase in flow velocity, the overall process is likely diffusion (or mass transfer) controlled. On the other hand, when a change in flow velocity does not affect biofilm accumulation, biofilm growth is controlled by the overall bioreaction rate. Some researchers (e.g. Lu *et al.* 1995) found the detachment of biofilms to be dependent on the shear force in a system. Rittmann (1982) reported that the significance of detachment or sloughing appeared to increase at higher flow velocities. Others reported that the shear stress was of minor significance for biofilm accumulation (e.g. Peyton *et al.*, 1993). Stewart (1993) has shown that under some conditions detachment was a growth-related phenomenon.

Information from drinking water utilities suggests that bacterial regrowth events tend to occur more frequently in the summer. During the summer, several water quality effects are seen. Surface water sources are impacted by run-off events and algal blooms in the warmer months, which may contribute to an increased assimilable organic carbon (AOC) concentration in both the source and treated water. Growth rates are also faster at warmer temperatures. Another significant factor is the increased consumer demand in the summer months, which generally results in an increase of (1) both nutrient and inhibitor (disinfectant) flux in distribution systems and, (2) the potential of a higher shear-induced biofilm detachment rate.

For the control of biofilm accumulation, a number of factors must be managed. Since conditions are continuously changing in actual distribution systems, most of the factors are dynamic in nature. Since full understanding of these highly complex biochemical relationships is difficult, most of the published biofilm results have tried to quantify steady-state conditions. Although the available information is increasing, results on cause-and-effect relationships between factors and bacterial accumulation, even at steady-state, are still limited. Modeling appears to be a potential avenue to summarize available knowledge about the subject matter. Unfortunately, the currently available biofilm accumulation models are too complex for practical applications. Therefore a user-friendly biofilm accumulation model with well defined input parameters would be an

immense value to the industry and would offer practical assistance for managing this issue in full-scale distribution systems.

CHAPTER 2: RESEARCH OBJECTIVES

The overall objective of this research is to develop a steady-state empirical biofilm accumulation model relating bacterial accumulation in distribution systems to chemical (biodegradable organic matter (BOM), disinfectant type and residual) and physical (temperature, shear stress and pipe material) conditions in the system.

The importance of the system variables will be determined and only the significant ones will be included in the final model. The model will be developed using data from synthetic waters and validated on actual real water data. This will be accomplished using strategically important water samples collected from actual water treatment facilities.

Two important sub-objectives are:

1. To optimize research efforts utilizing the Bayesian type of experimental design approach. Bayes' theorem describes in a fundamental way the process of learning from experience. The Bayesian experimental approach accomplishes this through the use of a sequential experimental design technique. In addition, results of others researchers can also be utilized.

2. To facilitate use of the model as an aid in system design or operation, an important goal is the development of a user-friendly model interface. This makes the model easily accessible to experts and also to users less familiar with biofilm terminology and/or research. To accomplish this goal an interface is developed using Visual Basic© version 4.0 programming language.

CHAPTER 3: BACKGROUND AND LITERATURE REVIEW

Most experimentation is done not for the purpose of pure investigation into the nature of the world, but to strengthen an opinion which is already held about the subject matter. Previous research results and/or 'real life' experience are often the sources of our opinions. Therefore, the prerequisite of well planned scientific experimentation must be the thorough knowledge of currently available results in the research area. This chapter introduces excerpts of disciplinary areas which directly or indirectly relate to the author's research. The foundation stone of biofilm research is microbiology. First, fundamental microbiological concepts will be reviewed. Section 3.2 introduces biofilm process principles, including both early and most recent biofilm concepts. A brief overview of rate limitation is also presented. Section 3.3 describes several bacterial quantification methods including microscopy, standard plate count, phospholipid analysis, and adenosine triphosphate (ATP) measurement. The availability of basic life supporting nutrients determines, in a fundamental way, the stability of our drinking waters. Biodegradable substances in general or biodegradable organic matter (BOM) in particular are introduced in Section 3.4. For about two decades great strides have been made in modeling biofilm kinetics. A review of the available deterministic biofilm accumulation models is presented in Section 3.5. Another important water stability parameter is the concentration of a bactericide in an aquatic system. Chlorine based disinfection chemistry is the focus of Section 3.6. The practical subject of microbiologically influenced corrosion (MIC) is introduced in Section 3.7. Basic bench-scale and pilot-scale models of distribution systems are introduced conceptually in Section 3.8. Lastly, but very importantly the Bayesian approach to experimentation is described in Section 3.9. Opponents of this design technique generally find the inherent subjectivity involved in the method unacceptable. The actual design of the author's experimentation will, hopefully, prove the adequacy or even superiority of this design technique over more conservative approaches.

3.1 MICROBIOLOGICAL CONCEPTS

In the field of environmental engineering, biology has often been considered a major area of interest. The specific subject of this research, investigating accumulation of indigenous microorganisms in aqueous ecosystems, has a pronounced microbiological involvement. A brief review of relevant microbiological concepts is introduced below.

Microbial cells are 70-90% water by weight (Brock *et al.*, 1994), and all chemical reactions which occur in the cytoplasm of a cell take place in this aqueous environment. Dissolved substances are continually passed into and out of the cell through transport activities of the cytoplasmic membrane. Specific proteins, called enzymes, serve as catalysts to increase the rate of a reaction by lowering its activation energy.

The cytoplasmic membrane is a thin structure that completely surrounds the cell. Only 8 nm thick, this vital structure is the critical barrier separating the inside of the cell (cytoplasm) from its environment. Due to its structural weakness, the membrane cannot hold the cell together. It is the cell wall, located outside the membrane, which protects and strengthens the cell. However, if the membrane is broken, the integrity of the cell is destroyed, the internal contents leak into the environment, and the cell dies. The cytoplasmic membrane is also a highly selective barrier, enabling a cell to concentrate specific metabolites and excrete waste materials. The general structure of most biological membranes is a phospholipid bilayer. Berg *et al.* (1982) speculated that bacterial resistance towards inhibitors was due to changes in the cell membrane permeability. Most transport processes are linked to the expenditure of energy and result in a much higher concentration of the transported molecule inside than outside the cell. Adenosine triphosphate (ATP) is the key energy carrier in cell function.

The energy source is obtained from light or chemicals. There are two basic kinds of chemical transformation processes, the building-up processes, called anabolism and the breaking-down processes, called catabolism. Metabolism is thus the collective result of anabolic and catabolic reactions.

Many prokaryotic organisms secrete on their surfaces polysaccharide (or glycocalyx) layers. These glycocalyx layers serve several functions, such as facilitating attachment and resistance to desiccation.

The small size of prokaryotes dictates a number of their biological properties. For example, the rate at which nutrients and waste products pass into and out of a cell, a factor that can greatly affect cellular metabolic rates and growth rates, is in general inversely proportional to cell size. This is because transport rates are to some degree a function of the amount of membrane surface available, and relative to cell volume, small cells have more specific surface available than do large cells. This advantage of the small cell frequently translates into more rapid growth rates and larger population densities. Furthermore, the evolutionary process, driven by mutation and natural selection, can proceed more quickly in small size microorganisms.

Gram-negative and Gram-positive cells differ markedly in the appearance of their cell walls. The Gram-negative cell wall is a quite complex multilayered structure and typical for organisms which are present in oligotrophic environments. On the other hand, Gram-positive cell wall consists of primarily a single type of molecule and is often much thicker.

Many prokaryotes are motile, and this ability to move to a new location may mean the difference between survival and death. Chemotaxis is the movement of an organism toward or away from a chemical. While positive chemotaxis refers to movement toward a nutrient, negative chemotaxis is a movement away from a repellent.

Nutrients can be divided into two classes: macronutrients which are required in large amounts and micronutrients which are required in small or trace amounts. After carbon, the next most abundant element in the cell is nitrogen. A typical bacterial cell is 12 to 15 percent nitrogen (by dry weight) (Brock *et al.*, 1994). While autotrophs (i.e. autotrophic microorganisms) use carbon dioxide, heterotrophs use organic compounds as source of energy.

Bacteria occasionally contain so called inclusions consisting of storage material made up of compounds of carbon, nitrogen, sulfur and/or phosphorus. Such inclusions can be formed when these nutrients are in excess in the environment and serve the cells as repositories of these nutrients when limitations occur. Interestingly, LeChevallier *et al.* (1988a) reported that nutrient limitation increased bacterial resistance to various disinfectants.

For organic compounds, when existing alone, there is a minimum substrate concentration (S_{min}) that cannot be reduced to sustain bacterial activity because the cell's net growth rate is always negative for concentrations below S_{min} (Rittmann and McCarty, 1980). However, microorganisms can metabolize the compound in the presence of another substrate that supplies energy and carbon for the cell's long term growth and maintenance (co-metabolism). The species with $S < S_{min}$, have been termed secondary substrates. The species with $S > S_{min}$, termed primary substrates. This terminology was proposed by Rittmann and his co-workers (Namkung *et al.*, 1983) and has been accepted in the literature.

Endospores are very resistant to heat and cannot be destroyed easily by chemical disinfectants. Endospores can remain dormant for many years, but they can convert back into a vegetative cell. This process involves three steps: activation, germination and outgrowth. Bacteria may cease vegetative growth and begin sporulation when a key nutrient, such as carbon, becomes limiting ($S < S_{min}$). de Beer *et al.* (1994b), LeChevallier *et al.* (1988b) and numerous other researchers observed the 'phenomenon' of rapid regrowth after a biocide treatment (e.g. superchlorination). This is possibly the result of endosporadic cell activity.

The temperature can affect organisms in either of two opposing ways. As temperature rises, chemical and enzymatic reactions in the cell proceed at more rapid rates and growth becomes faster. However, above a certain temperature, proteins, nucleic acids, and other cellular components may be irreversibly denatured (Brock *et al.* 1994). Thus, as the temperature is increased within a given range, growth and metabolic function increase up to a point where inactivation reactions set in. The growth rate of psychrophilic organisms is positive in the range of 0°C to 20°C with an optimum of about 15°C. Corresponding values for mesophiles are 12°C,

45°C, and 38°C. Psychrotolerant micoorganisms tolerate a wider temperature range (0°C to 48°C) with the highest growth rate between 20°C to 40°C. The investigated range of 8°C to 26°C in the overall research may support the continuous existance of mesophile and/or psychrotolerant bacteria.

3.2 BIOFILM PROCESSES

Early biofilm concepts assumed that bacterial cells, single or in microcolonies, were embedded in a homogeneous polysaccharide matrix. This concept failed to elucidate how cells deep within a thick biofilm could have access to nutrients from the bulk fluid. Nor was this concept appropriate to clarify how antibacterial agents could penetrate rapidly deep into the matrix without killing bacteria in the more superficial regions of the biofilm. The recent availability of confocal scanning laser microscopy (CSLM) provided, for the first time, accurate high resolution images of living, fully-hydrated microbial biofilms. CSLM studies revealed the marked heterogeneity and remarkable structural complexity of microbial biofilms.

Ridgway and Olson (1981) verified these physical attributes by analyzing the structural characteristics of biofilms using scanning electron microscopy (SEM). Bacterial cells were found to be predominantly located in discrete microcolonies embedded in a matrix permeated by well-defined channels within which convective flow has been clearly demonstrated (Costerton *et al.*, 1994). It was postulated that solutes have access to the water channels, and so they may contact microcolonies deep within the biofilm, even at the colonized surface itself (Costerton *et al.*, 1994). It appears safe to conclude that, at higher flow rates, convective transport within the film becomes increasingly important for nutrient, inhibitor and waste product exchange.

Although it has been hypothesized that the biofilm structure is not a chance occurrence but represents an optimal arrangement for the influx of nutrients (de Beer, 1994b), this may be less significant for thin biofilms in distribution systems. Substrate conversion rates in biofilms are controlled by growth kinetics and mass transport processes. The overall rate of reaction is equal

to the rate of the slowest therefore rate limiting step in the mechanism. The following steps may represents the overall diffusion with reaction process for nutrients (Fogler 1992):

1. mass transfer of the nutrients from the bulk liquid to the external surface of the biofilm
2. diffusion of nutrients from the external biofilm surface to a specific cell in the matrix
3. adsorption of nutrients onto the cell surface
4. cell metabolism
5. desorption of waste products
6. diffusion of waste products from the matrix interior to the biofilm surface
7. mass transfer of waste products from the biofilm surface to the bulk liquid

de Beer *et al.* (1994a) measured the diffusion coefficients of chlorine and glucose and found that, under equal conditions, the maximum possible glucose flux is two orders of magnitude below the lowest measured chlorine flux. The Center for Biofilm Engineering at Montana State University (MSU) integrated CSLM and microelectrode techniques to access the relationship between the internal structure of biofilms and oxygen and/or chlorine residual concentration profiles. The profiles consistently showed that the concentration at the substratum was higher than in the middle of the cell cluster. Consequently, the presence of a biocide and/or nutrient at the base of a biofilm does not necessarily mean that the cell clusters are entirely penetrated.

A useful way of modeling diffusive transport is to treat the fluid layer next to the biofilm boundary as a stagnant film. Rittmann *et al.* (1981) hypothesized that all the resistance to mass transfer is found within this stagnant film, the so called diffusion layer. The thickness of the diffusion layer is thought to be inversely proportional to the bulk liquid velocity. Since this thickness is essentially proportional to the diffusional resistance, the efficacy of a biocide treatment may be improved when higher liquid velocities are applied (de Beer *et al.* 1994a). Since larger size particles generally have thicker diffusion layers, bioreaction limitation may be more pronounced in the initial transient biofilm development where smaller size clusters are present, and diffusional limitation is more likely at well developed steady-state biofilms. If flow conditions have an impact on the reaction rate, the global reaction is likely to be diffusion limited. Otherwise bioreaction limitation is likely to occur.

It was found that, in certain cases, the concentration of the, presumably, rate limiting nutrient did not decline below a minimum (S_{min}) even when convective flow velocities were substantially reduced. Rittmann *et al.* (1981) developed the theory of dual limitation to explain this 'phenomenon'. According to his theory, in lieu of the nutrient, the concentration of dissolved oxygen (DO) became rate limiting and controlled the reaction. Consequently, a precise knowledge of the rate limiting substance is of utmost importance.

Since all bioreactions are of an exothermic nature (Brock *et al.*, 1994), the temperature on the surface of the extracellular matrix is less then the temperature at the cluster center. Consequently, the bioreaction, within the biofilm, occurs at a different temperature from the one measured in the bulk liquid phase. For exothermic reactions, diffusional and thermal resistance have opposite effects on reaction rate. The effect of temperature on bioreaction rate is generally considered to be negligible due to the relatively low thermal energy yield of microniches and the high thermal conductivity of water. Therefore this temperature difference appears to be of little practical importance.

The Monod model is considered to adequately describe the kinetics of biodegradation in oligotrophic environments by attached-growth biofilm. In this model, $\mu = \mu_{max} S / (K_S + S)$ where S denotes the nutrient concentration, μ is the specific growth rate, μ_{max} is the maximum growth rate achievable when $S >> K_S$ and the concentration of all other essential nutrients are unchanged. K_S refers to that value of the limiting nutrient concentration at which the specific growth rate is half its maximum value; roughly speaking, it is the division between the lower concentration range, where μ is strongly (linearly) dependent on S, and the higher range, where μ becomes independent of S (Bailey *et al.*, 1986). Camper *et al.* (1991a) found that μ increased in a near-linear fashion with increasing temperature.

3.3 MEASUREMENT OF BACTERIAL ACCUMULATION

Growth is defined as an increase in the mass or number of microbial cells in a population, which can also be measured as an increase in microbial mass. Growth rate is the change in cell number or mass per unit time. During this cell-division cycle, all the structural components of the cell double (binary fission). The interval for the formation of two cells from one is called a generation, and the time required for this to occur is called the generation time. From knowledge of initial (N_0) and final (N) cell numbers and the time of exponential growth (t), the generation time of the cell population (g) can be calculated directly (Brock *et al.*,1994):

$$g = \frac{0.301(t)}{\log(N) - \log(N_0)}$$

There are two basic methodologies for removing biofilm from a supporting surface (e.g. coupons), sonication and scraping. Block and co-workers (Mathieu *et al.*, 1993) reported the use of sonication in their sample preparation for microscopic direct counting. The attached bacteria were released from the coupons by 2 minutes of sonication (Vibra Sonic Cells - 10W - 20 KHz). A minimum of 80% removal efficiency and 'guaranteed' viability were reported. de Beer *et al.* (1994b) assayed attached bacteria by scraping biofilm from sample slides into 100 mL of phosphate buffer. Stewart *et al.* (1994) reported that the scraping procedure removed 95 to 98% of biofilm organisms. After homogenization (Camper *et al.*, 1985), the total cells can be enumerated by any of the techniques introduced in this chapter.

The viable biomass of a microbial community can be determined by measuring a cellular component that is common to all cells of the microbiota and quickly degraded upon cell death (Brock *et al.*, 1994). Based on this principle, numerous techniques have been developed, which include direct microscopic techniques as well as indirect measurements such as ATP and phospholipid analysis. Direct enumeration of bacteria fall into two broad groups, enumeration by microscopy and plate counting.

Microscopy

The number of cells in a population can be measured by enumerating under the microscope, a method called the 'direct microscopic count'. It is the resolution and not magnification that ultimately defines the limits of what we are able to see with a microscope. Microscopic examination of microorganisms makes use of either a light microscope or the electron microscope. The light microscopes, e.g. (1) bright-field (2) phase-contrast and (3) fluorescence, have been of crucial importance for the development of microbiology as a science. Direct microscopic counting is a quick way of estimating microbial cell number. However it has certain limitations: (1) dead cells are not distinguished from living cells, unless an advanced staining technique is used, (2) small cells may be difficult to visualize, and (3) contaminants (e.g. corrosion products) makes cell counting difficult.

Electron microscopes are widely used for studying the detailed structure of cells. To study the internal structure of cells, a transmission electron microscope (TEM) is essential. In the TEM, electrons are used instead of light rays and electromagnets function as lenses, with the entire system operating in a high vacuum.

If specifically the external features of an organism need to be observed, thin sections are not necessary, and intact whole cells can be examined directly with the scanning electron microscope (SEM). In the SEM, even fairly large specimens can be observed and the depth of field is extremely good. However, with SEM only the surface of an object can be visualized.

Using advanced procedures in staining viable bacteria, epifluorescence microscopy (EFM) is appropriate for viable cell counting (Brock *et al.*, 1994).

The introduction of confocal scanning laser microscope (CSLM) provided, for the first time, accurate high-resolution images of living, fully hydrated microbial biofilms. de Beer *et al.* (1994b) reported the use of fluorescence exclusion with a fluorescein (0.1 mM) in conjunction with CSLM to enhance visualization of internal cell structure.

Standard Plate Count Procedures

Hesse (1881) suggested the use of agar as a solidifying agent in culture media and the procedure has subsequently been a standard technique in isolation of microbiological cultures. It is assumed that each viable cell will yield one colony. McFeters *et al.* (1986) reported that injured coliforms were largely undetected by the use of accepted analytical media. Stewart *et al.* (1994) postulated that plate counts may seriously overestimate biocide efficacy if the culture technique fails to detect injured organisms. The homogenized cell suspension is typically plated in duplicate (or triplicate) on 10% plate count R2A non-selective medium and/or MT7 agar, which is selective for coliforms. After plating by either (1) spread, (2) pour or (3) streak plate methods (Brock *et al.*, 1994), the plates are typically incubated at room temperature for 7 days and the colonies are enumerated (APHA, AWWA, and WEF, 1992).

With all the plate count methods, it is important that the number of colonies developing on the plates not be too large or too small. To achieve this several ten-fold dilutions of the sample are used, if needed, before plating. Plates containing 30 and 300 colonies should be selected for enumeration. The most commonly used measure bacterial accumulation is colony forming units (CFU).

Phospholipid Analysis

Phospholipids are contained within membranes of living cells. The principle of most phospholipid measurement techniques is that the total lipid cell content can be extracted from the cell membrane by a chloroform-methanol-water based mixture (Findlay *et al.*, 1989). The extracted phospholipid is then digested with an oxidant (e.g. potassium persulfate) to release phosphate which in turn is complexed with ammonium molybdate and a dye (e.g. malachite green) and measured colorimetrically. The technique is quantitative, sensitive and relatively simple.

Measurement of Adenosine Triphosphate (ATP)

ATP is found as a relatively constant proportion of all living cells and is typically not present in detritus or dead cells. The principle of the method is that in the presence of ATP, the enzime

luciferase reacts with luciferin to generate light. The amount of light generated is then directly proportional to the concentration of ATP in the sample. Stanfield *et al.* (1987) reported that as the concentration of sodium acetate increased so did the ATP yield, indicating that the latter can be used as a measure of the assimilable organic carbon (AOC) content of the water.

3.4 BIODEGRADABLE ORGANIC MATTER (BOM)

3.4.1 BOM SOURCES

Substances which can be broken down or utilized by living organisms are called biodegradable. Organic material and ammonium are two major electron donor sources in natural aquatic environments. Other sources of biological instability are Fe^{2+}, Mn^{2+}, NO_2^-, dissolved H_2 gas, and the several reduced species of sulfur, especially including the bisulfide ion (HS^-), hydrogen sulfide (H_2S), and thiosulfate ($S_2O_3^{2-}$) (Rittmann and Huck, 1989). Both natural surface and ground waters may support the metabolism of living cells. Generally, ground water supplies contain less organic material and so their treatment is often easier and the potable water in the distribution system is more stable. However, the presence of ammonia is often a problem regardless of the source of the supply water.

Organic Material

There is some inconsistency in the literature with regard to defining the ingredients of the general term, organic material. Undoubtedly, a significant portion of dissolved organic carbon (DOC) in natural surface and aerobic groundwaters is biodegradable. This biodegradable portion, which can be mineralized by heterotrophic microorganisms, has been termed BDOC (Servais *et al.*, 1987). A generic term which includes both easily and slowly biodegradable organic carbon is biodegradable organic matter (BOM). BOM can be split into two parts: easily assimilable organic carbon (AOC), which can be converted to cell mass in a relatively short time (Huck, 1990; van der Kooij *et al.*, 1982) and slowly degradable largely macromolecular organic

carbon (SDOC), which biodegradation may last up to one month. Zhang and Huck (1996a) have suggested that important components of AOC could be acetate, formate, glyoxylate and oxalate.

BOM, which may consist of humic and fulvic acids, polymeric carbohydrates, proteins and carboxylic acids, has been implicated as a major factor in the accumulation of bacteria in distribution systems (Rittmann and Huck, 1989). AOC measurements were initially used to assess the removal of dissolved organic matter by a treatment process to predict the potential of finished water to support the accumulation of microorganisms (Huck *et al.*, 1989). van der Kooij (1989) has shown that concentrations of AOC below 10 µg acetate carbon (C) equivalent per liter do not lead to the accumulation of heterotrophic organisms (HPC) in waters distributed without a disinfectant residual. Many water supplies in the Netherlands meet this criteria and in some cases drinking water is distributed without a disinfectant residual (van der Kooij *et al.*, 1993).

Ammonia

The presence of ammonia, usually in the form of the ammonium ion (NH_4^+), can enhance bacterial accumulation in the distribution system (Rittmann and Snoeyink, 1984). Consequently, elimination of ammonia produces more bacteriologically stable drinking water and reduces the costs associated with additional disinfectant requirements. Ammonia is converted sequentially to nitrite (NO_2^-) and nitrate (NO_3^-) (nitrification) by two groups of chemolithotrophic nitrifying organisms, the ammonia-oxidizing bacteria (*Nitrosomonas*) and the nitrite oxidizing bacteria (*Nitrobacter*). The bacterially mediated anoxic reduction of NO_3^- to nitrogen gas (N_2) is termed denitrification. In contrast to the benefits of complete nitrification, incomplete or partial nitrification in chlor(am)inated distribution systems can adversely affect water quality (Wolfe *et al.*, 1990). An important issue is the high chlorine demand of the intermediate products.

3.4.2 MEASUREMENT OF BOM

There are essentially two categories of measurement methods for biodegradable organic matter (BOM): biomass-based methods and dissolved organic carbon (DOC) based methods. All of the biomass-based methods are predicated on BDOC being the limiting nutrient material for growth (Huck 1990). Huck (1990) has stated that if the concern is with bacterial regrowth generally or growth of coliforms specifically, the parameter which should be measured is bacterial biomass. Most of the current BOM measurement methods are established on this biomass basis. In this case the appropriate term used to express the organic carbon concentration is AOC. On the other hand, if the concern is the reduction in chlorine demand or disinfection by-product formation potential (DBPFP), then a more closely related parameter is DOC. In this case the appropriate term to express the organic carbon concentration is biodegradable DOC, i.e. BDOC (Huck, 1990).

Another way of distinguishing the methods is on the basis of inoculum used consisting of either (1) one or more known organisms or (2) the indigenous bacteria from the natural environment being tested. The method of van der Kooij *et al.* (1982) uses known cultured organisms, whereas Servais *et al.* (1987) and Werner (1985) use indigenous organisms.

Method of van der Kooij

AOC in a water sample is determined by measuring the growth of *Pseudomonas fluorescens* strain P17. A single pure culture may not be able to utilize all the organic matter, consequently the use of the *Pseudomonas* alone is likely to underestimate the AOC content. *Spirillum* strain NOX, known to grow primarily on carboxylic acid (Stanfield *et al.*, 1987) may also be used for growth measurements. van der Kooij *et al.* (1982) have reported a 'good' correlation between the maximum number of P17 (CFU/mL) and the concentration of sodium acetate added to tap water.

Method of Werner

The innovative feature in this method is that the AOC measurement is automated. Instead of using colony counting, the procedure measures the increase in turbidity as a response of change in sodium acetate feed. Turbidity is thought to correlate with microbial accumulation.

Method of Billen - Servais (biomass based analysis)

The filtered sample is inoculated with 1 percent by volume of water which has been passed through a 2 μm filter to eliminate protozoa. Following inoculation, the sample is incubated at room temperature for at least 4 weeks. Aliquots are taken from the sample daily for the first week and then less frequently. Bacterial numbers and total bacterial volume are determined by epifluorescence microscopy and converted to biomass by means of a conversion coefficient (Servais *et al.*, 1987).

Method of Billen - Servais (DOC based analysis)

This variation of the Billen-Servais procedure can be used for BDOC levels greater than 0.2 mg/L (Servais *et al.*, 1989). The initial sample preparation is the same as in their biomass based method. The sample is then inoculated and kept at approximately 20°C in the dark for four weeks. The DOC levels are measured at the beginning and the end of the incubation period and the difference is taken as the amount of BDOC (Servais *et al.*, 1987).

3.4.3 BOM COMPONENTS

The major BOM components found in treated drinking waters are: aldehydes, amino acids, and carboxilic acids (e.g. Gagnon *et al.*, 1997a). Most investigations have studied BOM components during drinking water treatment. Information regarding BOM components in distribution systems is not readily available. Aldehydes, such as formaldehyde and acetaldehyde, are easily removed by biological filtration, whereas the fraction of glyoxal removal is typically lower (Krasner *et al.*, 1993). Following nanofiltration, amino acids, the major nitrogen-containing compounds, represent approximately 63% of the biodegradable dissolved organic carbon (BDOC) (Agbekodo *et al.*, 1996).

In bench scale studies, Urfer and Huck (1997) found that carboxylic acids, such as acetate and formate, could be removed during biological filtration. A full scale investigation showed that complete removal of some carboxylic acids did not occur during biological filtration (Niquette *et al.*, 1998; Gagnon *et al.*, 1997a). This may affect the regrowth potential of heterotrophic bacteria in the filtrate.

3.5 REVIEW OF MODELS FOR BIODEGRADABLE ORGANIC MATTER (BOM) UTILIZATION AND BIOFILM ACCUMULATION

For about two decades great strides have been made in understanding and modeling biofilm kinetics. Although mathematical algorithms are practical and economical tools for process design and control, few researchers have attempted to model the fate of BOM and biofilm accumulation in distribution systems. The dynamic nature of biofilm accumulation and the fact that BOM represents a surrogate for a complex chemical mixture are two fundamental obstacles. The most advanced specific biofilm modeling approaches are: (1) the steady-state biofilm model, (2) SANCHO, (3) AQUASIM, and (4) PICCOLO QUALITY. Common characteristics of the developed models is their complexity, which necessitates simplifying assumptions for practical applications. A recent review of BOM utilization and biofilm accumulation models was published by Gagnon *et al.* (1997b). Although an empirical biofilm accumulation model will be developed in this research, the most common mechanistic models are reviewed in this section.

Steady-state Biofilm Model

The processes substrate utilization, molecular diffusion, and mass transport are often idealized as simultaneous differential equations for a homogeneous layer of bacteria. Rittmann & McCarty (1980a) established a steady-state biofilm model which included substrate diffusion from the bulk liquid to the biofilm surface through an effective diffusion boundary layer. Based

on kinetic and energetic constraints, the model couples substrate utilization to biofilm accumulation to predict, for a single substrate, that a bulk concentration, S_{min}, exists below which a steady-state biofilm cannot survive. The one-dimensional model assumes that substrate concentration within a biofilm changes only perpendicular to the substratum without impacting the bacterial density in the same direction.

The steady-state biofilm model was validated by calculating substrate removal in biological reactors (Rittmann & McCarty, 1980b). Recent application of the steady-state biofilm model includes developing a plug flow form of the model to predict AOC removal through a biologically active filter (Zhang *et al.*, 1996b), and modeling microbial accumulation in water pipes (Lu *et al.*, 1995; Dukan *et al.*, 1996).

SANCHO Model

Within the last decade or so Servais and co-workers have developed algorithms (e.g. SANCHO, Charbol, H3SB) which describe bacterial accumulation and substrate utilization in oligotrophic environments. The basis for both the SANCHO and Charbol model is the H3SB model (Billen *et al.*, 1988; Servais 1989). The biological and chemical processes SANCHO addresses are:

1. The exoenzymatic hydrolysis of dissolved organic matter by bacteria and the growth of free and fixed bacteria on the hydrolysis products; bacterial mortality which releases organic matter is also considered.
2. The reversible adsorption and biological attachment of bacteria to the inner pipe surface.
3. Chemical consumption of free chlorine and the impact of free chlorine on free and fixed bacterial activity.

In SANCHO, BDOC is the limiting nutrient for microbiological growth in distribution systems (Servais *et al.*, 1987). SANCHO assumes that some or most of the BDOC compounds are too large for direct utilization by bacteria. As a result, BDOC is hydrolyzed by exoenzymatic processes to form smaller monomeric substrate. Hydrolysis of BDOC is assumed to occur both rapidly and slowly. Following hydrolysis of the rapidly and slowly hydrolysable BDOC, the monomeric substrate is utilized by the heterotrophic bacteria by Michaelis-Menten kinetics.

Chlorine consumption is attributed to two sources: chlorine demand of dissolved organic carbon (DOC) and chlorine demand due to fixed bacteria.

SANCHO simulations were tested in North American and European distribution system and a good agreement of model predictions with experimental data was found (Laurent *et al.*, 1997b; Servais *et al.*, 1995a). A recent sensitivity analysis of SANCHO by Prévost and co-workers (Cigana *et al.*, 1997) identified the most influential model parameters of SANCHO.

AQUASIM Model

The multispecies biofilm model, AQUASIM, evolved from BIOSIM which was developed by Wanner & Gujer (Wanner *et al.*, 1986). The major objective of a multispecies approach is to allow for a general treatment of microbial interactions in a fixed biomass.

In the multispecies approach, substrate and microorganisms may exist in two distinct phases: bulk liquid (water) and biofilm. The multispecies model predicts changes in biofilm thickness and describes the spatial distribution of obligate aerobic heterotrophic microorganisms as well as substrates in the biofilm. The bulk phase is assumed to be completely mixed and absent of any concentration gradients. In the biofilm phase, however, a concentration gradient exists in the direction perpendicular to the substratum. The biofilm phase is assumed to be homogeneous and continuous in the direction parallel to the substratum.

A distribution system algorithm which uses BIOSIM as a building block for modeling biofilm growth is the Biofilm Accumulation Model, or BAM (Camper *et al.*, 1994a). BAM was specifically designed for modeling biofilm accumulation and substrate utilization in model distribution systems.

In their current forms, all of the available biofilm models are relatively complex. This means that they are most likely to be used on large systems and/or by investigators with a considerable understanding of biofilm phenomena. The water industry could benefit greatly from user-friendly biofilm models with readily available input parameters. This could be achieved by either

the simplification of the existing models or the development of user-friendly empirical or semi-mechanistic models. The first approach could be achieved if detailed analyses of the existing models identified redundant parameters which might ultimately be eliminated from the model. The second approach, the development of a user-friendly empirical model, was the major objective of the author's research.

3.6 CHLORINE-BASED DISINFECTION

Since many water supplies are not biologically stable, the stability-related problems usually are minimized by the application of chlor(am)ine doses great enough to keep a residual throughout the treatment plant and the distribution system. In this section, the basic chemistry of chlor(am)ination and its use and efficiency as a disinfectant are briefly reviewed.

Historical Review

Disinfection, the other means of providing water stability, is the process by which pathogenic microorganisms are inactivated. The demanding need for eliminating pathogens from potable water supplies was first realized after the 1854 London, England cholera epidemic. At first slow sand filtration was employed. Of course, it was not realized, at that time, that this seemingly primitive method represented a 'high-tech' biological water treatment process which reduced the level of biodegradable substances in the filtrate and provided a certain degree of water stability. In 1881, Koch demonstrated that chlorine could kill bacteria. Following an outbreak of typhoid fever in London, continuous chlorination of a public water supply was used for the first time in 1905. Chick first advanced her famous theory of disinfection in 1908. Chloramines were first purposefully used in the water treatment field by Race in Ottawa, Canada in 1918. Ammonia-chlorine disinfection, which enjoyed great popularity for 20 years, fell into disfavor shortly after the 1940 discovery of breakpoint chlorination and the simultaneous consensus that the germicidal efficiency of free-chlorine residuals was many times greater than that of chloramines. The detection of trihalomethanes (THMs) (New Orleans distribution system, 1974; Rook

distribution system, 1974) as byproducts of chlorination made post-chlorination less attractive for controlling regrowth (van der Kooij *et al.*, 1993). The biological stability of finished drinking water has become of increasing importance over the last few decades, as the water industry copes with even stricter regulations on disinfection by-products while maintaining or enhancing disinfection capacity. Maximum contaminant levels (MCL) for THM: Canada 350 µg/L current and 100 µg/L proposed, US 100 µg/L current and 80 µg/L proposed, Germany 10 µg/L (Hambsch and Werner 1993). Since byproducts of chloramination are less of a concern from a public health perspective and because of the relatively low cost (Kreft *et al.* 1985) implication, chloramination is becoming once again an attractive means of disinfection.

Chemistry

The ability to oxidize biological molecules and to diffuse through cell walls are requirements of any effective disinfectants. The major modes of disinfection are: (1) destruction or impairment of cellular structure (2) interference with energy-yielding metabolism (3) interference with biosynthesis and growth (Brock *et al.*, 1994). One measure of a disinfectant's ability to oxidize organic material is the standard reduction potential. The higher the oxidation potential, the easier that compound is able to oxidize organic matter. Although a good deal of work has been done on modeling disinfection, the principal disinfection theory used today is still the Chick model or a modification of it. Chick's law expresses the rate of destruction of microorganisms as a first-order chemical reaction in the form of $\ln(N/N_0)=-kt$ where N/N_0 is the survival rate at time t and k is the rate constant.

Disinfectant capabilities of chlorine depend on its chemical form in water, which in turn is dependent on pH, temperature, organic content of the water and other factors. Gaseous chlorine (Cl_2), when added to water, rapidly hydrolyzes to hypochlorous acid (HOCl) according to

$$Cl_2 + H_2O = HOCl + H^+ + Cl^-$$

The HOCl is then subject to additional reaction which can include dissociation to hydrogen (H^+) and hypochlorite ions (OCl^-), disinfection, or reaction with various organic and inorganic compounds.

$$HOCl = H^+ + OCl^-$$

Both HOCl and OCl^- are known as 'free chlorine'. While HOCl is dominant below pH 6, above pH 9 the prevailing species is OCl^-. Since germicidal properties of the different chlorine species are substantially different (Montgomery, 1985) and because of the normal pH levels in actual systems is within this transitional range, pH should be specified in systems where a disinfectant residual is present. The effect of temperature is such that at a given pH, the fraction of HOCl will be lower at higher temperatures.

When excess HOCl and ammonia (NH_3) are both present in water, they react to form chloramines. As opposed to free chlorine, the chloramines are referred to as 'combined chlorine'. The principal reactions for chloramine formation are shown below (Kreft *et al.*, 1985):

$$NH_3 + HOCl = NH_2Cl + H_2O$$
$$NH_2Cl + HOCl = NHCl_2 + H_2O$$
$$NHCl_2 + HOCl = NCl_3 + H_2O$$

The products are monochloramine, followed by dichloramine and trichloramine (nitrogen trichloride). Similarly to HOCl/OCl^- formation, the formation of chloramines is also a pH dependent reaction. Shull, K.E. (1981) reported that while acidic conditions (pH < 4.5) favored NCl_3 formation, high (pH > 8.5) values supported the formation of NH_2Cl. Between these pH values, mono and dichloramines form. Snoeyink and Jenkins (1980) suggested that maximum NH_2Cl formation typically occurs in the 8.3-8.4 pH range. The 'total chlorine residual' is the term to characterize the sum of the combined and free chlorine residuals. When ammonia is present, either naturally occurring or deliberately added to form chloramines, the hump-shaped

breakpoint curve is produced. As the chlorine dose increases, the chlorine residual first rises to a maximum and then declines to a minimum (breakpoint). The point where the residual reaches a maximum is roughly a chlorine to ammonia-nitrogen weight ratio of 4:1 to 6:1. While NH_2Cl is dominant in the increasing portion of the 'hump' curve, $NHCl_2$ prevails near the breakpoint. The ratio of combined chlorine, consisting mainly of NCl_3, to free chlorine beyond the breakpoint is determined by the organic nitrogen content of the water.

In distribution systems with long residence times or where disinfection by-product (DBP) formation is a concern, changing from free chlorination to chloramination is often beneficial. It is important, however, to restrict the combined chlorine formation to NH_2Cl by a proper Cl_2 to ammonia-nitrogen ratio adjustment since both di- and trichloramines have offensive odors (Kreft *et al.*, 1985). Another drawback of chloramination is that chloramines have been found to be mutagenic in bacteria and toxic to fish at microgram-per-liter (μg/L) levels (Kreft *et al.*, 1985). In addition, ammonia-oxidizing bacteria (AOB) may establish and proliferate in chloraminated systems, causing the rapid decay of the disinfectant by oxidizing the ammonia (NH_3) content to nitrite (NO_2^-), which in turn has a strong chlorine demand. In a survey, Stewart *et al.* (1997) found that 63% of the studied utilities experienced nitrification related water quality concerns. Odell *et al.* (1994) recommended breakpoint chlorination and the reduction of hydraulic detention time in a distribution system as the two most important means of controlling nitrification. Laurent *et al.* (1997a) investigated, at both pilot and full-scale, nitrification in biological filters.

Indicator Organisms

Because of the large number of pathogens known to occur in drinking water, a system of indicators has been adopted, in which selected groups of bacteria are used to indicate the potential for pathogen contamination. The ideal pathogen indicator for the evaluation of bacteriological water quality would: (1) always be present when pathogens are present (2) always be absent when pathogens are absent (3) be non-pathogenic and (4) be more resistant to disinfection and environmental stress than the pathogens (Montgomery 1985). In distribution

networks, the presence of an indicator organism reflects the potential but not necessarily the presence of pathogens.

The total coliform group includes bacteria from many species, including soil and enteric bacteria. The fecal coliforms, a subgroup of the total coliform group, are used to represent organisms of fecal origin. *Escherichia coli* is the predominant indicator organism in the fecal coliform group. The HPC bacteria are often used as indicators of general microbial activity in waters. The presence of a high number of HPC is considered to indicate the presence of opportunistic pathogens, such as *Pseudomonas* spp., *Aeromonas* spp., *Legionella* spp., and *Moraxella* spp. Means *et al.* (1986) found that HPC data was a more rigorous measure of overall disinfection efficiency than coliforms due to their lower sensitivity to disinfectants. The coliform group of organisms have traditionally been used as indicators and coliform limits are still the basis of most drinking water regulations. However, several recent studies (Payment *et al.*, 1993; Rose, 1988) and outbreaks (*Cryptosporidium parvum* episodes, Milwaukee 1992, Waterloo 1993) demonstrated that coliforms are inadequate to indicate the presence of pathogens, especially viruses and parasites. Payment *et al.* (1993) postulated that bacteriophages and *Clostridium perfringens* would be better suited as indicators of general drinking water quality.

Impact of Pipe Material on Disinfection Efficiency

Stewart *et al.* (1994) used stainless steel slides for biofilm colonization and sampling. Rogers *et al.* (1994) and LeChevallier *et al.* (1990) examined the impact of various disinfectants on biofilm behavior on the surfaces of different plumbing materials. In their pilot scale pipeloop system they found that bacteria grown on galvanized, copper or PVC pipe surfaces were readily inactivated by a 1 mg/L residual of free chlorine or monochloramine. Biofilms grown on iron pipes, however, did not respond appreciably to 3 mg/L free chlorine over a period of two weeks. If treated with 4 mg/L monochloramine for two weeks, these biofilms exhibited a more than 3-log die-off on the iron surface. LeChevallier *et al.* (1988a) also reported that HPC bacteria grown on metal coupons were 2,400 times more resistant to free chlorine than were suspended cells. For suspended bacteria, however, the effectiveness of free chlorine was

superior over chloramine (LeChevallier *et al.*, 1988b). These findings may be supported by the theory which postulates that, due to its great potential, free chlorine reacts rapidly with electron donors of less redox potential, such as corrosion products and may be exhausted at an early stage of diffusing into cell clusters (LeChevallier *et al.*, 1993). Monochloramine, on the other hand, being a weaker oxidizing agent, reacts slower with electron donors and is able to penetrate deeper into the matrix before being consumed. This theory speculates that, if a long enough period is allowed for the diffusion-bioreaction process, attached bacteria are controlled more effectively by monochloramine than free chlorine or other higher standard reduction potential agents. In contrast, Block and co-workers (Mathieu *et al.*, 1993) reported a higher bactericidal efficiency of free chlorine.

Numerous municipalities reported a declining chlor(am)ination efficiency during the course of disinfection of distribution networks. It was hypothesized that, due to their mutation, certain bacterial populations could adopt to potentially lethal environments, such as high levels of disinfectants. Mathieu *et al.* (1993) and LeChevallier *et al.* (1990) examined the impact of changing disinfectants on bacterial response. They switched from free chlorination to chloramination and *vice versa* in their controlled laboratory experiments on a regular 2 to 5 week basis. Contrary to expectations, statistically significant benefits could not be reported.

Kiene *et al.* (1993) reported that chlorine consumption of biofilms was significant only for pipes with a high surface-to-volume ratio (small diameter). The critical pipe diameter was determined to be 75mm (3"). They also found that bulk flow velocity had a significant impact on the rate of pipe wall chlorine consumption (mass transfer limitation), being low under laminar flow conditions and high at turbulent flow regimes. Kiene *et al.* (1993) results are completely supported by the findings of de Beer *et al.* (1994a).

3.7 MICROBIOLOGICALLY INFLUENCED CORROSION FUNDAMENTALS

Although microbiologically influenced corrosion (MIC) has been studied extensively (e.g. Borenstein, 1994), information directly related to drinking water distribution systems is relatively sparse. Recent evidence suggests that biofilm accumulation in drinking water systems is closely associated with corrosion (Abernathy and Camper, 1997). The biochemical reactions in this interaction are inherently complex. Since corrosive supporting surfaces (e.g. ductile iron) were investigated in this research, a brief review of corrosion fundamentals is provided below.

The thermodynamics of metals exposed to water describe the underlying relationships of free energy that drive the corrosion reaction. As for any chemical reaction, the oxidation of a metal has a standard free energy ($\Delta G°$). For electrochemical reactions, $\Delta G°$ is usually translated into a standard potential ($E°$). Faraday's law ($\Delta G° = -nFE°$) describes this relationship, where n is the number of electrons transferred in the reaction and F is Faraday's constant. When metal is oxidized, it generates electrons. Since electrons cannot accumulate, the oxidative half-reaction must always be coupled to a reduction half-reaction, which consumes electrons. The half-reaction with the more positive $E°$ is referred to as more 'noble' and proceeds as a reduction and the half-reaction with the more negative $E°$ proceeds as an oxidation. Bacteria often experience positive chemotaxis towards sites of redox chemical reactions for the support of their metabolism (Brock, *et al.*, 1994).

MIC or biocorrosion of metal surfaces is due to biological and electrochemical processes and is associated with discontinuities in extracellular polymeric substances (EPS) (Borenstein, 1994). The two main influences of biofilms on corrosion are contrasting, either retarding or accelerating metal dissolution (Videla, 1994). Retardation or passivation means a reduction in the metal chemical reactivity and may be due to a 'barrier effect' of a homogeneous biofilm. As biofilms are rarely uniform, the opposite effect of enhanced metal reactivity is prevalent.

Several inorganic sulfur compounds are important electron acceptors in anaerobic respiration of microorganisms. Sulfate (SO_4^{2-}), the most oxidized form of sulfur, is often one of the major anions in natural waters. SO_4^{2-} is very stable chemically and its reduction does not occur spontaneously (Brock *et al.*, 1994). The ability to utilize SO_4^{2-} as an electron acceptor for energy-generating processes is restricted to a very special group of obligately anaerobic bacteria, the sulfate reducing bacteria (SRB). SRB, including *Desulfovibrio, Desulfobacter* and *Desulformaculum*, may establish in anaerobic aquatic microniches where SO_4^{2-} is the thermodynamically favored electron acceptor and adequate concentration of metabolizable organic/inorganic electron donors are present.

The respiration of aerobic bacteria near the EPS surface scavenges oxygen and produces usable carbon, thus creating favorable growth conditions for SRB, which in turn produce sulfides (S^{2-}) and hydrogen sulfide (H_2S). H_2S is a reductant that reacts with oxygen. Thus, once established, SRB consortia can protect themselves against oxygen. There is a competition between methanogenic and sulfate-reducing bacteria for available electron donors, especially H_2 and acetate (H_3COO^-), and as long as SO_4^{2-} is present the sulfate reducing bacteria are favored (Brock *et al.*, 1994). Characklis and co-workers (Lee *et al.*, 1994) reported that under totally anaerobic conditions, the corrosion rate of mild steel was not controlled directly by the SRB activities. Instead, it followed first-order kinetics with respect to suspended ferrous sulfide (FeS) concentration.

Biofilms influence the corrosion processes by changing the local chemistry near the metal surface (Lee *et al.*, 1994). Areas under respiring cell clusters become anodic. Conversely, surrounding areas become cathodic where the opposite of oxygen reduction occurs. The typical redox reactions for the corrosion of iron surfaces are:

anodic reaction	$4Fe$	$\rightarrow$	$4Fe^{2+} + 8e^-$
dissociation of water	$8H_2O$	$\rightarrow$	$8H^+ + 8OH^-$

cathodic reaction	$8H^+ + 8e^-$	$\rightarrow$	$8H$
depolarization by SRB	$SO_4^{2-} + 8H$	$\rightarrow$	$S^{2-} + 4H_2O$
corrosion products	$Fe^{2+} + S^{2-}$	$\rightarrow$	FeS
	$3Fe^{2+} + 6OH^-$	$\rightarrow$	$3Fe(OH)_2$

The overall reaction can be written as

$$4Fe + SO_4^{2-} + 4H_2O \rightarrow 3Fe(OH)_2 + FeS + 2OH^-$$

The cathodic depolarization theory postulates that SRB can remove hydrogen from a cathodic area by the hydrogenase enzyme. Without the presence of SRB, the process stops at the cathodic reaction step, because the surface would be covered by a layer of hydrogen.

Some dissolved oxygen (DO) is always present in water treatment plant (WTP) final effluents and so the question is whether the typically occurring process of DO reduction in actual distribution networks ever proceeds to such an extent which favors the existence of SRB. Even if complete exhaustion of DO is not reached in the macroscale, localized anaerobic conditions may exist in the EPS.

The presence of iron-oxidizing bacteria, such as *Gallionella, Sphaerotilus, Leptothrix* and *Crenothrix*, may also contribute to severe corrosion damage. These bacteria oxidize ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), which in turn readily reacts with the chloride ion (Cl^-) and produces a highly aggressive ferric chloride ($FeCl_3$).

The effect of temperature on submerged metal corrosion is twofold. Increasing temperature increases diffusivity of oxygen to the metal surface as well as the rate of corrosion reaction at the surface according to the Arrhenius equation resulting in enhanced corrosion. On the other hand, increased temperature leading to a lower solubility of oxygen, reduces corrosion. The former effect is often dominant (Borenstein, 1994) and so an increase of corrosion activity would generally be expected at higher temperatures. Since corrosion products support the

growth of specific microorganisms, and higher steady-state HPCs associated with higher temperature (Ollos *et al.*, 1996), an increased amount of biofilm is expected to be present at elevated temperatures.

pH is important in governing the rate of corrosion. A drop in pH is a remarkable indication of imminent corrosion in a system (Borenstein, 1994). The actual pH at a submerged metal surface can differ greatly from that in the bulk liquid phase. Neutral pH is often favored from a corrosion control viewpoint (Borenstein, 1994). While low pH increases the corrosion of most construction materials, a basic environment (pH > 7) may have a positive or negative impact depending on the material. Metals such as iron, nickel, cadmium and magnesium corrode to a lesser extent at elevated pH values, whereas other elements like aluminum, zinc and lead corrode excessively at high pH. The corrosion rate of noble metals such as gold and platinum is not affected by pH.

An increase in flow velocity will improve mass transfer and increase corrosion rates when diffusion is the rate limiting step. Mechanical corrosion of the supporting substratum is influenced by the kinetic energy of the flow. Erosion corrosion and cavitation are the main destruction processes. Borenstein (1994) suggested 1.2 m/s (4 fps) flow velocity in a water-steel system which leads to an excessive corrosion of the metal surface. Actual distribution system flow velocities are generally restricted to an upper limit of 1 m/s (3.2 fps), primarily to eliminate excessive energy losses. This limit value is also favorable from a mechanical corrosion viewpoint.

The concentration of cations and anions in the liquid phase has a significant impact on corrosivity (Borenstein, 1994). The hardness ions, calcium (Ca^{2+}), magnesium (Mg^{2+}) and bicarbonate (HCO_3^-), tend to be inhibitive and suppress corrosion. Chloride (Cl^-) and sulfate (SO_4^{2-}) ions, on the other hand, promote the rate of corrosion.

Cathodic protection is a method of controlling corrosion by making the protected metal a cathode by means of (1) an impressed direct current or (2) attachment to a sacrificial anode. The exact mechanism of MIC is still being debated and many important questions are not answered at all or answered only by speculation.

3.8 MODELS OF DISTRIBUTION SYSTEMS

Due to the limited control of operational variables (and no control of the environmental variables) in full scale distribution system, pilot and/or bench scale models of distribution systems are better suited for research which attempts to identify cause-effect relationships.

Pilot-scale systems are pipe networks which are smaller in distance and complexity than actual distribution systems but may operate at flow rates typical in actual networks. The two most common design of pilot scale distribution systems are the once-through system (Holden *et al.*, 1995; LeChevallier *et al.*, 1990) and the recirculating system (Camper, 1996; Piriou and Levi, 1994). The once through system is essentially a plug flow reactor (PFR) where BOM is continuously consumed (utilized) as it flows down the length of the reactor. The recirculating system can be idealized as a continuously stirred tank reactor (CSTR) which offers spatial uniformity through complete mixing and identical coditions at the outlet and the inside of the reactor. The once-through system more realistically represents conditions in actual distribution systems. However, data analysis and kinetic evaluation are simplified with the recirculating design. Once-through systems also require substantially more water and space than recirculating system.

Bench scale systems, such as annular recators (ARs), are more often used for research because of their smaller size and lesser cost. ARs are essentially CSTRs and are assumed to represent a finite portion of a distribution system (Characklis, 1988). Recent applications of ARs in biofilm

research include: examining the effect of corrosion inhibitors (Rompré *et al.*, 1996) and evaluating biocide efficacy (Camper, 1996).

3.9 A BAYESIAN APPROACH TO EXPERIMENTATION

Statistical design of experiments was first introduced by Fisher in the 1920's. Factorial experiments often involve several variables examined at multiple design levels. The completion of a large number of experiments is generally not possible, or at least not practical, due to time and material constraints. The later-developed fractional factorial design technique necessitates a reduced number of experimental trials, but also introduces the disadvantage of confounding between potentially important main effects and/or interactions. Strictly speaking, (complete or fractional) factorial experiments should be designed when nothing is known about a process. In fact, some prior knowledge is almost always available (about everything) which allows design according to a Bayesian type of experimentation (Reilly, 1993).

Reverend Thomas Bayes (a Presbyterian minister) developed his famous theorem in the 1750's. After his death (1761), the Bayesian approach to experimentation was published by his friend, Richard Price, in 1762. For almost two centuries, the Bayesian design concept was not widely accepted. Opponents of the theorem argued that the input of prior information influences the outcome of the design and involves subjectivity. A counter argument to this may be that most experimentation is done not for the purpose of pure investigation into the nature of the world, but to strengthen an opinion which is already held about a process being investigated (Reilly, 1993). Before an experiment is performed, the scientist or engineer has a certain level of knowledge about the result which will be obtained. This knowledge may stem from (1) his/her own previous experience in the subject area or (2) equally if not more importantly from the findings of other researchers. Bayes' theorem describes in a fundamental way the process of learning from experience. Besides easy management of common problems, such as dropped or altered design levels during the course of experimentation, the Bayesian experimental approach

also minimizes experimental efforts, i.e. provides the most new information with the least amount of experimental trials. This is accomplished by the use of a sequential design technique and the typical update of prior covariances (i.e. assumed knowledge) before the design of each new segment. Unlike in conventional factorial design, the number of Bayesian-designed experiments is not restricted. Typically, the variances in a Bayesian design are higher than those of a fractional factorial design experiment. On the other hand, no complete confounding of the various factors exists in a Bayesian designed experiment.

Mathematical Basis of the Bayesian Approach

The Bayesian design approach involves sequential updating of the posterior covariance matrix. This can be mathematically described by the following expression (Bayes' theorem) for the conditional probability:

$$Df(\theta^*/y) \propto Df(\theta^*)Df(y/\theta)$$

where θ^* is the true value of the parameter and y is the observation vector. The posterior probability [$Df(\theta^*/y)$] is thus proportional to the prior probability [$Df(\theta^*)$] and the likelihood function [$Df(y/\theta)$] (Smith *et al.*, 1993).

For simplicity, the principles will be described here in terms of two level factorial experiments though the approach is by no means limited to them. The model (3-1) is a linear regression one and includes all possible interactions.

$$\underline{y} = \underline{X\theta}^* + \underline{\varepsilon} \qquad (3\text{-}1)$$

which may be expanded; for example, for a 2^2 factorial experiment as:

$$y_i = \theta_0^* + \theta_1^* x_{1i} + \theta_2^* x_{2i} + \theta_{12}^* x_{1i} x_{2i} + \varepsilon_i ; \qquad i = 1, 2, ...,n \qquad (3\text{-}2)$$

where y_i is the ith of n observations and is the ith element of the $n \times 1$ vector $\underline{y}$. The elements of the $n \times p$ matrix $\underline{X}$ are the coefficients of the parameters and have the values +1 and -1 in coded form. The number of parameters, equal to the number of effects, is p. For a complete factorial experiment with m factors and all interactions being considered, p is 2^m. The symbol θ represents the parameter identified by its subscripts. The corresponding effect is numerically twice the parameter. The superscript * denotes the true value of the parameter. The symbol ε_i represents the experimental error at the ith trial. The basic assumptions of this regression are: (1) the model perfectly describes all observations, (2) the independent variables are perfectly known and (3) the error vector is assumed normally distributed with mean zero and covariance matrix $\underline{I}\sigma^2$. The prior knowledge about $\underline{\theta}^*$ is represented by

$$\underline{\theta}^* : N[\underline{\alpha}; \underline{U}] \tag{3-3}$$

That is, the knowledge held about $\underline{\theta}^*$ before the experiment can be expressed by the multivariate normal distribution with mean $\underline{\alpha}$ and covariance matrix $\underline{U}$, where $\underline{\alpha}$ is a $p \times 1$ known vector and $\underline{U}$ is a $p \times p$ known positive definite matrix. The diagonal $\underline{U}$ values shows the assumed prior knowledge about the mean, main effects, and interactions. The magnitude of the off-diagonal elements of $\underline{U}$ shows the strength of correlation between the parameters.

Application of Bayes's theorem gives the posterior distribution of $\underline{\theta}^*$ as

$$\left(\underline{\theta}^* / \underline{y}\right) : N\left\{\left[\underline{U}^{-1} + \left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}\left[\underline{U}^{-1}\underline{\alpha} + \left(1/\sigma^2\right)\underline{X}'\underline{y}\right]; \left[\underline{U}^{-1} + \left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}\right\} \tag{3-4}$$

Upon the design of each new trial, the prior mean ($\underline{\alpha}$) is replaced by the posterior parameter estimates $\left[\underline{U}^{-1} + \left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}\left[\underline{U}^{-1}\underline{\alpha} + \left(1/\sigma^2\right)\underline{X}'\underline{y}\right]$, and the prior covariance matrix ($\underline{U}$) is updated by the posterior covariance matrix of $\underline{V} = \left[\underline{U}^{-1} + \left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}$. In order to minimize

uncertainty $|\underline{V}|$, which is often called the generalized variance, must be minimized. This can be done before commencing actual experimentation, since $\underline{V}$ does not contain the observation vector of $\underline{y}$.

The Design Criterion

The design problem is that of choosing an n-trial fraction of a 2^m factorial experiment. Let the p x p ($p=2^m$) matrix $\underline{F}$ represent the regression matrix for the full factorial experiment with m factors and let $\underline{X}$ be the n rows of $\underline{F}$ which are chosen as the experiment. Thus $\underline{X}$ is n x p and in nontrivial cases n will be smaller than p. The chosen experiment is fully described by the m columns of $\underline{X}$ which correspond to the main effects.

The uncertainty, in the Bayesian design, is measured by a 'posterior hyper volume' (HV). The HV, which is a multidimensional analog of a joint confidence region of two parameters, can be mathematically described as:

$$HV = \frac{(\pi K)^{p/2}|\underline{V}|^{1/2}}{\Gamma(1+p/2)} \tag{3-5}$$

where Γ denotes the gamma function and K is a constant which depends on the number of parameters and the probability level. At the design stage all quantities in (equation 3-5) are constants except $\underline{V}$, and so HV is proportional to $|\underline{V}|$. Since $\underline{V}$ is always positive definite and therefore $|\underline{V}|$ is positive, it follows from equation 3-4 that HV may be minimized by choosing the experiment as that choice of n rows of $\underline{F}$ which maximizes

$$G = \left|\underline{U}^{-1} + (1/\sigma^2)\underline{X}'\underline{X}\right| \tag{3-6}$$

The determinant of G is p x p and can be expressed as

$$G = \left|\underline{U}^{-1}\right| M \tag{3-7}$$

where

$$M = \left|\underline{I} + \left(1/\sigma^2\right)\underline{XUX}'\right| \tag{3-8}$$

In equation 3-7 the first determinant is constant and so the optimality problem reduces to that of choosing $\underline{X}$ so as to maximize *M*. This is a determinant of dimension *n x n* which in nontrivial cases is smaller than the *p x p* determinant in equation 3-6. If n=1, i.e. the experiment is being designed sequentially one trial at a time, equation 3-8 leads to choosing the single trial so as to maximize $\underline{X}'\underline{UX}$, where $\underline{X}'$ is the chosen row of $\underline{F}$. This criterion has logic of its own in that it places the new trial where there is maximum prior uncertainty about its outcome.

Choosing the Optimal Experiment

In the experimental design procedure *n* rows of $\underline{X}$ are chosen, one at a time, to be locally optimal with respect to the rows previously chosen. For a particular row this is done by first assigning +1's and -1's at random as levels of the factors. Then each of these signs, one at a time in random order, is changed and the effect on the design criterion is noted. If the change does not produce an increase in the criterion, the next sign is changed. If no improvement is seen by any of the sign changes the row is accepted as locally optimal and either a new row is started or if *n* rows have already been chosen the design is considered complete. If the change in sign produces an improvement in the criterion, that change is made in the row and the testing of sign changes in random order is started again. The optimal experiment is the one which gives the best criterion value from many repetitions of this cycle. The Bayesian design concept is described in additional detail elsewhere (Reilly, 1993).

CHAPTER 4: MATERIALS AND METHODS

The bench-scale experiments employed two annular reactor (AR) systems which were fed with either synthetic or real waters. The operation with these distinct water sources was similar but not identical. Section 4.1 introduces the physical setup and operation of the AR systems with both synthetic and real waters. Biofilm accumulation was investigated on the surface of four different pipe materials. Section 4.2 introduces these, so called, biofilm coupons or substrata. All the considered bacterial enumeration techniques required the biofilm to be removed from the substrata and be suspended in a liquid phase. HPC sampling and biofilm removal protocols, as well as the description of the applied bacterial quantification and HPC reporting techniques are introduced in Section 4.3. The background BOM concentration of synthetic waters was intentionally minimized before feeding into the ARs so that the nutrient level could be controlled in the experiments by the introduction of known amounts of a BOM cocktail directly into the reactors. BOM cocktail components and preparation protocols are described in Section 4.4. The disinfectant type and residual were two of the investigated variables in the experimental design. Section 4.5 introduces the applied disinfectants and their preparation protocols.

4.1 EXPERIMENTAL SETUP

Bench-scale experiments employed two annular reactors (ARs), as developed by the Center for Biofilm Engineering at Montana State University (MSU) and provided by BioSurface Technologies Corporation (Bozeman, Montana). Full descriptions of the reactors have been provided elsewhere (Characklis, 1988). The bench scale AR assembly (prior to the installation

of a temperature control system, insulation etc.) is shown photographically in Figure 4.1. Briefly, each reactor consists of a stationary outer cylinder which holds twelve flush-mounted removable coupons (beneath the rubber stoppers). The rotational speed of the inner drum determines the shear stress at the inner wall of the outer cylinder. The total liquid volume in each reactor is 685 mL and the hydraulic detention time (HDT) is controlled by the influent feed rate. Thus, the HDT and the shear stress are independently controlled parameters (dilution rate of 0.5 h^{-1} was used in all the experiments). Each reactor performs essentially as a continuously stirred tank reactor (CSTR), representing a finite portion of the distribution system. Consequently, relating results from bench-scale experiments to full-scale systems involves considerable extrapolation (Gagnon *et al.*, 1997b). Gjaltema *et al.* (1994) critically evaluated the AR and found that non-homogeneous flow patterns existed which affected biofilm accumulation on the polycarbonate coupon surface. It was concluded that the ARs are less suited to quantitative physiological studies.

In general, the ARs were operated in parallel, to allow two conditions to be evaluated simultaneously. Typically, two coupons (i.e. duplicate) were aseptically removed from an AR for biofilm HPC quantification upon a sampling procedure. The removed coupons were replaced by the same presterilized substratum to maintain consistent shear conditions in the ARs. Excluding the preliminary experimental phase (Chapter 6), liquid phase temperature control was provided in each AR system by means of a single refrigerating circulator unit. The Lauda RM6-S circulator (Brinkmann Instruments Ltd., Mississauga, Ontario) had a temperature control range of -20 to 120°C with an accuracy of ±0.02°C. The AR was fitted with PharMed® tubing (Norton CO.). All exposed surfaces (e.g. AR, tubing, feed tank) were covered by black plastic to reduce the potential for phototrophic growth in the bench scale reactor system. An online data acquisition system recorded physico-chemical system conditions including liquid phase temperature in the AR.

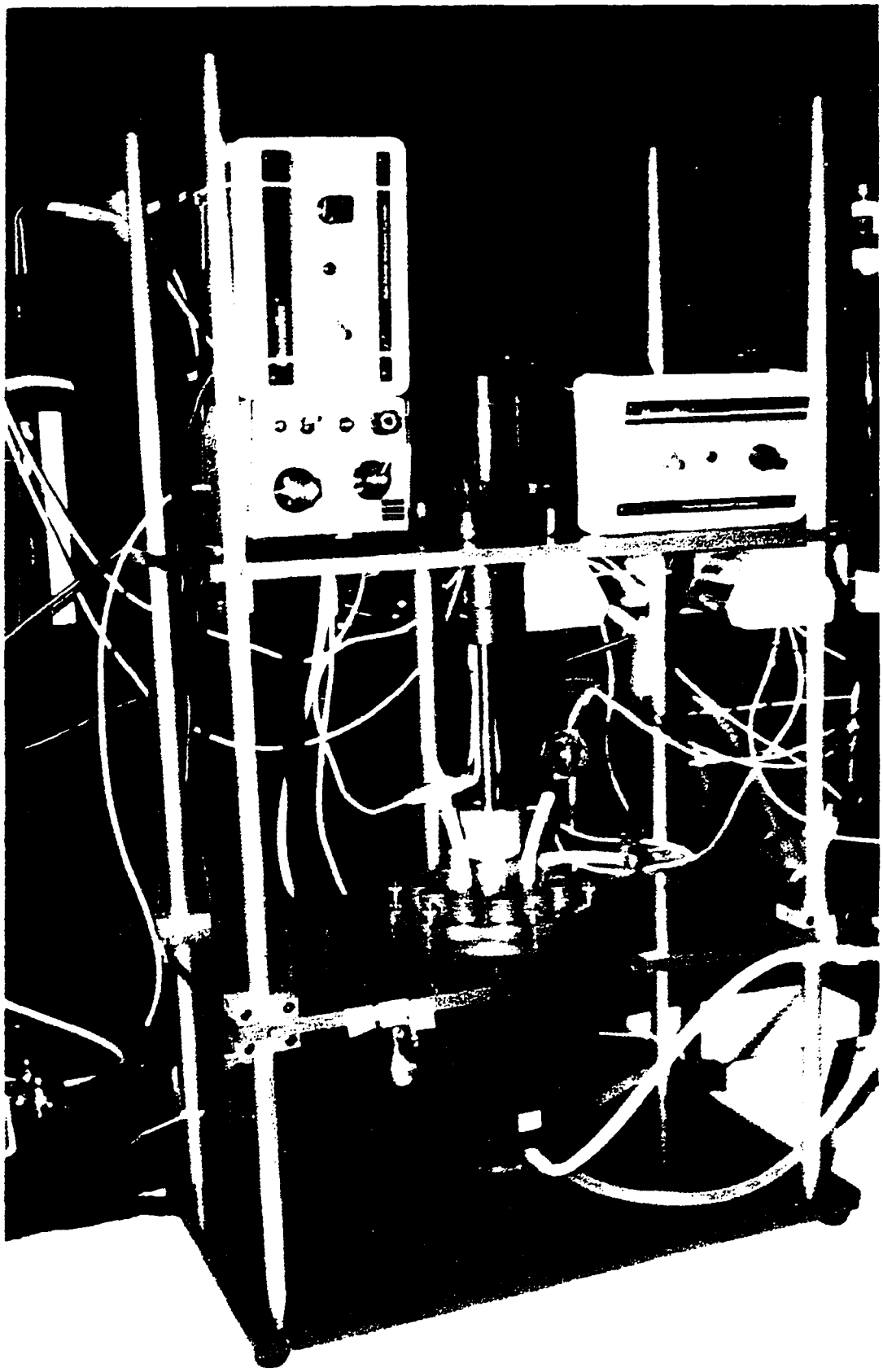


Figure 4.1: Annular reactor (photo)

Prior to use the AR was washed with hot double distilled water in a dishwasher. The AR was then fitted with tubing and autoclaved at 121°C and 101 kPa for an exposure time of 15 minutes. After cooling, the AR assembly was put in operation expeditiously to maintain sterile integrity.

In terms of feed water, the ARs were operated by utilizing either (1) synthetic water or (2) real waters. The operation with these distinct water sources is different, therefore discussed separately in the following sections. The AR effluent was discharged to waste.

4.1.1 OPERATION WITH SYNTHETIC WATER

The bench scale experimental setup (flow diagram) for one AR operating with synthetic water is shown in Figure 4.2. The synthetic feed water, one or two nutrient cocktails and a chlor(am)ine solution could be fed separately to each reactor as required. The influent flow rate of other than synthetic water influent flows was selected so that the synthetic water feed rate (5.1 mL/min) contributed about 90% of the total reactor throughput. This was necessary to maintain a consistent ratio of synthetic and non-synthetic water sources throughout the experiments which, according their design (Section 5.2), were performed in the presence or absence of an inhibitor and/or a BOM supplement.

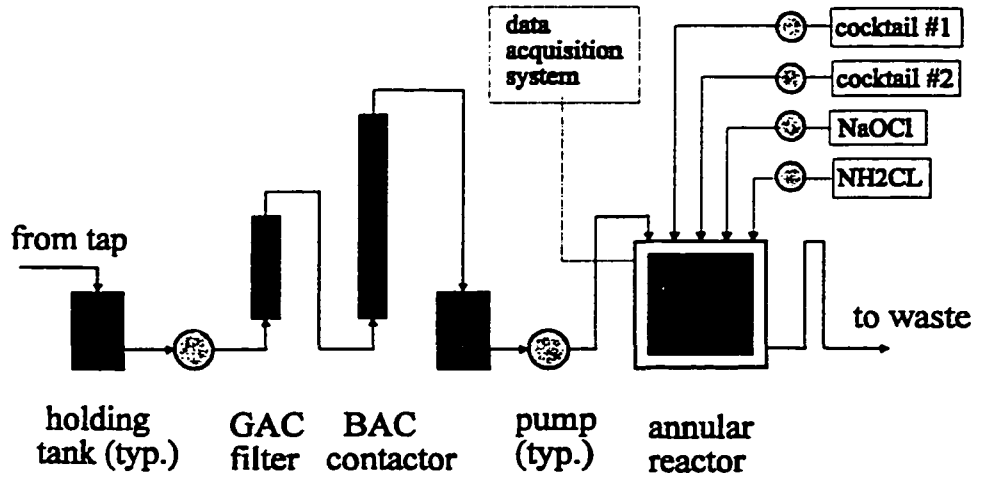


Figure 4.2: Bench scale experimental setup - synthetic water experiments

The feed water used in these experiments was a groundwater with a free chlorine residual of less than 0.3 mg/L (tapwater at the NSERC Chair for Industrial Research in Water Treatment laboratories at the University of Waterloo). This was treated prior to use to remove residual chlorine and minimize background BOM concentration by passing the feedwater through a granular activated carbon (GAC) contactor and a biologically active carbon (BAC) filter in series with a combined empty bed contact time (EBCT) of 30 minutes (Figure 4.2). Both units were operated in the upflow mode. The media of the GAC contactor was replaced by new media on a calculated three-month basis to prevent the state of complete exhaustion of the column. No replacement of media in the BAC column was necessary throughout the experimental period. Recirculated temperature control liquid was supplied to the jacketed carbon filter units and the submerged ARs (temperature control system is not indicated in Figure 4.2 for clarity).

4.1.2 OPERATION WITH REAL WATER

A flow diagram of the experimental system for one AR is shown in Figure 4.3. These experiments were performed with real waters supplied from two water treatment plants in Ontario. WTP 'E' (Appendix B/4) utilized an agriculturally and municipally impacted river of moderate total organic carbon (TOC) and hardness as its water source. The treatment applied chemically enhanced sedimentation, ozone oxidation, rapid gravity biologically active carbon (BAC) filtration and final chlorine-based disinfection. The two sampling locations for the water supplied to the ARs were the influent and effluent of one of the BAC filters of the surface WTP. BOM concentrations were about 600 µg/L and 150 µg/L in the partially treated filter influent and effluent, respectively. These values represent carboxylic acid concentration (Emelko *et al.* 1997), which were the major identified BOM components at this plant. WTP 'C' was supplied with a low iron concentration (0.03 mg/L) groundwater and applied prechlorination and direct filtration in its treatment process. Samples in this site were collected from the raw groundwater with an estimated BOM concentration of 50 µg/L.

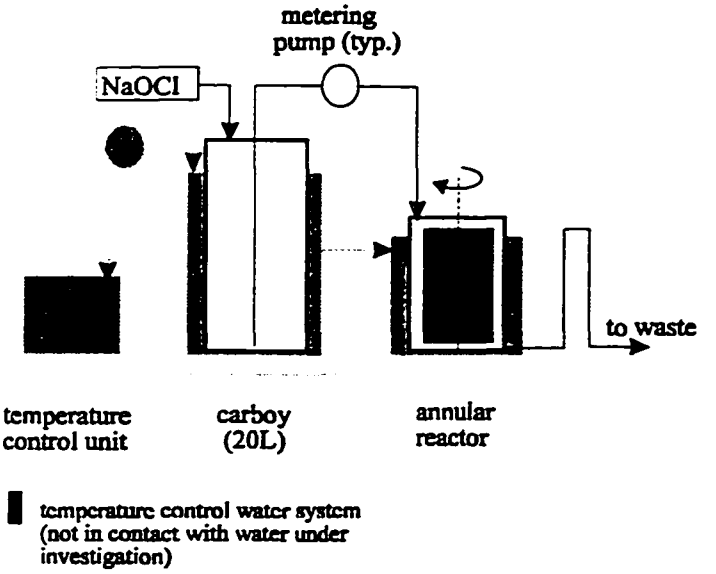


Figure 4.3: Bench scale experimental setup - real water experiments

Kaplan *et al.* (1994) surveyed 109 treated waters across North America and found that assimilable organic carbon (AOC) concentrations ranged from 18 to 322 µg/L. In another full-scale survey, LeChevallier *et al.* (1996) found that AOC levels ranged from 1 to 1300 µg/L in finished waters. The above AOC values were reported in acetate carbon (C) equivalents.

After 20 minutes flushing of the sampling ports at both facilities, water samples were collected in 20 L carboys every second day throughout each experiment. The carboys were transported to the NSERC Chair for Industrial Research in Water Treatment laboratories at the University of Waterloo, where they replaced in-place carboys upon depletion, allowing an essentially continuous feed of the water to the ARs.

A calculated shear level of 1.2 N/m^2 was applied at the outer wall. This corresponded to a flow velocity of about 0.9 m/s in a 100 mm smooth pipe. The actual water-holding carboys and both ARs were submerged in the recirculated temperature control liquid. Additional operating considerations specific to individual experiments are presented in Chapter 8.

4.2 SUPPORTING SURFACES

A recent survey, conducted in the UK, suggests that plastic accounted for at least 81% of newly-laid pipes in drinking water distribution systems (Bennett, 1996). Although plastic pipes are typically used in North-America for distribution systems since the 1960's, corrosive pipes still represent a significant portion of networks in particular cities. The four main types of plastic pipe used in the water industry are: (1) polyethylene (PE), (2) polyvinyl chloride (PVC), (3) polypropylene (PP) and (4) glass-reinforced plastic (GRP). Nevertheless, PVC is, by far, the most often used plastic pipe material. Iron based pipe materials in distribution systems include (1) unlined ductile iron, (2) cement lined ductile iron, (3) white cast iron, and (4) gray cast iron. Copper is a common household plumbing material. Stainless steel (SS 304) pipes are occasionally used for indoor piping at water treatment/distribution facilities. Ductile iron (DI) represents the majority of iron based service lines (Bennett, 1996).

Biofilm supporting characteristics of four different materials, (1) polycarbonate (PC), (2) mild steel (MS), (3) stainless steel (SS 304), and (4) unlined ductile iron (DI), were investigated in the bench scale research program. With the understanding that they are seldom or hardly ever used in actual distribution systems, PC and MS were selected to conform to common practice of other researchers and, in case of MS, to represent a worst case scenario for the water industry. SS 304 and especially DI, being actual pipe materials, were of significant interest for the research.

All removable flat coupons were manufactured to a size of 180 x 200 mm (36 cm^2 surface area) with a thickness of 1/16" to fit into the grooving of the ARs. Since they were readily available in sheets, the fabrication of polycarbonate, mild steel and SS 304 coupons was relatively simple (and also inexpensive). The manufacturing of DI coupons was more difficult (and expensive) since this material is available only in tubular form. The Engineering Machine Shop at the University of Waterloo had the capacity to cut out the required coupons from an 1220 mm (48") diameter actual unlined ductile iron pipe ring as supplied by Canada Pipe Company Ltd. (Hamilton, Ontario). The exposed DI coupon surface is that of the undisturbed original inner

pipe surface. While PC, MS, and SS 304 coupons were available right from the beginning, DI surfaces, due to their more difficult manufacturing process, became available later in the preliminary experimental phase.

4.3 BACTERIAL SAMPLING AND ENUMERATION PROCEDURES

4.3.1 BIOFILM SAMPLING AND REMOVAL TECHNIQUES

With favorable environmental conditions, biofilms can establish on submerged surfaces in a short period of time. Biofilms are generally quantified from surfaces of known surface area. In AR experiments, this is typically achieved by the removal of flush mounted biofilm coupons. A randomly chosen rubber cap is aseptically removed from the reactor (Figure 4.1) and the accessible coupon is removed using 70% ethanol sterilized pliers in the vicinity of a gas flame. The biofilm must then be removed from the coupon surface as expeditiously as possible to prevent potential contamination of the samples. The investigated biofilm removal techniques were: (1) utility knife (later referred to as 'knife'), (2) scoopula and (3) stomacher. Most researchers (e.g. Camper *et al.*, 1996; Baribeau *et al.* 1996) removed attached bacteria from biofilm coupons with a utility knife.

Utility Knife Biofilm Removal

After removal from the AR, the removed coupon is held in the vicinity of a gas flame over a 250 mL beaker which contains 100 mL sterile double distilled water. By scraping ten times, biofilm is first removed from the upper part of the coupon using a 70% ethanol sterilized utility knife. Between each scraping the knife is rinsed in the double distilled water. Then, biofilm from the lower coupon surface is removed in a similar way. The knife is typically held perpendicular to the surface with a gentle applied pressure towards it. Biofilm is intentionally removed only from the inner coupon surface. After scraping, the beaker is covered with aluminum foil, homogenized by simple shaking for 1 minute and enumerated by a standard plate count technique later described. The elapsed time between sampling and plating is typically less than

45 minutes. The advantage of this method is its simplicity, however scraping could compromise sterility and be subject to variation due to human error.

Scoopula Biofilm Removal

After removal from the AR, the removed coupon is held in the vicinity of a gas flame over a 250 mL beaker which contains 100 mL sterile double distilled water. By ten times scraping, biofilm is first removed from the upper part of the coupon by a 70% ethanol sterilized scoopula. All biofilm removal and enumeration procedures are identical to the one described for the utility knife technique.

Stomacher Biofilm Removal

The randomly selected biofilm coupon is aseptically removed from the AR, using flame sterilized pliers with 70% ethanol, into a sterile stomacher bag (177 x 304 mm) in the vicinity of a gas flame. Close to the gas flame, 100 mL of sterile double distilled water is poured into the stomacher bag without contacting the edge or the inside of the stomacher bag. After sealing, the bag holding both the coupon and the liquid is placed into a Stomacher 400 Lab Blender (Seward Ltd.; London, UK) where simultaneous biofilm removal and homogenization of the biofilm sample takes place. Although both the intended and the back side of the biofilm coupons were exposed to stomaching, viable cells originating from the back side of coupons was shown to represent a consistent but relatively insignificant portion of removed total cells (Gagnon, 1997). The Stomacher was operated on normal speed (230 rpm ± 5%) for 2 minutes. A full description of the stomacher removal technique has been provided elsewhere (Gagnon *et al.*, 1998).

4.3.2 LIQUID PHASE SAMPLING

In order to get a suspended HPC profile of a reactor system, liquid phase samples were typically taken from three different locations of each train: GAC filtrate, AR influent and AR effluent (Figure 4.2). Upon sampling, the tubing is disconnected (or a three-way valve is adjusted) in the vicinity of a gas flame and the liquid is discharged into a 25 mL pre-sterilized test tube. After

capturing approximately 15 mL of liquid sample, the test tube is capped and homogenized using a vortex mixer (Fisher Vortex, Genie 2™; Fisher Scientific Corp.). Bacterial enumeration technique of the homogenized suspended HPC sample is identical to the procedure for quantifying biofilm HPCs.

4.3.3 BACTERIAL QUANTIFICATION BY STANDARD PLATE COUNT PROCEDURE

After mechanical removal from the substratum, the microbiological samples were homogenized (by Stomacher 400 Lab Blender) and the amount of viable biofilm material is typically quantified in duplicate using the spread plate technique on R2A agar (BBL Products; Becton Dickinson Microbiology Systems; Cockeysville, MD), as outlined in Standard Method 9215 C (APHA, AWWA, and WEF, 1992) and described in Section 3.3.

Heterotrophic plate counts (HPCs) were determined by serially diluting the sample into sterile double distilled water and spreading 100 μL of diluent onto R2A agar. The plates were incubated for 7 days at room temperature (20 ± 2°C). Plates counting between 30 and 300 colonies were enumerated and reported as CFU/cm^2 (CFU - colony forming unit), based on a coupon surface area of 36 cm^2. The minimum biofilm HPC level which could be reliably detected using this approach is approximately 10^3 CFU/cm^2. Double distilled water was prepared from a Milli-Q system (Millipore Corp.; Bedford, MA).

4.4 BOM COCKTAIL COMPONENTS

Since background BOM concentration was intentionally minimized in the GAC/BAC filtered synthetic water feed lines, a known amount of essential nutrients could be dosed separately into the ARs. For balanced growth in drinking water, carbon (C), nitrogen (N), and phosphorus (P) must be present in a ratio of approximately 100:10:1 (Camper, 1994b). Table 4.1 introduces

four BOM cocktail 'recipes' used by other researchers. To ensure the system was carbon limiting, ARs were dosed with an intended C:N:P ratio of 100:25:4 (molar basis). This ratio was established on the basis of a survey of literature and was confirmed in shake flask experiments. The nutrient cocktails, containing C, N, and P sources, were contained in two 4 L amber glass bottles (cocktail #1 containing the C source and cocktail #2 the N and P sources) and pumped into the ARs (Figure 4.2). By separating the nutrient solutions, the potential for unwanted bacterial accumulation in the feed tanks and/or feed lines could be significantly reduced. As an extra precaution, so called, backflow preventers were also installed in the nutrient feed lines prior to entry into the ARs. Supplemental BOM dosage was not required in real water experiments.

Table 4.1: BOM Cocktail 'Recipes'

Chemical	Researcher			
	Camper *et al.* (1996)*	LeChevallier *et al.* (1990)**	de Beer *et al.* (1994b)	Rittmann and Snoeyink (1984)
K_2HPO_4	7.0 g/L	0.7 mg/L	4 mM	21.8 mg/L
KH_2PO_4	3.0 g/L	0.3mg/L	2.2 mM	8.5 mg/L
$(NH_4)_2SO_4$	1.0 g/L	0.01mg/L	0.76 mM	-
$MgSO_4*7H_2O$	0.1 g/L	0.01mg/L	-	-
$MgSO_4$	-	-	$4.1x10^{-2}$ mM	11.0 mg/L
glucose	-	1.0 mg/L	2.2 mM	-
NaCl	-	0.01 mg/L	-	-
$CaCl_2$	-	1.0 µg/L	-	-
$FeSO_4$	-	0.1 µg/L	-	-
$FeCl_3$	-	-	-	0.15 mg/L
Na_2HPO_4	-	-	-	17.9 mg/L**
NH_4Cl	-	-	-	1.7 mg/L
$CaCl_2$	-	-	-	27.4 mg/L**
D-galactose	-	-	-	0.03-3.0 mg/L

Notes: g/L (mg/L) refers to gram (milligram) per liter of final concentration
* Carbon source: acetate, sodium benzonate, propionaldehyde, parahydroxybenzoic acid, and ethanol (concentrations not published)
** uncertain values

The nutrient cocktails consisted of filter sterilized (0.2 µm) stock solutions of 3 M sodium acetate ($C_2H_3NaO_2$) as the sole carbon source, 8 M sodium nitrate ($NaNO_3$) as the nitrogen source, and 6 M potassium hydrogen phosphate (K_2HPO_4) and 1 M potassium dihydrogen phosphate (KH_2PO_4) as phosphorus sources. The stock solutions were kept refrigerated at 4°C and diluted with sterile double distilled water for cocktail preparation.

4.5 DISINFECTANTS AND DISINFECTION PROCEDURES

To facilitate maintaining a stable disinfectant residual in the ARs, the reactors were superchlorinated for a period of about 24 hours prior to the commencement of an experiment. Following this superchlorination period, the chlorinated water was replaced with sterile double distilled water until the total residual chlorine concentration in the reactor was reduced below 0.1 mg/L. At this point regular operating conditions were established, designating time zero of the experimental run. The effects of both free and combined chlorine residuals on biofilm accumulation was investigated. Since disinfection practice for experiments with synthetic and real waters were different, these procedures will be discussed separately.

Experiments with Synthetic Water

A predetermined concentration of a disinfectant was maintained in the AR effluent by the continuous introduction of known amount of liquid chlorine or monochloramine solution directly into each AR (Figure 4.2). The disinfectant was typically applied from day 4 of an experiment when pseudo-steady state conditions (i.e. essentially constant HPC numbers) were established.

For experiments involving chlorine, a NaOCl solution, obtained from commercially available Javex (5.6% w/v), was dosed directly into the ARs at a rate of 0.25 mL/min. For experiments with monochloramine (NH_2Cl) disinfection, a preformed NH_2Cl solution was prepared with a 1.4:1 molar ratio of 1 M NH_4Cl and NaOCl (5.6% w/v). As in actual distribution systems, the pH was not adjusted. At the pH conditions experienced (7.0-7.6), the theoretical amount of NH_2Cl was formed in the 4 L amber glass dosage bottle within 1 hour, and no significant amount of other combined chlorine species were present. Approximately 50% of the NH_2Cl was converted to $NHCl_2$ in the 4 L amber glass dosage bottle within 48 hours. The amperometric titration indicated that no detectable free chlorine was present in the preformed solution. To minimize the presence of other than the required chlorine species in the AR system, the preformed disinfectant solutions were prepared daily.

Experiments with Real Water

For experiments involving the presence of a free chlorine residual, commercially available Javex (5.6% w/v) was typically added directly into the 20 L carboys (Figure 4.3) to maintain the predetermined residual in the AR effluent. Residual in the AR effluent was monitored daily and adjustments in the carboy were made if required. With the exception of two trials, the disinfectant addition was initiated after 96 hours of system start-up and residual concentrations in the AR effluent were checked daily and adjustment in the carboys was applied as needed.

For experiments with a monochloramine residual, commercially available Javex (5.6% w/v) was added directly into the 20 L carboys. This disinfection practice was adapted only with the groundwater source (Section 4.1.2) where the natural ammonia content reacted with the applied NaOCl, eliminating the need for an external ammonia supply. As in actual distribution systems, the pH was not adjusted. At naturally occurring pH conditions (7.2-7.6), the theoretical amount of monochloramine (NH_2Cl) was formed in the carboy within 1 hour, and no significant amount of other combined or free chlorine species were detected. In general, residual concentrations were checked daily and additional chlorine added to the carboys if needed to maintain the target residual in the AR effluent.

CHAPTER 5: EXPERIMENTAL DESIGN

There are numerous factors which have a significant or potentially significant impact on the growth of heterotrophic microorganisms. In such cases one of the main objectives of an experimental design is to screen the large number of potential variables and select the most important ones for detailed analysis. From among the numerous potentially important operational, environmental and water quality variables, 6 system variables were selected for detailed investigation (based on a survey of the literature). These variables or design factors were introduced in Section 2.0. For convenience, they are repeated here:

- BOM supplement (a),
- disinfectant type (b),
- disinfectant residual (c),
- shear stress (d),
- temperature (e) and,
- substratum (f).

Design factors will be referred by their designating letter (in brackets) in later parts of the thesis. The sole system response is the steady-state net accumulation of heterotrophic microorganisms (HPCs) reported as CFU/cm^2.

There were a total of 72 experiments conducted throughout the research program. This large number of trials may be grouped such as (1) preliminary experiments, (2) experiments with synthetic water, and (3) experiments with real water. The above grouping represents also the chronological order of conducting the experiments.

5.1 PRELIMINARY EXPERIMENTS

A series of preliminary trials were conducted to investigate several specific research issues. The outcome of these preliminary trials answered some fundamental questions with pronounced impact on later model building and testing trials. In particular, the preliminary experiments were concerned with: (1) familiarization with the bench scale AR system, (2) establishment of growth kinetics of heterotrophic microorganisms with special interest to the time required to establish steady-state net accumulation of HPCs, (3) the study of both HPC-supporting and corrosion characteristics of different supporting surfaces (substrata), (4) comparison of different biofilm removal and sample homogenization methods, (5) establishment of sampling reproducibility (variance), (6) reporting correlation results between biofilm HPC numbers and other environmental factors, (7) recommendation of supplementary BOM concentrations for a later research phase, (8) using real waters for bench scale experiments to develop recommendation for (real) water source(s) for use in the third research phase, and (9) establishment of the bactericidal effect of different disinfectants on investigated substrata and recommendation of 'reasonable' residual levels to be used in later research phase(s).

Due to the heterogeneous (i.e. multiple purpose) nature of the preliminary trials, an overall experimental design could not be set up in advance. Design concepts and the setup of new experiments, a few at a time, were developed as the experimentation progressed. Coded system variables along with the adopted biofilm removal method for all 38 preliminary experiments are shown in Table 5.1 according to their objectives. Some of the preliminary experiments were conducted for multipurpose analysis. For example, experiment P8 was analysed in three different cathegories; substrata, regression, and corrosion. All these preliminary experiments are assigned with the capital letter 'P' followed by the trial number.

Table 5.1: Preliminary Synthetic Water Experiments

Objective	Exp #	Variable: BOM supplement - none 0 250 µg/L 00 250 mg/L ++ 500 mg/L	Variable: Disinfectant type - Chlorine + Monochloramine	Variable: Disinfectant residual (mg/L)	Variable: Shear stress - 0.4 N/m² 0 1.2 N/m² + 2.0 N/m²	Temp (°C)	Substratum	Biofilm removal method
Familiarization with bench scale AR system								
	P1	++	-	0.2	-	24	polycarbonate	scoopula
	P2	++	-	0.1	-	24	mild steel	scoopula
	P3	++	N/A	none	-	24	mild steel	scoopula
	P4	++	N/A	none	-	24	polycarbonate	scoopula
Substrata								
	P6	-	N/A	none	-	24	mild steel	stomacher
	P7	-	N/A	none	-	21	polycarbonate	knife
	P8	-	N/A	none	-	21	mild steel	stomacher
	P9	-	N/A	none	-	19	SS 304	stomacher
	P10	-	N/A	none	-	19	polycarbonate	stomacher
	P13/1	-	N/A	none	-	19	polycarbonate	knife
	P13/2	-	N/A	none	-	19	polycarbonate	stomacher
	P14/1	-	N/A	none	-	19	mild steel	knife
	P14/2	-	N/A	none	-	19	mild steel	stomacher
	P15	-	N/A	none	-	17	polycarbonate	stomacher
	P16	-	N/A	none	-	17	mild steel	stomacher
Biofilm removal method								
	P11/1	-	N/A	none	-	19	polycarbonate	knife
	P11/2	-	N/A	none	-	19	polycarbonate	stomacher
	P12/1	-	N/A	none	-	19	polycarbonate	knife
	P12/2	-	N/A	none	-	19	polycarbonate	stomacher
	P13/1	-	N/A	none	-	19	polycarbonate	knife
	P13/2	-	N/A	none	-	19	polycarbonate	stomacher
	P14/1	-	N/A	none	-	19	mild steel	knife
	P14/2	-	N/A	none	-	19	mild steel	stomacher
Sampling reproducibility								
	P11/1	-	N/A	none	-	19	polycarbonate	knife
	P11/2	-	N/A	none	-	19	polycarbonate	stomacher
	P12/1	-	N/A	none	-	19	polycarbonate	knife
	P12/2	-	N/A	none	-	19	polycarbonate	stomacher
Regression analyses								
	P6	-	N/A	none	-	24	mild steel	stomacher
	P7	-	N/A	none	-	21	polycarbonate	knife
	P8	-	N/A	none	-	21	mild steel	stomacher
	P10	-	N/A	none	-	19	polycarbonate	stomacher
	P11/2	-	N/A	none	-	19	polycarbonate	stomacher
	P12/2	-	N/A	none	-	19	polycarbonate	stomacher
	P13/2	-	N/A	none	-	19	polycarbonate	stomacher
	P14/2	-	N/A	none	-	19	mild steel	stomacher
	P15	-	N/A	none	-	17	polycarbonate	stomacher
	P16	-	N/A	none	-	17	mild steel	stomacher
	P19	00	N/A	none	0	17	polycarbonate	stomacher
	P20	-	N/A	none	+	17	polycarbonate	stomacher
	P21	++	N/A	none	+	17	polycarbonate	stomacher
	P25	++	-	0.25	+	18	polycarbonate	stomacher
	P26	++	N/A	none	-	18	polycarbonate	stomacher

Table 5.1 cont'd: Preliminary Synthetic Water Experiments

Objective	Exp #	BOM supplement - none 0 250 µg/L 00 250 mg/L ++ 500 mg/L	Variable Disinfectant type - Chlorine + Monochloramine	Variable Disinfectant residual (mg/L)	Shear stress - 0.4 N/m² 0 1.2 N/m² + 2.0 N/m²	Temp (°C)	Substratum	Biofilm removal method
BOM level								
	P4	++	N/A	none	-	24	polycarbonate	scoopula
	P13/2	-	N/A	none	-	19	polycarbonate	stomacher
	P17	-	N/A	none	0	17	polycarbonate	stomacher
	P18	++	N/A	none	0	17	polycarbonate	stomacher
	P20	-	N/A	none	+	17	polycarbonate	stomacher
	P21	++	N/A	none	+	17	polycarbonate	stomacher
Effect of increasing disinfectant residual on net accumulation of HPCs on different substrata								
	P27	++	-	increasing	0	18	polycarbonate	stomacher
	P28	++	+	increasing	0	18	polycarbonate	stomacher
	P29	0	-	increasing	0	18	polycarbonate	stomacher
	P30	0	+	increasing	0	18	polycarbonate	stomacher
	P31	0	-	increasing	0	18	ductile iron	stomacher
	P32	0	+	increasing	0	18	ductile iron	stomacher
Corrosion								
	P8	-	N/A	none	-	21	mild steel	stomacher
	P9	-	N/A	none	-	19	SS 304	stomacher
	P14/1	-	N/A	none	-	19	mild steel	knife
	P14/2	-	N/A	none	-	19	mild steel	stomacher
Real waters								
	P33	50 µg/L*	+	0-0.02	0	14	polycarbonate	stomacher
	P34	50 µg/L*	+	0.02-0.13	0	14	polycarbonate	stomacher
	P35	50 µg/L*	+	0.18-42	0	18	polycarbonate	stomacher
	P36	50 µg/L*	+	0.03-0.4	0	18	polycarbonate	stomacher
	P37	600 µg/L**	N/A	none	0	22	polycarbonate	stomacher
	P38	150 µg/L**	+	0.15-0.5	0	22	polycarbonate	stomacher
Miscellaneous								
	P5	-	N/A	none	-	24	mild steel	knife
	P21	++	N/A	none	+	17	polycarbonate	stomacher
	P22	-	-	1.0	-	17	polycarbonate	stomacher
	P23	++	-	1.0	-	17	polycarbonate	stomacher
	P24	-	-	0.5	+	18	polycarbonate	stomacher

Note:
P33 WTP 'A' final effluent
P34 WTP 'B' final effluent
P35 WTP 'C' final effluent
P36 WTP 'D' final effluent
P37 WTP 'E' intake
P38 WTP 'E' final effluent

* estimated value
** Emelko *et al.*. (1997)
N/A not applicable
SS 304 stainless steel 304

5.2 EXPERIMENTS WITH SYNTHETIC WATER

After establishing certain groundwork with the preliminary experiments, the objective of experimentation with synthetic water was to generate data which could be used for modeling the biofilm accumulation process.

If designed according to conventional factorial design principles, the 6 variables investigated at two levels would have required 2^6=64 trials. This number of trials was excessive. Another possibility was to plan the synthetic water trials according to a conventional fractional factorial design. While a conventional fractional factorial design is a useful and elegant design tool, it assumes no prior knowledge about the subject matter. A further disadvantage of conventional fractional factorial design is that complete confounding between potentially important factors is always present. Since biofilm research during the past two decades has generated a great amount of published data (Section 3.2 and Table 5.2), it was decided to use another experimental design technique, the Bayesian approach. The principles of the Bayesian approach, which allows prior knowledge to experimentation are outlined in Section 3.9 and described in detail elsewhere (Reilly, 1993). The capital letter 'S' followed by the trial number are assigned to each experiment with synthetic water. The design of experimentation with synthetic water is described below.

As discussed previously, the Bayesian type of experimentation is based on the principle of learning from experience (Section 3.9). It is common practice to design approximately 25% of the anticipated number of trials at a time. The 26 trials with synthetic water were designed in 5 segments. Preliminary experimental results and a review of published literature (Table 5.2) provided the prior information for the design of the first segment of 7 trials. After completing the first segment of experiments, the results were evaluated and supplemented to the previous prior distribution resulting in improved prior information for the design of the second segment. The second segment consisted of 6 experiments. Since ductile iron coupons were not available for experiments in the second segment, the design was restricted to polycarbonate substrata.

Table 5.2: Prior Information from Published Literature

Source	Description	Value
Accumulation		
van der Kooij *et al.* (1993)	limiting accumulation of HPC bacteria (in the presence of a disinfectant residual)	10 µg acetate C/L
LeChevallier *et al.* (1990)	limiting accumulation of coliform bacteria (in the absence of a disinfectant residual)	54 µg AOC/L
	limiting accumulation of HPC bacteria (in the absence of a disinfectant residual)	50 µg AOC/L
	length of microbial accumulation before application of a disinfectant (in pilot pipeloops)	2 weeks
	time for steady-state biofilm to establish on pipeloop surface	2 weeks
Servais *et al.* (1992)	maximum surface colonization density of fixed biomass	2 µg acetate C/cm^2
Camper *et al.* (1994)	growth rate at 35°C per growth rate at 10°C	7
Hydraulics		
LeChevallier *et al.* (1990)	typical slow flow velocity (applied in pilot model)	0.04 m/sec
Camper *et al.* (1991b)	Residence time in AR to eliminate planktonic growth of bacteria	< 20 min
Disinfectants		
LeChevallier *et al.* (1990)	effective dosage of NH_2Cl on iron pipe surface for biofilm control	2 mg/L (residual)
	effective dosage of free chlorine on iron pipe surface for biofilm control	> 4 mg/L
	effective dosage of NH_2Cl for significant reduction in biofilm viable count	2.0 mg/L (residual) or 3.24 mg/L (dose)
	effective dosage of free chlorine for biofilm control at corrosion rates of 12-19 mils/year	> 4 mg/L (residual)
Kiene *et al.* (1993)	Chlorine concentration ↓ ⇒ rate of chlorine consumption ↑	-
Kiene *et al.* (1993)	pipe diameter ↑ ⇒ pipe wall reactivity ↓	-
Nutrients		
Servais *et al.* (1992)	S_{min} for biological stability	0.16-0.20 mg BDOC as acetate C/L
	growth rate of fixed and suspended bacteria	~ same
van der Kooij (1993)	AOC/DOC ratio for biologically stable organic carbon	1.4 µg C/mg DOC
	S_{min} for aeromonas multiplication at AOC/DOC < 1.4	< 10 µg acetate C/L
Woolschlager *et al.* (1994)	S_{min} for easily degradable organic matter	34.5 µg/L
	S_{min} for slowly degradable organic matter	259 µg/L
	BDOC/BOM	50-60%
Camper *et al.* (1994)	AOC values do not correlate in any way with growth rate	-

Table 5.2 cont'd: Prior Information from Published Literature

Source	Description	Value
Biomass		
Servais *et al.* (1992)	biomass (C) vs. bacterial number	1 μg ~ $4x10^7$
	fixed vs. free bacteria number in < 100 mm dia. pipes	53-77
	biofilm density	25 kg/m^3 or 10^{16} cells/m^3
Servais (1989)	biofilm coverage of inner pipe surface	10%
Woolschlager *et al.* (1994)	cells per μg acetate C	$4.1x10^6$
Characklis (1989)	dry cell weight / wet cell weight	0.22
	gram carbon / bacterial cell	$1x10^{-13}$
	surface area per cell	4.2-4.5 μm^2/cell
	bacterial density	1.07 kg/dm^3
	cellular carbon - ATP ratio	250 : 1
Stewart (1993)	cell surface density	$5x10^{12}$
	HPC - coliform ratio	10^4

Similarly, after completing the second segment, the results were evaluated and supplemented to the existing prior data resulting in a further improved prior knowledge for the design of the third segment. The third design segment consisted of 4 trials. Due to oversights, three experiments in the first two segments were conducted with factor level(s) contradictory to those suggested by the Bayesian design, i.e. experiment S18 adopted 0.5 mg/L monochloramine instead of the same concentration of chlorine, and experiments S19 and S20 were performed, unintentionally, in the absence of a free chlorine residual. The 6 experiments of the fifth segment were 'borrowed' from the preliminary trials. The experiments of the last two segments, although sub-optimal, were still included for modeling.

With the exception of the first segment, logarithmic steady-state biofilm HPC values were used in the experimental design. A good example for the flexibility of the Bayesian design technique is that it allows the usage of either arithmetic or logarithmic values in its design segments.

The coded design matrix of the 26 experiments with synthetic water is shown in Table 5.3. One or two variables in segment 5 of Table 5.3 were designed at a medium level (1.2 N/m^2 shear stress and 17°C temperature) providing information for possible quadratic effects. Experiments

S21, S22, S23 and S24 are replicates. The design of the third segment was more complicated due to design constraints than the design of the other segments, therefore the design of this segment is introduced in detail below. A similar design procedure was adapted for the design of the first three segments of Table 5.3.

Table 5.3: Coded Design Matrix - Synthetic Water Experiments

Design segment	Experiment #	Variable: BOM supplement - none + 500 µg/L	Disinfectant type - chlorine + monochloramine	Disinfectant residual - none + 0.5 mg/L	Shear stress - 0.4 N/m² 0 1.2 N/m² + 2.0 N/m²	Temp - 8°C 0 17°C + 26°C	Substratum - polycarbonate + ductile iron
#1	S1	-	-	-	-	+	-
	S2	-	+	+	-	-	-
	S3	+	+	+*	-	+	+
	S4	-	-	+	+	+	+
	S5	+	+	-	+	+	-
	S6	+	-	+	+	-	-
	S7	+	-	-	-	-	+
#2	S8	+	+	+	+	+	-
	S9	+	-	-	-	-	-
	S10	+	-	+	-	+	-
	S11	-	-	+	+	+	-
	S12	-	+	-	-	-	-
	S13	-	-	-	+	-	-
#3	S14	-	+	-	+	+	+
	S15	-	+	+	-	+	-
	S16	+	-	+	-	-	+
	S17	-	-	+	-	-	-
#4	S18	+	+	+	+	-	-
	S19	+	-	-	-	+	-
	S20	-	-	-	+	+	-
#5	S21 (P 10)	-	-	-	-	0	-
	S22 (P 12/2)	-	-	-	-	0	-
	S23 (P 13/2)	-	-	-	-	0	-
	S24 (P 15)	-	-	-	-	0	-
	S25 (P 17)	-	-	-	0	0	-
	S26 (P 20)	-	-	-	+	0	-

Note: * 2.0 mg/L

Optimal Design of the Third Segment

As described in Section 4.1, the two available AR systems were operated typically in parallel throughout the research project. A single refrigerating circulator unit provided the control of

liquid phase temperature in both of the parallel operated trains as introduced in Section 4.1. The limited number of available ductile iron coupons (18) did not allow simultaneous ductile iron operation of the two ARs. These physical constraints must be considered in the design of the third segment, necessitating the design of: (1) identical temperature levels for simultaneously conducted trials, and (2) different substrata for simultaneously conducted trials.

The 64 x 42 $\underline{F}$ (Appendix A/1) matrix contains all possible experiments (2^6=64) in natural order in its rows. Four-factor and interactions of higher order were assumed to have no effect on the response. From among the possible 64 experiments, 16 had already been completed at the time of designing the third segment, allowing the elimination of the completed rows from $\underline{F}$. The matrix of the remaining trials, $\underline{H}$, is therefore 48 x 42 (Appendix A/2).

The objective is to select 4 trials ($\underline{X}$) out of the 48 remaining possible trials ($\underline{H}$). This can be done 194,580 possible different ways. A code, written by P.M. Reilly for a general selection problem, was used to select the particular design of the third segment (4 additional trials). The result showed the existence of 16 equally optimal selections of $\underline{X}$. One of these was randomly selected.

The shaded rows in $\underline{F}$ indicate already completed experiments, which are identified next to the regression matrix (Appendix A/1). Matrix $\underline{H}$ is arranged in 4 sets of rows, according to the following levels of temperature and substratum respectively: + +, + -, - +, and - -. The +ve sign designates 26°C or ductile iron substratum. The corresponding values for the -ve sign are 8°C and polycarbonate. Columns *e* and *f*, corresponding to temperature and substratum, are shaded in matrix $\underline{H}$ (Appendix A/2). The third segment consists of a pair of rows, one chosen from set 1 and the other from set 2, or one from set 3 and the other from set 4, along with another similarly chosen pair. The selection pattern of the three feasible design alternatives is shown in Table 5.4.

Table 5.4: Design Alternatives of the Third Segment - Synthetic Water Experiments

Alternative #1		Alternative #2		Alternative #3	
temperature - 8°C + 26°C	substratum - polycarbonate + ductile iron	temperature - 8°C + 26°C	substratum - polycarbonate + ductile iron	temperature - 8°C + 26°C	substratum - polycarbonate + ductile iron
26°C	ductile iron	26°C	ductile iron	8°C	ductile iron
26°C	polycarbonate	26°C	polycarbonate	8°C	polycarbonate
8°C	ductile iron	26°C	ductile iron	8°C	ductile iron
8°C	polycarbonate	26°C	polycarbonate	8°C	polycarbonate

The diagonal (42 x 42) prior covariance matrix ($\underline{U}$) was constructed by utilizing assigned importances of the mean, main effects, and interactions on its diagonal. Although the importances were assigned in a scale of 1 to 10, their entry into $\underline{U}$ was in the logarithmic form. Instead of 0, the logarithm of the assigned importance of '1', a somewhat higher, 0.0016, log value was entered into $\underline{U}$ to avoid potential mishandling of the zero value. The arithmetic value of low importance is designated by '1' and refers to a case where the prior parameter estimate is believed to describe the posterior distribution with great accuracy. In other words adequate knowledge is assumed necessitating little further investigation for the parameter in question. A high importance, on the other hand, refers to circumstances where little is known about a mean, main effect or an interaction. This requires further thorough investigation of the parameters. Published research data (Table 5.2), preliminary trial experience, and intuition were the basis of assigning importances.

An importance of '5' is typically assigned to the mean, i.e. first diagonal element of the prior covariance matrix. The main effects were assigned by an importance of '10'. Assigned importances for the two order interactions are shown in Table 5.5. For example, the *ab* interaction (i.e. BOM supplement vs. disinfectant type) in Table 5.5 was given the low importance of '1'. On the other hand, the interaction of BOM supplement with disinfectant residual (*ac*) was given a medium importance of '4'. An importance of '1' was assigned to third and higher order interactions.

Table 5.5: Assigned Importances of Two Factor Interactions

Interaction	Importance (1-low, 10-high)		Interaction	Importance (1-low, 10-high)		Interaction	Importance (1-low, 10-high)	
	arithmetic	log		arithmetic	log		arithmetic	log
ab	1	0	bc	5	0.6990	ce	2	0.3010
ac	4	0.6021	bd	1	0	cf	5	0.6990
ad	2	0.3010	be	2	0.3010	de	2	0.3010
ae	3	0.4771	bf	5	0.6990	df	2	0.3010
af	1	0	cd	2	0.3010	ef	2	0.3010

Note: a - BOM
b - disinfectant type
c - disinfectant residual
d - shear stress
e - temperature
f - substratum

Replicate steady-state biofilm HPC numbers of the preliminary experiments, along with the weighted (by the number of replicates) overall mean variance (σ^2=0.0189) are shown in Table 5.6. The calculated σ^2 logarithm value was used in the design criteria (equation 3-8). Individual replicate HPC numbers are presented in Appendix A/3. Established variances of 21 preliminary experiments are contrasted to corresponding biofilm HPC mean values in Figure 5.1. The highly correlated variables in the log-log coordinate system suggest an additive nature of the variance.

Table 5.6: Replicate Steady-State Biofilm HPC Numbers - Preliminary Experiments

Exp #	'Age' of substratum	# of replicate samples	Variance		Standard deviation		Coefficient of variation	
			arithmetic	log	arithmetic	log	arithmetic	log
P2	new	6	8.77E+12	0.0306	2.96E+06	0.175	0.3989	0.0256
P3/1	used	3	2.43E+13	0.0047	4.93E+06	0.0686	0.1640	0.0092
P3/2	used	3	2.52E+12	0.0004	1.59E+06	0.0201	0.0456	0.0027
P4/1	new	3	4.36E+15	0.0422	6.60E+07	0.2050	0.4738	0.0253
P4/2	new	3	3.94E+15	0.0390	6.27E+07	0.1980	0.4025	0.0242
P7/1	new	3	7.06E+07	0.0131	8.40E+03	0.1140	0.2477	0.0253
P7/2	new	3	1.46E+09	0.0777	3.82E+04	0.2790	0.6946	0.0596
P8/1	used	3	1.31E+12	0.0600	1.14E+06	0.2450	0.5622	0.0391
P8/2	used	3	8.17E+10	0.0025	2.86E+05	0.0498	0.1106	0.0078
P9	new	6	2.34E+08	0.0053	1.53E+04	0.0726	0.1645	0.0146
P10	new	6	1.50E+8	0.0099	1.23E+04	0.0994	0.2431	0.0212
P15/1	new	4	1.26E+07	0.0017	3.55E+03	0.0417	0.0956	0.0091
P15/2	used	4	4.73E+07	0.0055	6.88E+03	0.0740	0.1639	0.0160
P16/1	new	4	9.47E+09	0.0311	9.73E+04	0.1760	0.4522	0.0333
P16/2	used	4	1.05E+10	0.0692	1.03E+05	0.2630	0.4827	0.0499
P17	new	7	2.98E+10	0.0195	1.73E+05	0.1390	0.3187	0.0244
P18	new	7	5.25E+14	0.0089	2.29E+07	0.0943	0.2269	0.0118
P19	new	8	8.21E+11	0.0037	9.06E+05	0.0605	0.1433	0.0089
P20	new	8	5.1E+08	0.0048	2.26E+04	0.0692	0.1560	0.0134
P21	new	8	6.87E+15	0.0234	8.29E+07	0.1530	0.3839	0.0184
P22/1	new	3	1.00E+12	0.0003	1.00E+06	0.0174	0.0400	0.0023
P22/2	new	3	1.04E+14	0.0169	1.02E+07	0.1300	0.3159	0.0173

weighted (by # of replicate samples) average log variance: **0.0189**

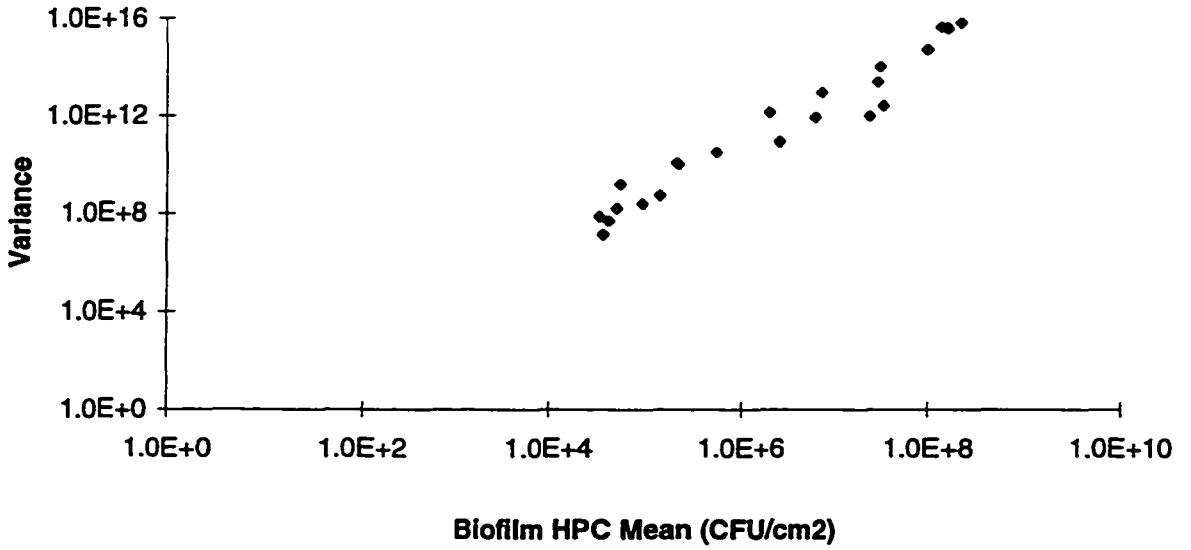


Figure 5.1: Biofilm variance vs. biofilm HPC mean
Analysed experiments: P2, P3/1, P3/2, P4/1, P4/2, P7/1, P7/2, P8/1, P8/2, P9/10, P15/1, P15/2, P16/1, P16/2, P17, P18, P19, P20, P21, P22/1, P22/2

The design criterion, as introduced in Section 3.9, can be summarized by the following simple statement: choose $\underline{X}$ optimally so as to maximize *M*

$$M = \left|\underline{I} + (1/\sigma^2)\underline{XUX}'\right| \tag{3-8}$$

where $\underline{I}$ identity matrix,

$\underline{X}$ regression matrix of chosen trials,

$\underline{U}$ prior covariance matrix (42 x 42), and

σ^2 variance

For each alternative the MATLAB codes, which calculate all possible 'M' along with the four corresponding rows of the $\underline{H}$, are shown in Appendix A/4. The letter *i* in the code stands for rows 1 to 14 (i.e. set 1), *j* for rows 15 to 23 (set 2), *k* for rows 24 to 38 (set 3), and *l* for rows 39 to 48 (set 4) of $\underline{H}$ (Appendix A/2).

From among the large number of possible determinants (194,580), the ones with the highest values (for each of the alternatives) are shown in Appendix A/5. The optimal 4 trials, designated

by the highest overall determinant value (4.92×10^{10}) of the three alternatives, are rows 10, 18, 28, and 41 of $\underline{H}$. The selected 4 rows are shaded in $\underline{H}$ (Appendix A/2). Elements 2 to 7 of the shaded rows of $\underline{H}$ show the coded design levels of the 6 independent variables in the third segment. These design levels are repeated in Table 5.3.

5.3 EXPERIMENTS WITH REAL WATER

The primary objective of the experimentation with real water was to obtain data for model testing. Another objective was to compare real waters to the synthetic water used in preliminary and synthetic water experiments. Two essential requirements governed the selection of real water sources: (1) a wide range of organic (BOM) content, and (2) disinfection by both chlorine and chloramine residuals. Experimental setup and operation with real water were introduced in Section 4.1.2.

An overview of the coded real water experimental design is presented in Table 5.7. The partially treated water 'E' (Appendix B/4), with a low ammonia content (Section 4.1.2), was spiked in the laboratory with liquid chlorine to maintain a free chlorine residual of 0.1 and 0.3 mg/L in the AR effluent. As a result of the addition of liquid chlorine, the higher ammonia content ground water, 'C', yielded a combined chlorine (mainly monochloramine) residual of 0.8 mg/L in the AR effluent. The adjusted liquid phase temperature levels in the bench scale experiments (ranging from 12 to 24°C) were the same as those of the real waters sampled. The 14 real water experiments were designed so that each condition was investigated using both polycarbonate and ductile iron substrata. The real water experiments are assigned with the capital letter 'R' followed by the actual number of the trial.

The real water experiments were built around a 2^3 factorial design. The 2^3 factorial design (experiments R1 to R8; Table 5.7) investigated the effects of (1) BOM concentration (i.e. sample obtained before or after biological filtration), (2) substratum, and (3) free chlorine residual on the steady-state biofilm HPC numbers. In conjunction with other experiments,

experiments R9 and R10 were designed to investigate potential temperature effects. Trials R11 and R12, utilizing a low organic but relatively high ammonia content groundwater, were designed to test the model for a low BOM condition and where a disinfectant residual was present in the form of mainly monochloramine. In general, a disinfectant residual was maintained after the establishment of pseudo-steady state system conditions (4 days) in experiments R1 to R12. Experiments R13 and R14 were designed to contrast the previous results to a condition where the disinfectant residual was maintained from the beginning of each trial.

Table 5.7: Coded Design Matrix - Real Water Experiments

Water source	Exp #	BOM -1 = zero +1 = 500 µg/L	Disinfectant type -1 chlorine +1 monochloramine	Disinfectant residual -1 = zero +1 = *	Shear stress -1= 0.4 N/m^2 0 = 1.2 N/m^2 +1=2.0 N/m^2	Temperature -1 = 8°C +1 = 26°C	Substratum -1 = polycarbonate +1 = ductile iron
filter effluent	R1	-0.4	-1	-0.6	0	-0.11	-1
filter effluent	R2	-0.4	-1	-0.6	0	-0.11	+1
filter effluent	R3	-0.4	-1	0.2	0	0.33	-1
filter effluent	R4	-0.4	-1	0.2	0	0.33	+1
filter influent	R5	1.4	-1	-0.6	0	0.55	-1
filter influent	R6	1.4	-1	-0.6	0	0.55	+1
filter influent	R7	1.4	-1	0.2	0	0.55	-1
filter influent	R8	1.4	-1	0.2	0	0.55	+1
filter effluent	R9	-0.4	-1	-0.6	0	0.78	-1
filter effluent	R10	-0.4	-1	-0.6	0	0.78	+1
ground water	R11	-0.8***	+1	-0.2	0	-0.11	-1
ground water	R12	-0.8***	+1	-0.2	0	-0.11	+1
filter effluent	R13 **	-0.4	-1	-0.6	0	0.55	-1
filter effluent	R14**	-0.4	-1	-0.6	0	0.55	+1

Note: Disinfectant is dosed after 96 hours of system start-up unless noted otherwise
* 0.5 mg/L free chlorine residual on both polycarbonate and ductile iron
0.5 mg/L monochloramine residual on polycarbonate
2.0 mg/L monochloramine residual on ductile iron
** Disinfectant is dosed from time zero
*** Estimated value

CHAPTER 6: RESULTS OF PRELIMINARY INVESTIGATIONS

This chapter is concerned with the presentation of research results from the preliminary investigations. System variables and responses of the 38 preliminary experiments are summarized in Table 6.1. Heterotrophic plate count (HPC) supporting characteristics of four investigated substrata will be compared in Section 6.1. Consistent biofilm removal from supporting surfaces is of utmost importance in biofilm research. Advantages and disadvantages of three different biofilm removal techniques are reported in Section 6.2. Sampling reproducibility of both biofilm and suspended HPC samples are reported in Section 6.3. A proven correlation between fixed and/or suspended HPCs and some other more readily measurable water quality parameters (e.g. turbidity) would be of importance for the water industry. Regression results are presented in Section 6.4. The possible effects of BOM concentration on the limitation of biofilm accumulation processes are shown in Section 6.5. Suppressive effects of increasing concentration of free and/or combined chlorine residuals on HPC numbers in polycarbonate and/or ductile iron supported systems are presented in Section 6.6. Corrosion behavior of the investigated substrata are introduced in Section 6.7. Annular reactor (AR) experimental results with real waters are introduced in Section 6.8. A brief summary of the preliminary investigations is presented in Section 6.9.

In the absence of a temperature control unit, seasonal effect based on liquid phase temperature variation (17°C to 24°C) was unavoidable in the preliminary trials. Although it is a potential effect, the investigated range was within a normal range for mesophilic accumulation by the expected indigenous population. Therefore, temperature was not considered as a variable.

Table 6.1: Preliminary Synthetic Water Experiments - System Variables and Response

Exp #	Variable						Steady-state biofilm HPC (CFU/cm^2)		Biofilm removal
	BOM suppl ($\mu g/L$ unless noted)	Disinfectant type	residual (mg/L)	Shear stress (N/m^2)	Temp (°C)	Substratum	arithmetic	log	method
P1	500 mg/L	chlorine	0.2	0.4	24	polycarbonate	1.7×10^1	1.2304	scoopula
P2	500 mg/L	chlorine	0.1	0.4	24	mild steel	5.0×10^7	7.6989	scoopula
P3	500 mg/L	N/A	0	0.4	24	mild steel	3.0×10^7	7.4771	scoopula
P4	500 mg/L	N/A	0	0.4	24	polycarbonate	8.3×10^7	7.9190	scoopula
P5	none	N/A	0	0.4	24	mild steel	2.5×10^6	6.3979	knife
P6	none	N/A	0	0.4	24	mild steel	8.3×10^5	5.9190	stomacher
P7	none	N/A	0	0.4	21	polycarbonate	1.0×10^5	5.0000	knife
P8	none	N/A	0	0.4	21	mild steel	3.0×10^6	6.4771	stomacher
P9	none	N/A	0	0.4	19	SS 304	1.0×10^5	5.0000	stomacher
P10	none	N/A	0	0.4	19	polycarbonate	4.6×10^4	4.6627	stomacher
P11/1	none	N/A	0	0.4	19	polycarbonate	1.0×10^4	4.0000	knife
P11/2	none	N/A	0	0.4	19	polycarbonate	4.0×10^4	4.4771	stomacher
P12/1	none	N/A	0	0.4	19	polycarbonate	1.0×10^4	4.0000	knife
P12/2	none	N/A	0	0.4	19	polycarbonate	4.0×10^4	4.4771	stomacher
P13/1	none	N/A	0	0.4	19	polycarbonate	3.0×10^3	3.4771	knife
P13/2	none	N/A	0	0.4	19	polycarbonate	4.0×10^4	4.6020	stomacher
P14/1	none	N/A	0	0.4	19	mild steel	9.0×10^5	5.9542	knife
P14/2	none	N/A	0	0.4	19	mild steel	9.0×10^5	5.9542	stomacher
P15	none	N/A	0	0.4	17	polycarbonate	3.7×10^4	4.5682	stomacher
P16	none	N/A	0	0.4	17	mild steel	2.0×10^5	5.3010	stomacher
P17	none	N/A	0	1.2	17	polycarbonate	5.5×10^5	5.7403	stomacher
P18	500 mg/L	N/A	0	1.2	17	polycarbonate	1.0×10^8	8.0000	stomacher
P19	250 mg/L	N/A	0	1.2	17	polycarbonate	$> 1.0 \times 10^7$	> 7.0000	stomacher
P20	none	N/A	0	2.0	17	polycarbonate	3.3×10^5	5.5185	stomacher
P21	500 mg/L	N/A	0	2.0	17	polycarbonate	2.1×10^8	8.3222	stomacher
P22	none	chlorine	1.0	0.4	17	polycarbonate	$< 1.0 \times 10^2$	< 2.0000	stomacher
P23	500 mg/L	chlorine	1.0	0.4	17	polycarbonate	$< 1.0 \times 10^2$	< 2.0000	stomacher
P24	none	chlorine	0.5	2.0	18	polycarbonate	$< 1.0 \times 10^2$	< 2.0000	stomacher
P25	500 mg/L	chlorine	0.25	2.0	18	polycarbonate	$< 1.0 \times 10^2$	< 2.0000	stomacher
P26	500 mg/L	N/A	0	0.4	18	polycarbonate	2.9×10^7	7.4623	stomacher
P27	500 mg/L	chlorine	increasing	1.2	18	polycarbonate	N/A	N/A	stomacher
P28	500 mg/L	monochloramine	increasing	1.2	18	polycarbonate	N/A	N/A	stomacher
P29	250	chlorine	increasing	1.2	18	polycarbonate	2.0×10^6	6.3010	stomacher
P30	250	monochloramine	increasing	1.2	18	polycarbonate	2.0×10^6	6.3010	stomacher
P31	250	chlorine	increasing	1.2	18	ductile iron	5.0×10^6	6.6989	stomacher
P32	250	monochloramine	increasing	1.2	18	ductile iron	5.0×10^6	6.6989	stomacher
P33	50*	monochloramine	0-0.02	1.2	14	polycarbonate	5.0×10^6	6.6989	stomacher
P34	50*	monochloramine	0.02-0.13	1.2	14	polycarbonate	1.0×10^7	7.0000	stomacher
P35	50*	monochloramine	0.18-42	1.2	18	polycarbonate	1.0×10^7	7.0000	stomacher
P36	50*	monochloramine	0.03-0.4	1.2	18	polycarbonate	1.0×10^7	7.0000	stomacher
P37	600**	N/A	0	1.2	22	polycarbonate	5.0×10^8	8.6989	stomacher
P38	150**	monochloramine	0.15-0.5	1.2	22	polycarbonate	1.0×10^7	7.0000	stomacher

Note:

P33	WTP 'A' final effluent	P38	WTP 'E' final effluent
P34	WTP 'B' final effluent	*	estimated concentration
P35	WTP 'C' final effluent	**	Emelko *et al.* (1997)
P36	WTP 'D' final effluent	N/A	not applicable
P37	WTP 'E' intake	SS	stainless steel

6.1 SUBSTRATA

As described in Section 4.2, the four investigated pipe materials (substrata) were: (1) polycarbonate (PC), (2) mild steel (MS), (3) stainless steel (SS 304), and (4) ductile iron (DI). Not only pipe material but also the 'age' of substrata were investigated in terms of HPC supporting characteristics. Age was defined as the actual condition of a coupon, either being used the first time or reused at the time of the investigation.

Figure 6.1 shows the net accumulation of biofilm HPCs in experiments P6 to P10. Error bars are typically not indicated for clarity. As stated in Section 5.2 the calculated average log variance was 0.0189. These preliminary experimental runs suggested that at a shear stress of 0.4 N/m^2 and in the absence of both a disinfectant and a BOM supplement, steady-state HPC accumulation was higher on MS surfaces than on either PC or SS 304 substrata. This suggests that, at low BOM levels, the ability of the biofilm to sequester nutrients is enhanced on the MS substratum. In terms of HPC supporting characteristics, SS 304 and PC exhibit similar behavior.

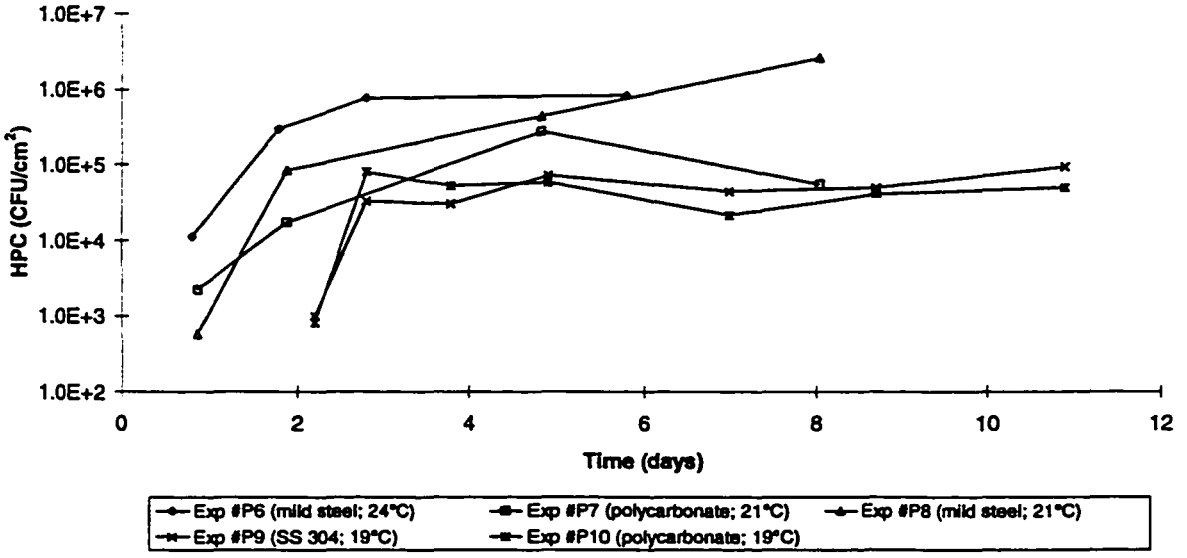


Figure 6.1: Net accumulation of biofilm HPCs in the absence of both a disinfectant and a BOM supplement at 0.4 N/m^2 shear stress (ref. Exps. P6, P7, P8, P9, and P10)
Note: biofilm is removed by stomacher

Comparison of MS and PC data (experiments P13 and P14) at 19°C liquid phase temperature in Figure 6.2 further strengthen the previous finding, i.e., regardless of the applied biofilm removal method, pseudo-steady state biofilm bacterial numbers were about two orders of magnitude lower on PC substrata. DI results are expected to be between the MS and PC data. Biofilm removal methods will be discussed in Section 6.2.

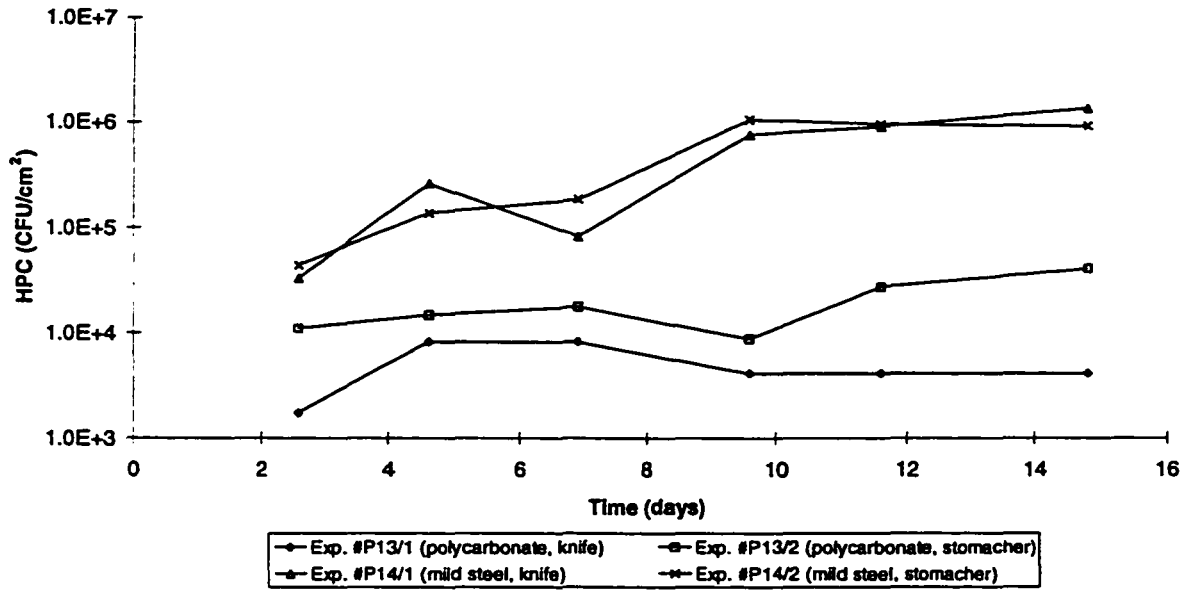


Figure 6.2: Net accumulation of HPCs on polycarbonate and mild steel substrata in the absence of both a disinfectant and a BOM supplement at 0.4 N/m^2 shear stress (19°C)
[ref. Exp. P13 and P14; Appendix B/1]

The primary objective of experiments P15 and P16 (Table 5.6 and Table 6.1) was to investigate the potential difference in HPC supporting characteristics of new and previously used surfaces. Half of the biofilm coupons in each reactor had never been used before and the other half had been used in earlier experiment(s). Sampling of the biofilm coupons was performed three times throughout the 6 day trials. Each of the data points in Figure 6.3 were generated by the duplicate analysis of each of the simultaneously removed coupon pairs of the same 'age'. A statistical analysis of the data suggested that the difference in net accumulation of HPCs on new and used substrata was not significantly large. This conclusion appears to be true for both MS and PC surfaces and based on a 5% significance level. Steady-state HPC numbers were about one order of magnitude higher on the mild steel surface.

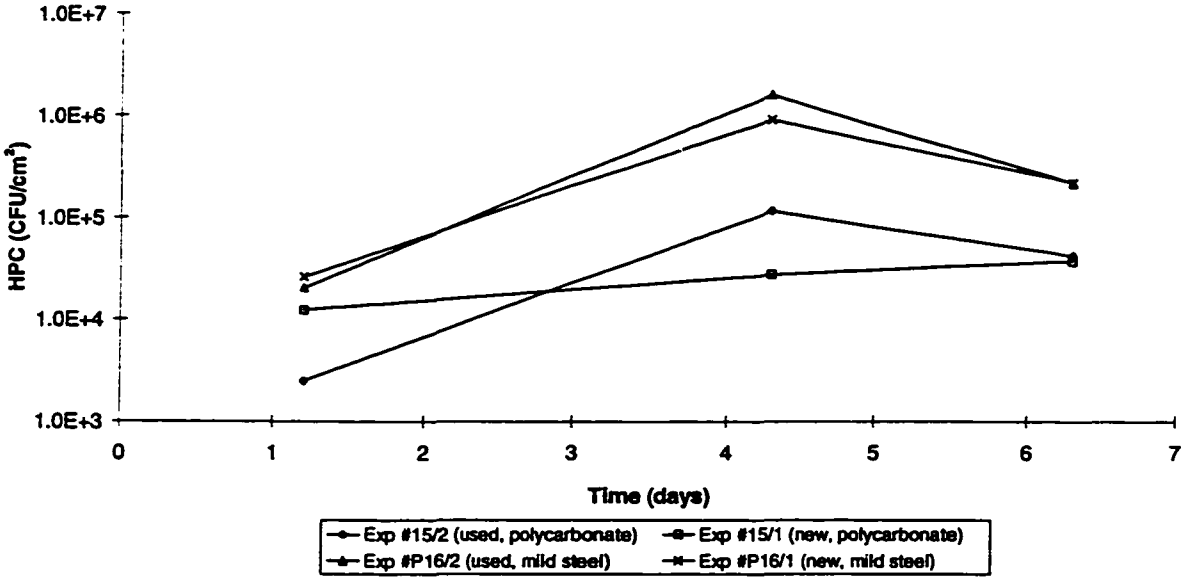


Figure 6.3: Net accumulation of biofilm HPC in the absence of both a disinfectant and a BOM supplement at 0.4 N/m^2 shear stress (ref. P15/1, P15/2, P16/1, and P16/2)
Note: biofilm is removed by stomacher

Summary

Strong evidence suggests that, in the absence of both a BOM supplement and a disinfectant at 0.4 N/m^2 shear stress, steady-state net accumulation of HPCs is at least one order of magnitude higher on mild steel than on either polycarbonate or stainless steel 304 substrata.

6.2 BIOFILM REMOVAL METHODS

The three pursued biofilm removal methods (1) utility knife, (2) scoopula, and (3) stomacher, were introduced in Section 4.3. This section is concerned with a 'statistically challenged' evaluation of the different biofilm removal techniques in terms of efficiency and consistency.

First, the utility knife and stomacher techniques were compared by a statistical significance test. The Student's t test (Reilly *et al.*, 1993) was used to assess if the sample means of these removal techniques were statistically different. Sample means were calculated by considering all data points along the net accumulation curves. The standard deviation of the population was

typically unknown, and so it was necessary to use the standard deviation of the samples as an estimate of the population standard deviation. Experiments P11 and P12 are replicates (Table 6.1) investigating the net accumulation of biofilm HPCs on polycarbonate substrata in the absence of both a disinfectant and a BOM supplement at 0.4 N/m^2 shear stress and 19°C. For sampling, two coupons were removed from an AR simultaneously. Biofilm was removed from these coupon surfaces by utility knife and stomacher, respectively. Net accumulation curves of the four sample series are shown in Figure 6.4. Statistical analytical results suggest that the difference between net accumulation of HPC numbers cannot be due to chance alone and so the biofilm removal efficiency of utility knife and stomacher are statistically different. This conclusion is based on a 5% significance level and supported by both replicate experiments. The pseudo-steady state biofilm bacterial numbers were consistently about half an order of magnitude higher with the stomacher removal technique.

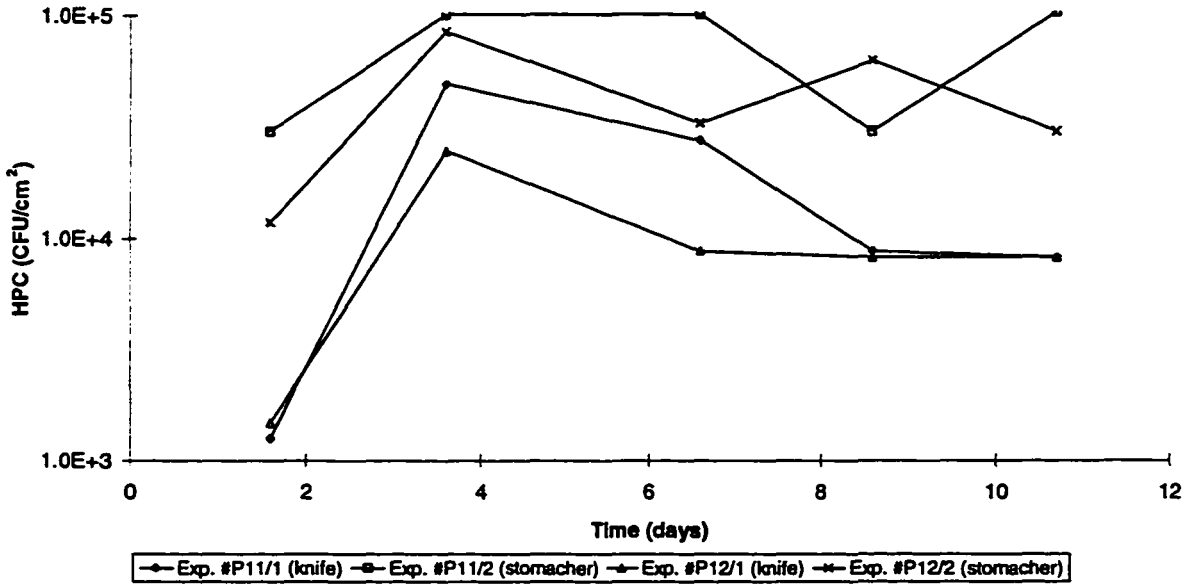


Figure 6.4: Net accumulation of HPCs on polycarbonate substrata in the absence of both a disinfectant and a BOM supplement at 0.4 N/m^2 shear stress (19°C) (ref. P11 and P12; Appendix B/2)

A similar analysis was conducted with data from experiments P13 and P14 (Table 6.1). All system conditions were identical to the ones applied in the previous experiments, except that experiment P13 was supplied with polycarbonate and experiment P14 with mild steel coupons. Figure 6.2 shows that the net accumulation of HPCs in these experiments were monitored for an

extended period (15 days). For sampling, two coupons were removed from each AR simultaneously. Biofilm was removed from the coupon surfaces by utility knife and stomacher, respectively. Statistical results (Appendix B/1) suggest that, for the polycarbonate material (P13), the difference between net accumulation of HPC numbers cannot be due to chance alone and so the utility knife and stomacher biofilm removal methods are statistically different. This conclusion is based on a 5% significance level. Similar to previous results, pseudo-steady state biofilm bacterial numbers were about half an order of magnitude higher using the stomacher removal technique.

On the other hand, for the mild steel material (P14), the difference between utility knife and stomacher biofilm removal methods could not readily be demonstrated (Appendix B/1). This may mean either that there really is very little difference between the two methods on mild steel (i.e. it could quite easily be chance that made the result seem different) or that there is a difference of some magnitude but there was not enough or good enough data to prove its existence. This conclusion is based on a 5% significance level.

The scoopula biofilm removal technique was adopted only in the first four preliminary experiments (P1 to P4; Table 6.1). It was decided that, without the rigorous statistical analysis of the data, this removal method would not be considered in further experiments. It may, however, be speculated that the removal efficiency and consistency of scoopula and utility knife is similar.

Summary of Statistical Significance Tests

On the polycarbonate substrata, biofilm removal efficiencies of the stomacher and utility knife were statistically different, being about half an order of magnitude higher by stomaching, in terms of HPC numbers. On mild steel surfaces, a statistical difference between utility knife and stomacher could not be demonstrated. In addition to these statistical results, an important aspect is that biofilm removal can be accomplished more consistently (and conveniently) by the mechanical stomaching device, thereby eliminating the 'human factor' which is always involved

when biofilm is removed by utility knife. Therefore, the stomacher biofilm removal method was chosen for use in future experiments.

6.3 SAMPLING REPRODUCIBILITY

First the biofilm data of experiments P11 and P12 (Table 6.1) will be compared. For sampling, two coupons were removed from an AR simultaneously. Biofilm from these coupon surfaces had been removed by utility knife and stomacher, respectively. Net accumulation of biofilm HPCs on polycarbonate substrata in the absence of both a disinfectant and a BOM supplement at 0.4 N/m^2 shear stress and 19°C temperature are shown in Figure 6.4. The Student's *t* test was the basis of the statistical analysis. Sample means were calculated by considering all data points along the net accumulation curves. The standard deviation of the distinct populations were typically unknown, and so it was necessary to use the standard deviation of the samples as an estimate of the population standard deviation. Results of detailed calculations (Appendix B/2) suggest that while biofilm accumulation means seem to be slightly lower in experiments P12/1 and P12/2, the difference between replicate biofilm HPC numbers of the same biofilm removal technique could not be demonstrated. This conclusion is based on a 5% significance level and supported by both of the biofilm removal techniques.

Net accumulation of suspended HPCs (both in AR influent and effluent) of experiments P11 and P12 is shown in Figure 6.5. Statistical analysis (Student's *t* test) of the data is attached in Appendix B/2. Results of this analysis suggest that the difference between net accumulation of suspended HPC numbers cannot be due to chance alone and so experiments P11 and P12 are statistically different in terms of their suspended HPC numbers. This conclusion is based on a 5% significance level and supported by corresponding suspended HPCs in AR influents and AR effluents. In AR effluents, the statistical difference in HPCs could be attributed to sloughing events (i.e. essentially randomly occuring detachment of biofilm HPCs from supporting surfaces).

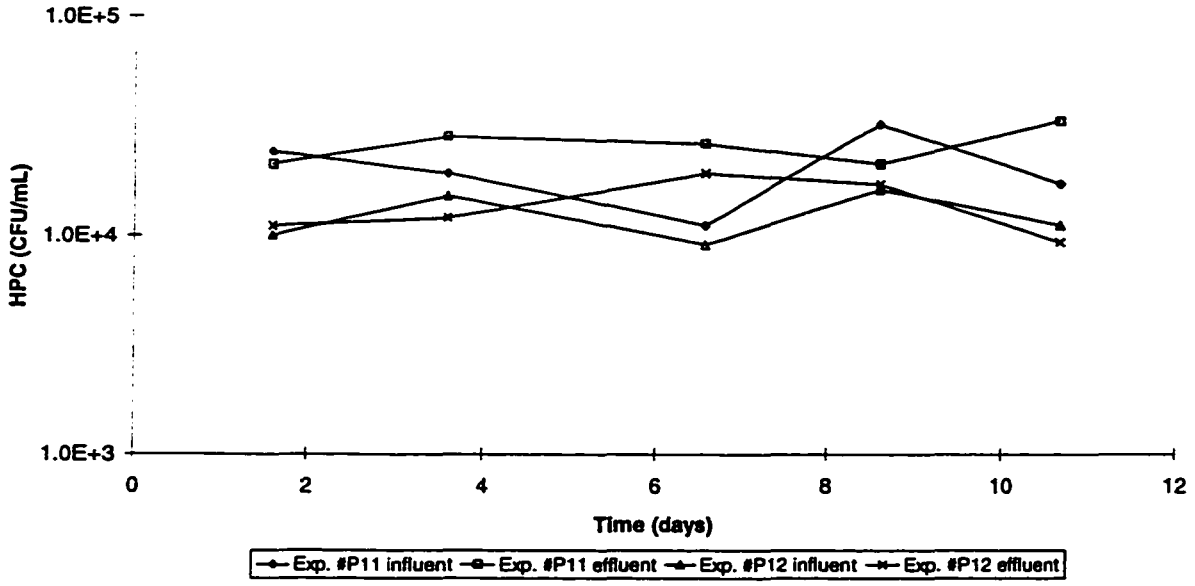


Figure 6.5: Net accumulation of suspended HPCs in the absence of both a disinfectant and a BOM supplement at 0.4 N/m^2 shear stress (19°C) [ref P11 and P12; Appendix B/2]

Summary of statistical significance tests

In terms of reproducibility of biofilm HPC numbers, the experiments failed to demonstrate a significant difference and suggest that removal is highly reproducible by either utility knife or stomacher. The reproducibility of suspended HPCs were statistically different. Both conclusions are based on a 5% significance level.

6.4 REGRESSION ANALYSIS

This section is concerned with reporting correlation levels between HPC numbers and other environmental/operational variables in the bench scale preliminary experiments. Both biofilm and suspended HPC numbers were calculated as the average of four bacterial numbers (duplicate plating of two simultaneous sampling). Since the bench scale reactors behave essentially as CSTRs (Section 4.1), HPC numbers in the reactor effluent are representative of suspended bacterial numbers in an operating AR. Six different variable pairs were analysed by linear regression. Experiment numbers, the number of data points considered in the analysis, and the strength of correlation expressed as R^2 are presented in the Table 6.2 and Table 6.3.

Twelve preliminary experiments were analyzed for the correlation strength between biofilm (CFU/cm^2) vs. AR effluent HPC numbers (CFU/mL). Results are reported in Table 6.2, supporting figures allocating these data are shown in Appendix B/3. The low or very low R^2 values (0.03-0.77 with a mean of 0.25) suggest a poor correlation between biofilm HPCs and their suspended counterparts.

Table 6.2: Regression Analyses of Biofilm HPCs versus AR Effluent HPCs (ref. Appendix B/3)

Exp #	Number of data	R^2	Reference Appendix B/3
P5	5	0.13	Fig B/3.1
P6	5	0.28	Fig B/3.2
P7	4	0.07	Fig B/3.3
P8	4	0.17	Fig B/3.4
P9	7	0.03	Fig B/3.5
P11	5	0.77	Fig B/3.6
P12	5	0.03	Fig B/3.7
P13/2	6	0.31	Fig B/3.8
P17	6	0.46	Fig B/3.9
P19	5	0.33	Fig B/3.10
P20	5	0.10	Fig B/3.11
P21	5	0.33	Fig B/3.12

mean: 0.25

Correlation results of different variable pairs are shown in Table 6.3 (supporting figures for these data are not shown). Liquid phase temperature (°C) versus steady-state biofilm HPC numbers (CFU/cm^2) on both polycarbonate (P7, P10, P11/2, P12/2, P13/2, P15) and mild steel (P6, P8, P14/2, P16) results suggest a relatively poor (mean R^2=0.43) correlation between these variables.

The turbidity of the stomached suspension (NTU) of four preliminary experiments (P19, P20, P21, P25) was compared with corresponding steady-state biofilm HPC numbers resulting in a poor correlation strength (mean $R^2 = 0.31$) for the investigated parameters.

Turbidity of the AR influent (i.e. BAC filtrate; Figure 4.2) of four experiments (P19, P20, P21, P26) was compared to suspended HPC numbers in the same AR influents. The correlation strength was found to be poor (mean $R^2 = 0.26$).

Turbidity of AR effluents of two experiments (P19, P20) was compared with suspended HPC numbers of the same AR effluents. R^2 values (0.80 and 0.81) suggest a good correlation between the turbidity and suspended HPC numbers in AR effluents.

Turbidity in a BAC influent (GAC effluent; Figure 4.2) was contrasted with suspended HPC numbers of the same sample. A single value ($R^2 = 0.45$) suggests a relatively poor agreement of the analysed data.

Table 6.3: Regression Analyses
(ref. P6, P7, P8, P10, P11/2, P12/2, P13/2, P14/2, P15, P16, P19, P20, P21, P25, P26)

Regression	Exp #	Number of data	R^2	Mean R^2
liquid phase temperature vs. SS biofilm HPC	P7, P10, P11/2, P12/2, P13/2, P15	6	0.62	
	P6, P8, P14/2, P16	4	0.24	0.43
turbidity of stomached suspension vs. SS biofilm HPC	P19	5	0.04	
	P20	5	0.17	
	P21	5	0.27	
	P25	5	0.76	0.31
AR influent turbidity vs. AR influent suspended HPC	P19	5	0.29	
	P20	5	0.09	
	P21	5	0.04	
	P26	5	0.63	0.26
AR effl. turbidity vs. AR effluent suspended HPC	P19	5	0.80	
	P20	5	0.81	0.805
BAC infl. turbidity vs. BAC influent suspended HPC	P26	5	0.45	0.45

Summary of regression analysis

Linear regression results between suspended and/or steady-state biofilm HPC numbers, and physical system conditions (e.g. temperature) showed typically poor correlation. The poor

agreement of the data was anticipated and could be explained by the complex nature of the biofilm phenomena. A higher correlation (R^2 = 0.81) exists between suspended HPCs and turbidity in AR effluents. This suggests the possibility of turbidity measurement and analysis as a surrogate of the more cumbersome techniques involved in quantification of suspended HPCs.

6.5 BOM LEVELS

BOM concentration in drinking water supply lines may range from 50 to 1000 µg/L. The three planned levels of BOM supplements in the experimental design were 0 µg/L, 250 µg/L, and 500 µg/L. Due to a calculation error, twelve preliminary experiments (P1, P2, P3, P4, P18, P19, P21, P23, P25, P26, P27, and P28) were conducted at three orders of magnitude higher BOM dosages than planned (Table 6.1), however, the results provide some useful information and are therefore presented.

Figure 6.6 shows the influence of BOM and shear on net accumulation of biofilm HPCs. The investigated BOM concentrations were 0 mg/L and 500 mg/L. The three applied shear levels were 0.4 N/m^2, 1.2 N/m^2, and 2.0 N/m^2. All these experiments were performed with polycarbonate substrata and in the absence of a disinfectant. The impact of BOM supplement is clearly evident. The increase of a BOM supplement from 0 to 500 mg/L typically resulted in a three order of magnitude increase in steady-state biofilm HPC numbers (Figure 6.6). The effect of shear appears to be more important at lower BOM levels. Without a BOM supplement, the increase of shear from low to medium level resulted in about one and a half order of magnitude increase in biofilm bacterial numbers. Without BOM supplement, the further increase of shear from medium to high level resulted in a slight decrease of steady-state HPC numbers. This suggests that at low BOM levels and low shear rates (or corresponding pipe velocity), the overall accumulation of a biofilm is mass transfer limited. Above a certain shear level, the biofilm accumulation may become bioreaction limited and/or the detachment process may become more important, thereby limiting net biofilm accumulation. At high BOM level, the

effect of shear appeared to be less important. Regardless of the applied shear conditions, steady-state biofilm HPCs were established at about 10^8 CFU/cm^2 at 500 mg/L BOM supplement.

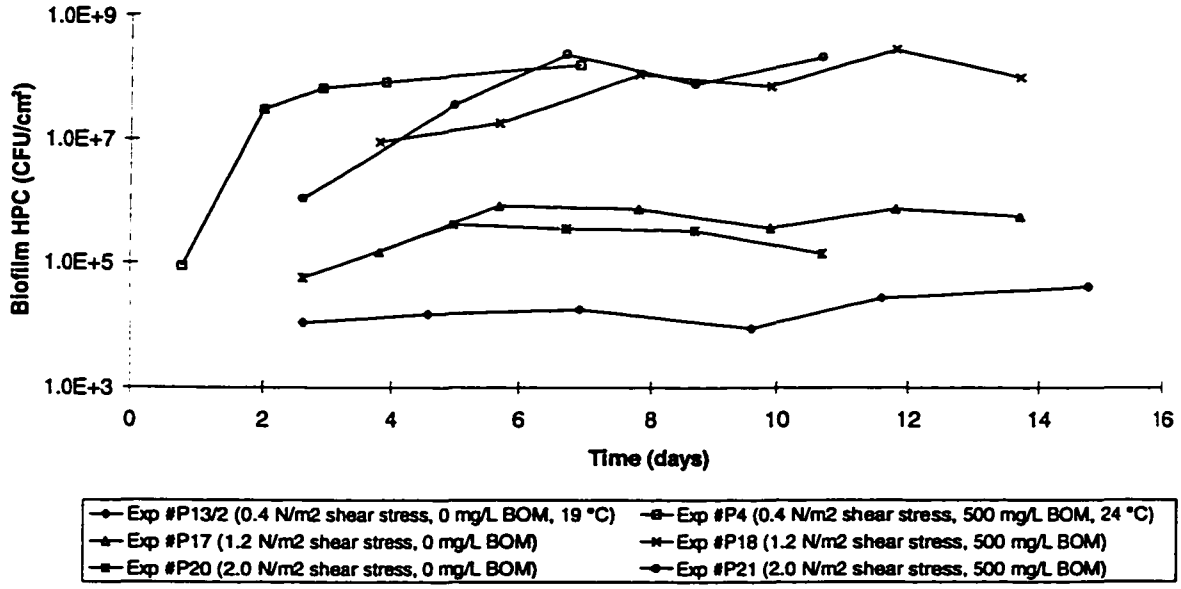


Figure 6.6: Net accumulation of HPCs on polycarbonate substratum in the absence of a disinfectant at 17 °C (unless noted otherwise) (ref. P4, P13/2, P17, P18, P20, and P21)
Note: supplemental BOM concentration in the *mg/L* level

Summary

Under the conditions tested and in the absence of both a BOM supplement and a disinfectant, the overall net accumulation of a biofilm appears to be mass transfer limited. With BOM supplement (500 mg/L) but still in the absence of a disinfectant, biofilm accumulation may become bioreaction limited and/or the detachment process may become more important.

6.6 EFFECT OF INCREASING DISINFECTANT RESIDUALS ON NET ACCUMULATION OF HPCs ON DIFFERENT SUBSTRATA

This section is concerned with the data analysis of those preliminary experiments (P29 to P32; Table 6.1) which involved increasing disinfectant dosages on different supporting surfaces. Two disinfectants (chlorine and monochloramine), and two supporting surfaces (polycarbonate and

ductile iron) were investigated (2^2 factorial design) using a 250 μg/L BOM supplement. As described in section 6.5, experiments P27 and P28 were conducted at higher than the designed BOM level, consequently they were repeated with the planned BOM concentration (P29 and P30). Preparation and dosage procedures of the employed disinfectants were introduced in Section 4.5. The objective of these experiments (P27 to P32) was to establish residuals of the investigated disinfectants which suppress biofilm HPC numbers to a level just above the detection limit of the enumeration method, so the established residuals can guide the design of later phases of the research.

Net Accumulation of Biofilm HPCs on Polycarbonate Substrata with Gradually Increasing Liquid Chlorine Dosage (Figure 6.7)

Results of experiment P29 are shown in Figure 6.7. Net accumulation of biofilm HPC numbers on polycabonate (PC) substrata with gradually increasing liquid chlorine dosage in the presence of 250 μg/L BOM supplement at 1.2 N/m^2 shear stress and 18°C liquid phase temperature were investigated. Previously conducted preliminary trial results suggested that pseudo-steady state system conditions were typically established well within the first 10 days of operation. Therefore, while maintaining normal operation conditions, the low level dosage of the disinfectant (0.7 mg/L influent free chlorine) was initiated at day 10. In the first six days of disinfection, a gradual build-up of free chlorine residual in the AR effluent was observed while free chlorine concentration in the AR influent was maintained essentially constant at 0.7 mg/L. At a low level (0.2 mg/L) of free chlorine system residual, half an order of magnitude increase of biofilm HPC numbers was observed. At and above a free chlorine system residual of about 0.3 mg/L, a monotone decline of HPC numbers was apparent. At about 0.5 mg/L free chlorine system residual (or 1.0 mg/L influent free chlorine concentration), the net accumulation of biofilm HPC numbers was 10^4 CFU/cm^2 (Figure 6.7) which was slightly over the reliable detection limit of about 10^3 CFU/cm^2 (APHA, AWWA, and WEF, 1992). Biofilm HPCs were completely suppressed at about 1.0 mg/L free chlorine system residual. The disinfection was abandoned at day 26 of the experiment, when the free chlorine system residual was about 1.6 mg/L. The system was left operating at otherwise normal conditions for another 4 days. At day 29 and 30 biofilm HPC samples were taken from the residual free system. Still growing HPC

numbers were recorded in the 10^6 CFU/cm^2 range, suggesting the possibility of an elevated accumulation of biofilm HPCs after the cease of disinfection. Experiment P29 was terminated at day 30.

While the influent combined chlorine concentration was negligibly small, a low concentration (0.2 mg/L) of combined chlorine residual was present in the system throughout most of the disinfection period. Based on these results, a free chlorine system residual of 0.5 mg/L on polycarbonate supporting surfaces appears to be a residual level to aim in later (model building/testing) experiments. Pursuant to this study the presence of 0.5 mg/L free chlorine residual significantly but not completely suppresses HPC bacteria which is generally the case in actual distribution systems where biological water stability is provided by disinfection.

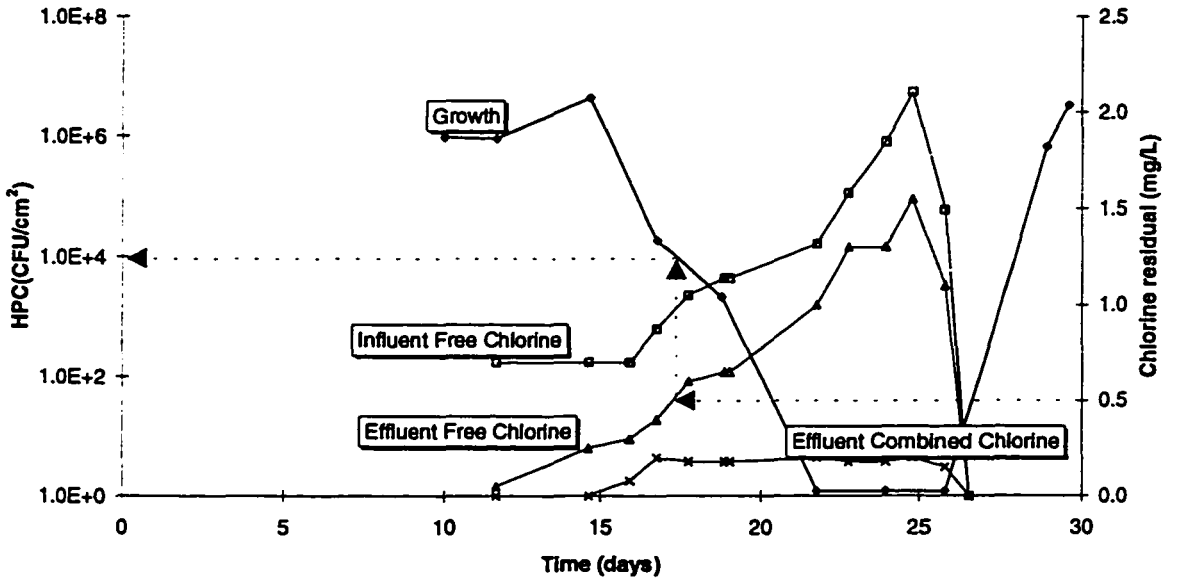


Figure 6.7: Net accumulation of HPCs on polycarbonate substrata with gradually increasing liquid chlorine dosage in the presence of 250 µg/L BOM supplement at 1.2 N/m^2 shear stress and 18°C (ref. P29) Notes:1. Influent combined chlorine concentration is negligible
2. HPC numbers below 10^3 CFU/cm^2 are estimates

Several studies have demonstrated the ability of bacteria to survive in drinking water after continued exposure to chlorine (e.g. Pedersen , 1994; LeChevallier *et al.*, 1990). Characklis and co-workers (Chen *et al.*, 1993) investigated the regrowth of biofilm cells after a 1 hour monochloramine treatment (4 mg/L [influent]). After the cessation of the disinfectant, a

calculated duration of 141 hours was required to reestablish bacterial numbers to their original level before the dosage of the disinfectant (Chen *et al.*, 1993).

Net Accumulation of Biofilm HPCs on Polycarbonate Substrata with Gradually Increasing Monochloramine Dosage (Figure 6.8)

Net accumulation of biofilm HPC numbers on polycabonate (PC) substrata with gradually increasing monochloramine dosage in the presence of 250 µg/L BOM supplement at 1.2 N/m^2 shear stress and 18°C liquid phase temperature were the operation conditions in experiment P30. Experiments P29 and P30 are identical designs with the exception of the employed disinfectant types.

Results of experiment P30 are shown in Figure 6.8. Low level dosage of the disinfectant (0.2 mg/L influent combined chlorine) was initiated at day 10 of experiment P30. Similarly to the accumulation behavior of HPCs in experiment P29, a further increase of biofilm bacterial numbers were observed at a low level (0.1 mg/L) of the combined chlorine system residuals. A further increase of the combined chlorine system residual resulted in a monotone decrease of biofilm HPC numbers. At about 0.5 mg/L combined chlorine system residual (or 1.0 mg/L influent combined chlorine concentration), the net accumulation of biofilm HPC numbers was about 5×10^3 CFU/cm^2 which was slightly over the reliable detection limit of about 10^3 CFU/cm^2. Biofilm HPCs were completely suppressed at about 1.5 mg/L combined chlorine system residual. The disinfection was abandoned at day 26 of the experiment, when the combined chlorine system residual was about 1.7 mg/L. The system was left operating at otherwise normal conditions for another 4 days. At day 29 and 30 biofilm HPC samples were taken from the residual free system. Still growing HPC numbers were recorded in the 5×10^5 CFU/cm^2 range, suggesting the possibility of an elevated accumulation of biofilm HPCs (with respect to steady-state HPCs before the commencement of the disinfection) after the cease of disinfection. Experiment P30 was terminated at day 30. There was no detectable level of free chlorine residual present in either the influent or the system on day 30. With respect to combined chlorine species, beside the dominating monochloramine, there were occasional trace amounts of di and trichloramines in the system. Based on these results, 0.5 mg/L

monochloramine system residual on polycarbonate supporting surfaces appears to be a reasonable disinfectant level to aim for in later model building and testing experiments.

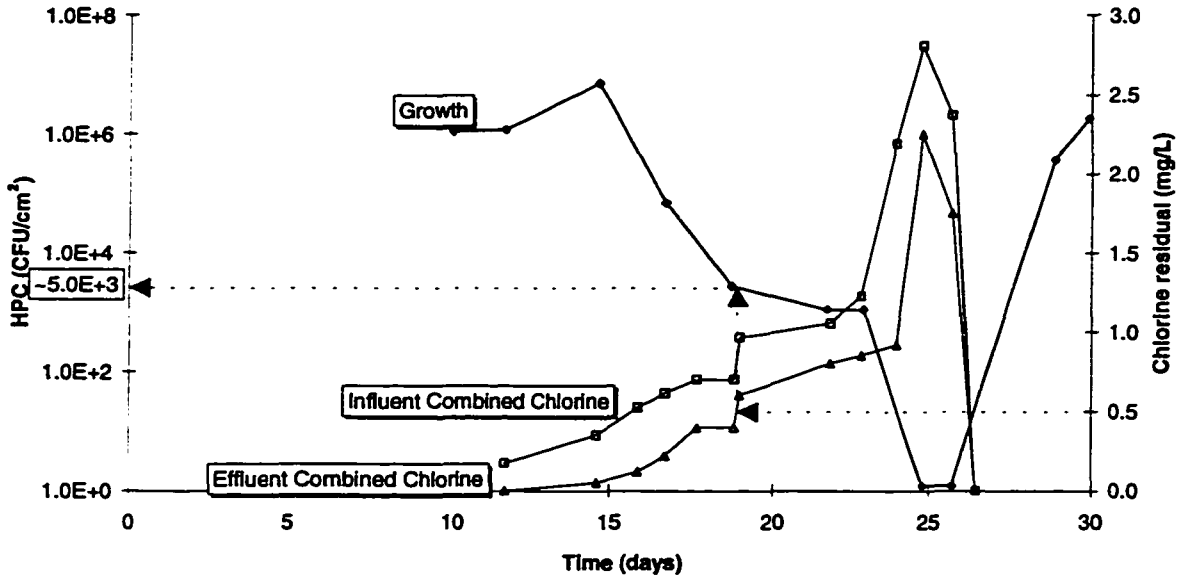


Figure 6.8: Net accumulation of HPCs on polycarbonate substrata with gradually increasing monochloramine dosage in the presence of 250 µg/L BOM supplement at 1.2 N/m^2 shear stress and 18°C (ref. P30) Notes: 1. free chlorine concentration is negligible in the system
2. HPC numbers below 10^3 CFU/cm^2 are estimates

Net Accumulation of Biofilm HPCs on Ductile Iron Substrata with Gradually Increasing Liquid Chlorine Dosage (Figure 6.9)

Experiments with ductile iron coupons required somewhat longer experimental runs. Experiment P31 investigated the net accumulation of biofilm HPC numbers on ductile iron (DI) substrata with gradually increasing liquid chlorine dosage in the presence of 250 µg/L BOM supplement at 1.2 N/m^2 shear stress and 18°C. Experimental results are shown in Figure 6.9. After establishing steady-state operation conditions, the dosage of the disinfectant was initiated at day 10. Early in the experiment disinfectant levels were checked daily. The gradual increase of free chlorine residual in the AR influent resulted in virtually no increase of system residuals,

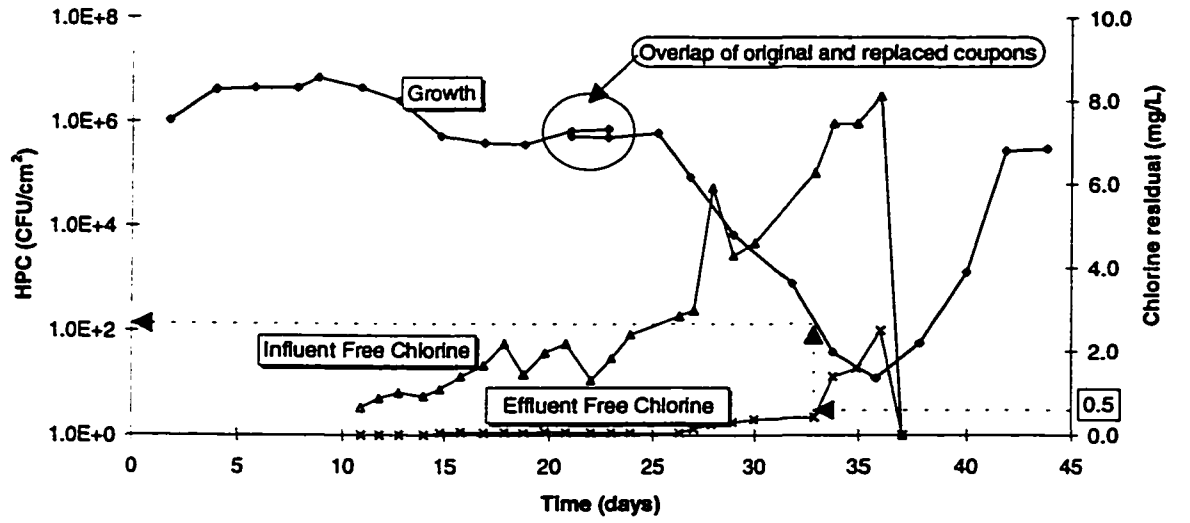


Figure 6.9: Net accumulation of HPCs on ductile iron substratum with gradually increasing liquid chlorine dosage in the presence of 250 µg/L BOM supplement at 1.2 N/m^2 sheer stress and 18°C (ref. P31) Notes:1. Combined chlorine concentration is negligible in the system
2. HPC numbers below 10^3 CFU/cm^2 are estimates

probably due to the high chlorine demand of the substrata. It took about two weeks to build-up a free chlorine residual of about 2.5 mg/L in the AR influent, at which concentration detectable levels of free chlorine system residuals were possible. Until the appearance of free chlorine residual in the system, only about a one order of magnitude decrease in biofilm HPC numbers was observed. Following the increasing presence of a free chlorine residual in the system, biofilm HPCs declined in a monotone fashion. At about 0.5 mg/L free chlorine system residual (or 6.5 mg/L influent free chlorine concentration), the net accumulation of biofilm HPC numbers was about 10^2 CFU/cm^2 (Figure 6.9) which was below the reliable detection limit of about 10^3 CFU/cm^2. Biofilm HPCs were essentially eliminated at about 2.5 mg/L free chlorine residual in the AR. The disinfection was abandoned at day 36 of the experiment. The system was left operating at normal conditions for an additional 8 days. Data of the four biofilm samples taken from the disinfectant free system suggest that pseudo-steady state biofilm HPC numbers are established at about 10^5 CFU/cm^2 level which is about one order of magnitude less than steady state HPCs before the start of the disinfection. Experiment P31 was terminated at day 44. There was no detectable concentration of combined chlorine residual in either the AR influent or the system. Upon sampling, the removed coupon is typically replaced by another

sterilized substratum of the same material. For example the second coupon in experiment P31 was removed on day 4, and the replaced coupon was submerged for 19 days before its removal at day 23. As shown in Figure 6.9, originally submerged and later replaced coupons were removed simultaneously at days 21 and 23 for reproducibility. HPC numbers were essentially the same on the originally and later submerged coupons, suggesting confidence for adequate continuity. Based on these results, a free chlorine system residual of about 0.5 mg/L on ductile iron supporting surfaces appears to be a reasonable disinfectant level to aim for in later (model building/testing) experiments.

Net Accumulation of Biofilm HPCs on Ductile Iron Substrata with Gradually Increasing Monochloramine Dosage (Figure 6.10)

Experiment P32 investigated the net accumulation of biofilm HPC numbers on ductile iron (DI) substrata with gradually increasing monochloramine dosage in the presence of 250 μg/L BOM supplement at 1.2 N/m^2 shear stress and 18°C. Experimental results are shown in Figure 6.10. The disinfectant dosage in this experiment was initiated on the seventh day of operation. A sharp decline in biofilm HPC numbers was observed as a result of an initial influent combined chlorine concentration of about 2.0 mg/L. Detectable levels of effluent combined chlorine residual appeared at day 17 of the experiment, when the influent combined chlorine residual was increased to about 5 mg/L. Net accumulation of biofilm HPCs appeared to be stabilized at a lower level (10^4 CFU/cm^2) until combined chlorine system residuals increased to about 2.0 mg/L, at which time another sharp decrease in HPCs was observed. At about 2.0 mg/L combined chlorine system residual (or 12.0 mg/L influent combined chlorine concentration), the net accumulation of biofilm HPC numbers was about 3×10^3 CFU/cm^2. Biofilm HPCs were reduced to zero at about 3.5 mg/L combined chlorine system residual. The disinfection was abandoned at day 32 of the experiment.

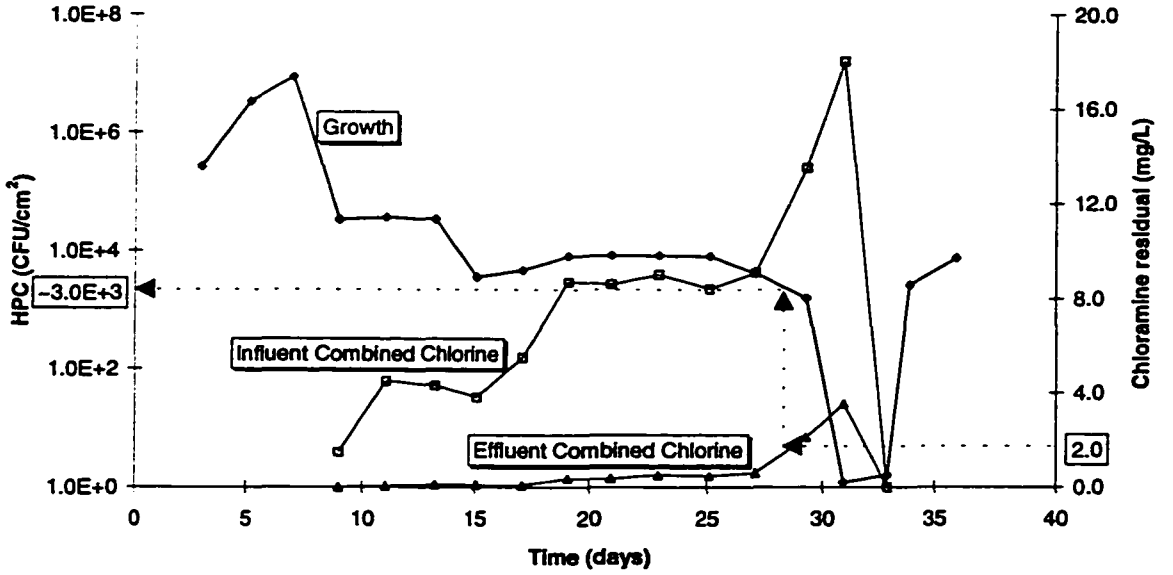


Figure 6.10: Net accumulation of HPCs on ductile iron substratum with gradually increasing monochloramine dosage in the presence of 250 μg/L BOM supplement at 1.2 N/m^2 sheer stress and 18°C (ref. P32) Notes: 1. free chlorine concentration is negligible in the system
2. HPC numbers below 10^3 CFU/cm^2 are estimates

The system was left operating at normal conditions for an additional 4 days. The three biofilm data points of the disinfectant free system shows a lower level but still increasing number of HPCs. Experiment P32 was terminated at day 37. There was no detectable concentration of free chlorine residual in either the AR influent or the system. Based on these results, a combined chlorine (mainly monochloramine) system residual of about 2.0 mg/L on ductile iron supporting surfaces appears to be a reasonable disinfectant level to aim for in later (model building/testing) experiments.

Net Accumulation of Biofilm HPCs with Gradually Increasing Disinfectant Dosage and 500 mg/L BOM Supplement

As described in section 6.5, experiments P27 and P28 were dosed, due to a calculation error, with a higher than planned BOM supplement. Experiments P27 and P28 investigated the net accumulation of biofilm HPC numbers on polycarbonate substrata in the presence of 500 mg/L BOM supplement at 1.2 N/m^2 shear stress and 18°C with gradually increasing free chlorine and monochloramine dosage. Due to both the nutrient rich environment in the ARs and the excessive chlorine demand of the system, biofilm bacteria were barely affected even at elevated

levels (15 mg/L) of disinfectant residuals in the AR influents. This suggest the need for superchlorination level of chemical oxidation in systems inadvertently exposed to such extreme levels of nutrients (i.e. groundwater infiltration into depressurized systems). Upon discovery of the error, the experiments were abandoned after about 60 days of operation. Due to their limited value these experimental results are not presented in detail.

Summary

Experiments with gradually increasing disinfectant residuals on different supporting surfaces suggested that to suppress biofilm HPC numbers to about 10^3 CFU/cm^2 under the conditions tested, the following disinfectant residuals must be present: 0.5 mg/L free chlorine residual on polycarbonate; 0.5 mg/L monochloramine residual on polycarbonate; 0.5 mg/L free chlorine residual on ductile iron; and 2.0 mg/L monochloramine residual on ductile iron. Experimental conditions included 250 µg/L BOM supplement, 1.2 N/m^2 shear stress, and 18°C liquid phase temperature.

6.7 CORROSION CHARACTERISTICS OF INVESTIGATED SUBSTRATA

Coupons were weighted before and after the experimental runs. Figure 6.11 (P8; Table 6.1) shows, in the absence of both a BOM supplement and a disinfectant at 0.4 N/m^2 shear stress and 21°C, an apparent linear weight loss of mild steel substrata (biofilm was removed by stomacher).

Under similar conditions but at 19°C, experiment P14 employed both utility knife and stomacher biofilm removal techniques (Table 6.1). Figure 6.12 suggests a linear weight loss of the mild steel substrata throughout the 15 day trial. In terms of corrosion related weight losses, statistical differences between the two mechanical biofilm removal methods could not be demonstrated. This conclusion is based on a 5% significance level (statistical calculation not shown).

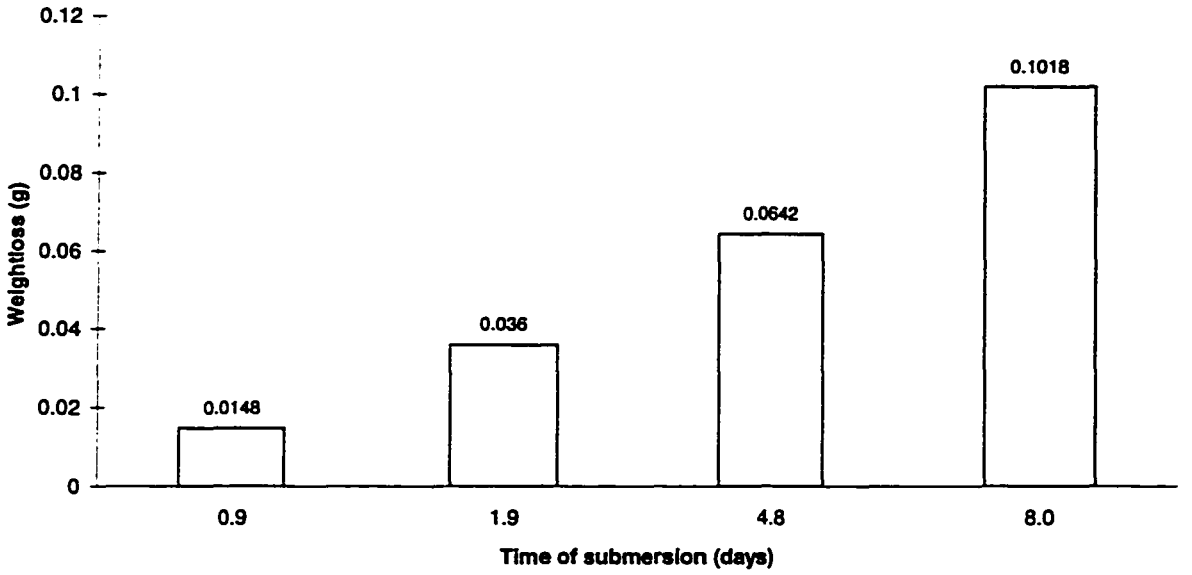


Figure 6.11: Corrosion related weightloss of mild steel substrata (1/2) (ref. P8)

Calculated relative weight loss (actual weight loss/weight before submergence) of these coupons suggest that, due to chemical and microbiologically enhanced corrosion, mild steel coupons would 'disappear' in approximately 5 years. Thus mild steel coupons likely represent an extreme case of corrosion, and caution should be used in extrapolating results obtained with mild steel coupons to actual distribution systems.

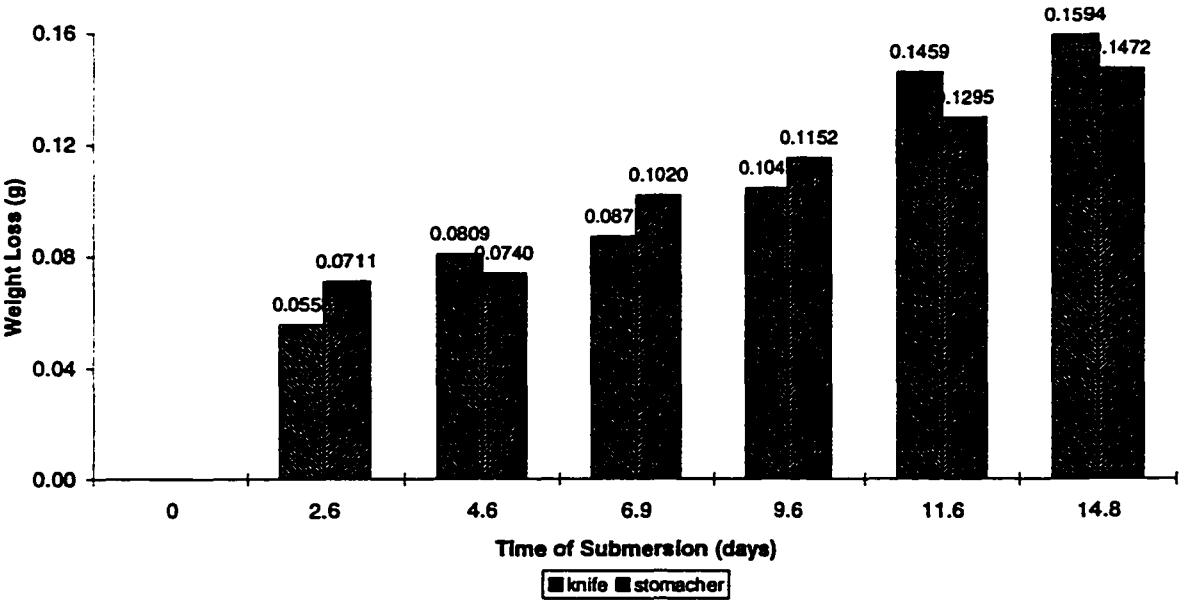


Figure 6.12: Corrosion related weightloss of mild steel substrata (2/2) (ref. P14/1 and P14/2)

Figure 6.13 shows a varying weightloss of stainless steel 304 substrata throughout the 11 day trial (P9; Table 6.1). Biofilm was removed by stomacher. The average corrosion related weight loss of SS 304 coupons was about 0.08 mg which corresponds to the weight loss of mild steel substrata after about 6 days of submergence. This suggests that SS 304 substrata have a low corrosion related mass loss. Polycarbonate coupons have not shown detectible weight loss.

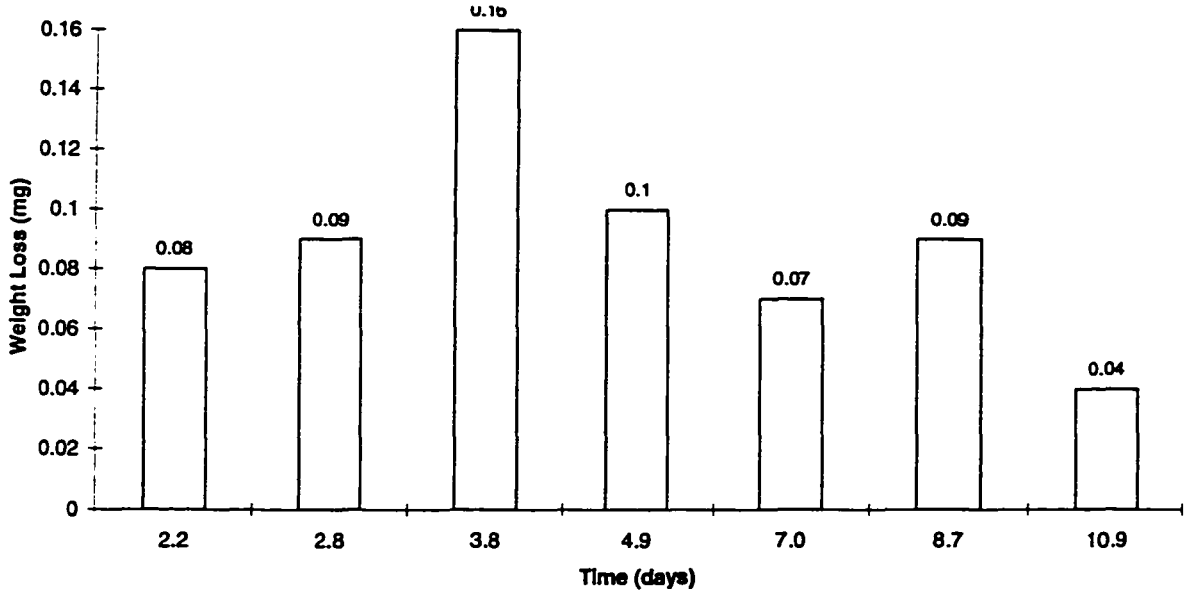


Figure 6.13: Corrosion related weightloss of stainless steel 304 substrata (ref. P9)
Note: sampling data of day 10.9 represents the average of 6 coupon analyses

Summary

Mild steel and polycarbonate substrata appear to bracket the corrosion behavior of stainless steel (SS 304) supporting surfaces.

6.8 REAL WATERS

Net accumulation of HPCs in six distinct real water sources was investigated, which were WTP 'A' final effluent (P33; Table 6.1), WTP 'B' final effluent (P34), WTP 'C' final effluent (P35), WTP 'D' final effluent (P36), WTP 'E' intake (P37), and WTP 'E' final effluent (P38). While experiments P33 to P36 were supplied with groundwaters, P37 was a surface water, and P38

was a partially treated surface water. Decoding of coded real water sources is shown in Table B/4. Experimental conditions are introduced in Table 6.1.

The primary objective of these trials was to compare the biofilm HPC supporting characteristics of actual waters, some of which might be used in model testing trials. As described in Section 4.1.2, instead of *in situ* analyses, actual water samples were carried to the NSERC Chair for Industrial Research in Water Treatment laboratories at the University of Waterloo where they were tested by the same reactor systems used for synthetic water analysis. Actual disinfectant residuals of the sampled real waters, without adjustment, were utilized in these experiments.

Prior to the commencement of trials P33 to P38, disinfectant residual decay was studied in batch experiments for final effluents from WTPs 'A', 'B', 'C', and 'D'. Samples were collected from these sites in 4L amber glass bottles. Samples from these bottles were taken five times during the 8 day study period for titration-based disinfectant residual analysis. The objectives of these side investigations were twofold. First, to recommend sampling frequency (i.e. replacement frequency of carboys) for acceptable levels of residual fluctuation in AR experiments with real waters (Section 4.1.2). Second, to facilitate the selection of real water(s) for further (model testing) analysis. The four batch experiments were not designated with distinct experimental numbers, instead referred as P33a, P34a, P35a, and P36a.

Batch Study of Disinfectant Residuals of Investigated Real Waters

Free chlorine was essentially the only disinfectant species present in the final effluent of WTP 'A' (Figure 6.14). The decay of free chlorine in the batch system is such that its concentration is reduced to half in about two days and only trace amounts of free chlorine residuals could be detected at the end of the experiment.

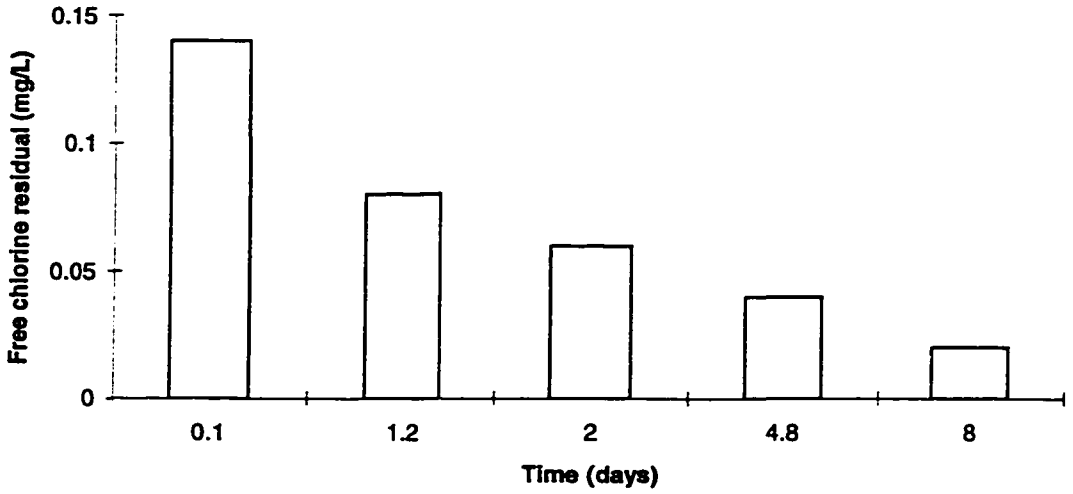


Figure 6.14: Disinfectant decay study - WTP 'A' final effluent (ref. Appendix B/4)

Figure 6.15 shows that monochloramine is the dominant chlorine species in the final effluent of WTP 'B'. The initial concentration of the monochloramine residual of 0.32 mg/L decayed to about 0.19 mg/L in the 8 day study period. The decay of the residual was essentially linear during this period. There was a trace amount of free chlorine residual in the system.

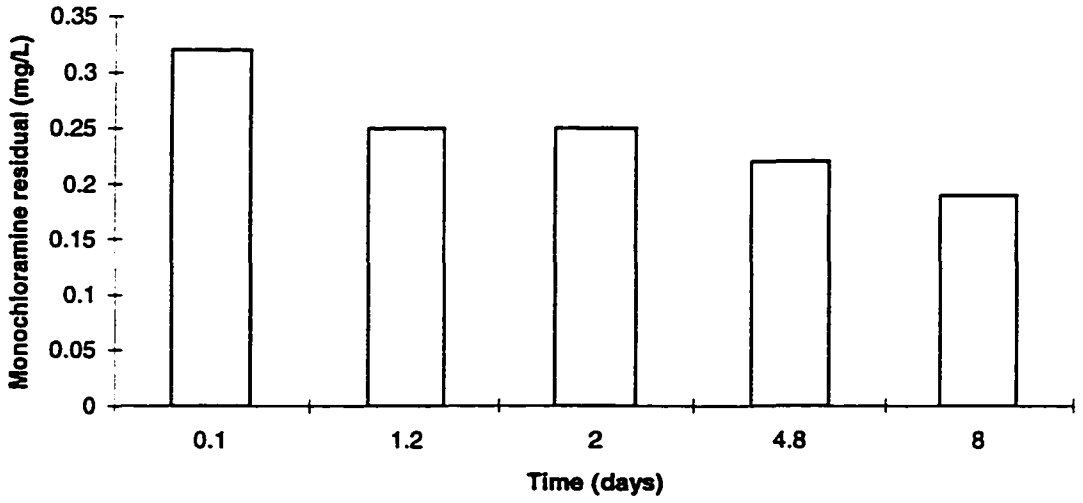


Figure 6.15: Disinfectant decay study - WTP 'B' final effluent (ref. Appendix B/4)

Figure 6.16 shows that the predominant combined chlorine species in WTP 'C' final effluent is monochloramine. Trace amounts of free chlorine residual was also present in the system. The initial concentration of monochloramine, measured within two hours after sampling, was 0.46 mg/L suggesting a combined chlorine residual of about 0.5 mg/L in the distribution system. The

concentration of monochloramine in the batch experiment was gradually reduced to half of the initial concentration in about 8 days. The residual of free chlorine (0.02-0.06 mg/L) changed little in the system.

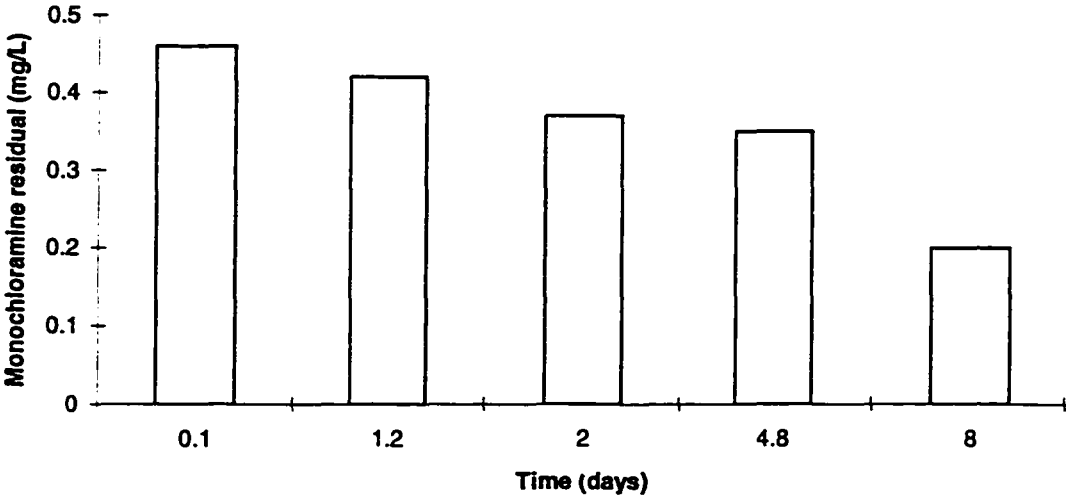


Figure 6.16: Disinfectant decay study - WTP 'C' final effluent (ref. Appendix B/4)

The most rapid reduction of chlorine residuals was observed in the final effluent of WTP 'D'. Figure 6.17 shows that the initial monochloramine concentration of 0.15 mg/L was reduced to 0.06 mg/L in about one day and only trace amounts of the residual could be detected at the 4.8 day sampling.

Summary of Batch Residual Analysis

With the exception of the rapidly dissipating residual in the final effluent of WTP 'D', the other investigated treated groundwaters show a slower decay of the disinfectant. While the half life of the residual is about two days in the final effluent of WTP 'A', only about 75% reduction of the residuals takes place in the final effluents of WTPs 'B' and 'C' within two days. Consequently, a two day sampling frequency appears to be optimal in future experiments with real waters.

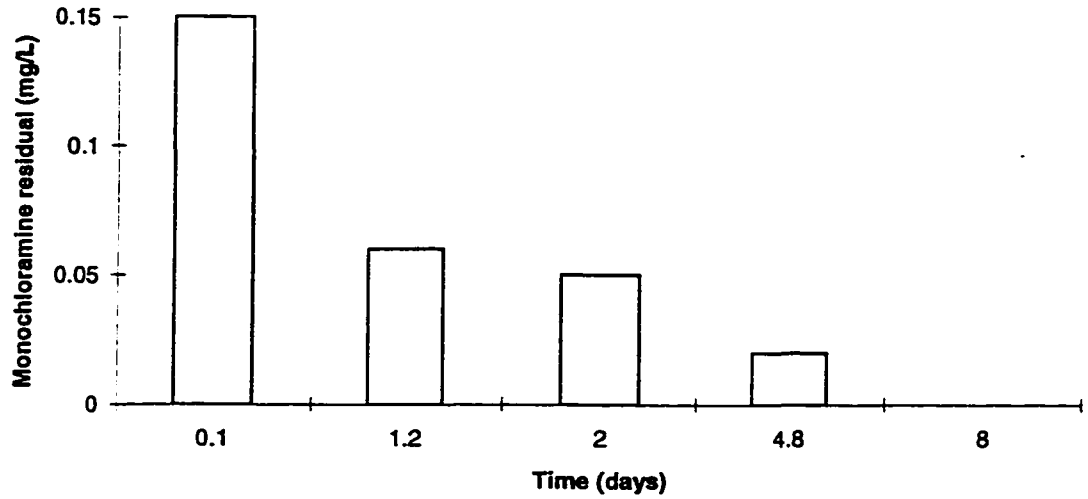


Figure 6.17: Disinfectant decay study - WTP 'D' final effluent (ref. Appendix B/4)

Biofilm HPC Numbers of Investigated Real Waters

Figure 6.18 shows that steady-state biofilm HPC numbers were typically established at the 10^6 CFU/cm^2 level with the exception of the surface water intake of WTP 'E' which showed about two orders of magnitude higher biofilm bacterial numbers. The comparison of experiments P37 and P38 suggests, in terms of steady-state biofilm HPCs, a 2 log (i.e. 99%) overall treatment efficiency of WTP 'E'. In Figure 6.18, the impact of temperature is evident. Establishment of the biofilm appeared to take about twice as long in experiments conducted at 14°C temperature.

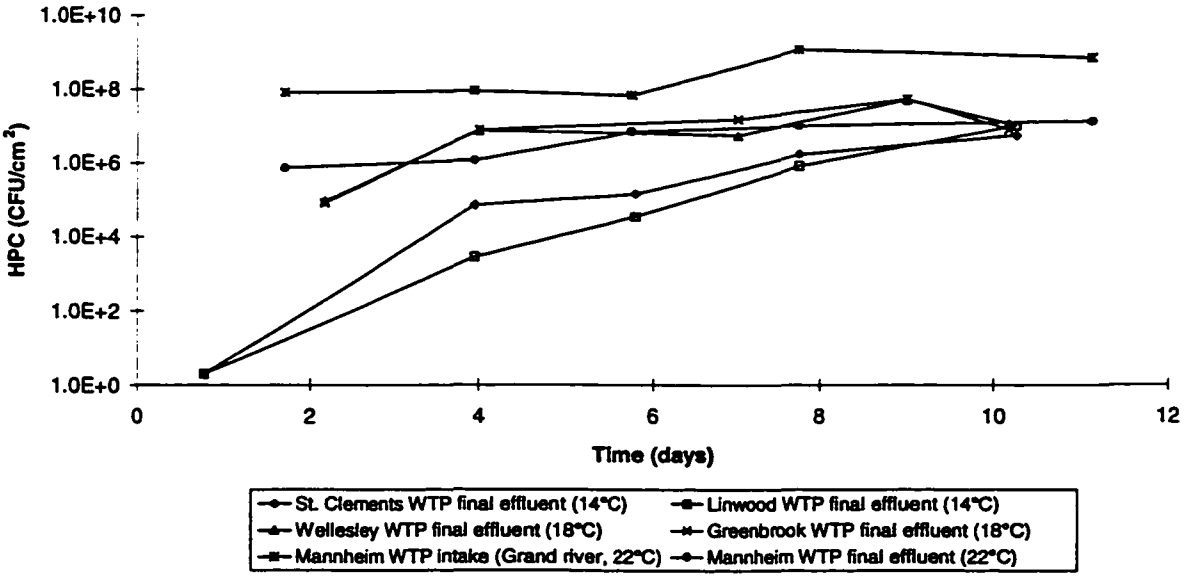


Figure 6.18: Net accumulation of biofilm HPCs in investigated real waters
(ref. P33, P34, P35, P36, P37, and P38)

Summary

Due to the asset of rapid establishment of steady-state accumulation conditions, the final effluents of WTPs 'C', 'D', and 'E' may be recommended for further study. In terms of the decay of the disinfectant, WTP 'C' exhibits more favorable characteristics than WTP 'D'. Therefore, water supplies of WTPs 'C' and 'E' are most recommended for further (model testing) analysis.

6.9 SUMMARY

Strong evidence suggests that, in the absence of both a BOM supplement and a disinfectant at 0.4 N/m^2 shear stress, steady-state net growth of HPCs is at least one order of magnitude higher on mild steel than on either polycarbonate or stainless steel 304 substrata. Mild steel and polycarbonate substrata appear to bracket the corrosion behavior of stainless steel (SS 304) supporting surfaces.

On polycarbonate substrata, biofilm removal efficiencies of stomacher and utility knife were statistically different, being about half an order of magnitude higher, in terms of HPC numbers, by stomaching. On mild steel surfaces, a statistical difference between utility knife and stomacher could not be demonstrated.

In terms of reproducibility of biofilm HPC numbers, experiments failed to demonstrate a significant difference and suggests that removal is highly reproducible by either utility knife or stomacher. The reproducibility of suspended HPCs were statistically different.

Linear regression results between suspended and/or biofilm HPC numbers, and physical system conditions (e.g. temperature, turbidity) showed typically poor correlation. Higher correlation strength ($R^2 = 0.81$) was demonstrated between suspended HPCs and turbidity in AR effluents.

Under the conditions tested and in the absence of both a BOM supplement and a disinfectant, the overall net accumulation of a biofilm appears to be mass transfer limited. With BOM supplement (500 mg/L) but still in the absence of a disinfectant, biofilm accumulation may become bioreaction limited and/or the detachment process may become more important.

Under the investigated conditions, experiments with gradually increasing disinfectant residuals on different supporting surfaces suggested that to suppress biofilm HPC numbers to about 10^3 CFU/cm^2, the following disinfectant residuals must be present: 0.5 mg/L free chlorine residual on polycarbonate; 0.5 mg/L monochloramine residual on polycarbonate; 0.5 mg/L free chlorine residual on ductile iron; and 2.0 mg/L monochloramine residual on ductile iron.

Experiments performed in this chapter were short term (<15 days) which implies the presence of very young biofilms which may not represent fully established biofilms of actual distribution systems.

CHAPTER 7: RESULTS OF TRIALS WITH SYNTHETIC WATERS

After setting the ground with the preliminary experiments, the objective of experimentation with synthetic water was to obtain data for a steady-state biofilm accumulation model. The Bayesian type of design of experiments with synthetic water was described in Section 5.2. The coded design matrix of the 26 trials with synthetic water was introduced in Table 5.3. The design factors were stated in Chapter 5. For convenience, they are repeated here (low and high levels of the design factors are bracketed):

- BOM supplement (0 - 500 μg/L)
- disinfectant type (chlorine - monochloramine)
- disinfectant residual (0 - 0.5 or 2.0 mg/L)
- shear stress (0.4 - 2.0 N/m^2)
- temperature (8 - 26°C)
- substratum (polycarbonate - ductile iron)

The sole system response is the steady-state net accumulation of heterotrophic microorganisms (HPCs) reported as CFU/cm^2.

After a general overview of the 26 synthetic water trials (Figure 7.1), this chapter will describe the individual effect of each variable on the response parameter (steady-state biofilm HPC numbers). Design factors will be evaluated by one-variable-at-a-time strategy which tacitly assumes that the effect of the investigated variable on the response is independent of the level of other variables. Consequently, this approach provides more limited information for assessing the effect of a single factor by directly comparing several experimental trials. What the approach does not establish is what might happen if the variables were changed, not individually, but

together. A more complex, joint functional dependence approach (i.e. combined effects of the variables on the response) will be introduced in Chapter 9.

As it was reported in Chapter 6, pseudo-steady state biofilm HPCs were typically established within 10 days in the AR experiments. Since actual distribution systems operate for an extended period with established biofilms, the primary research objective was to investigate and report steady-state biofilm HPC numbers. Since biofilm HPCs were typically sampled and quantified every second day throughout each AR experiment, net accumulation of biofilm HPCs are also introduced in Appendix C/1. These accumulation curves may be of value for readers who are interested not only in steady-state system conditions but also in dynamic systems where growth kinetics of HPCs are of importance.

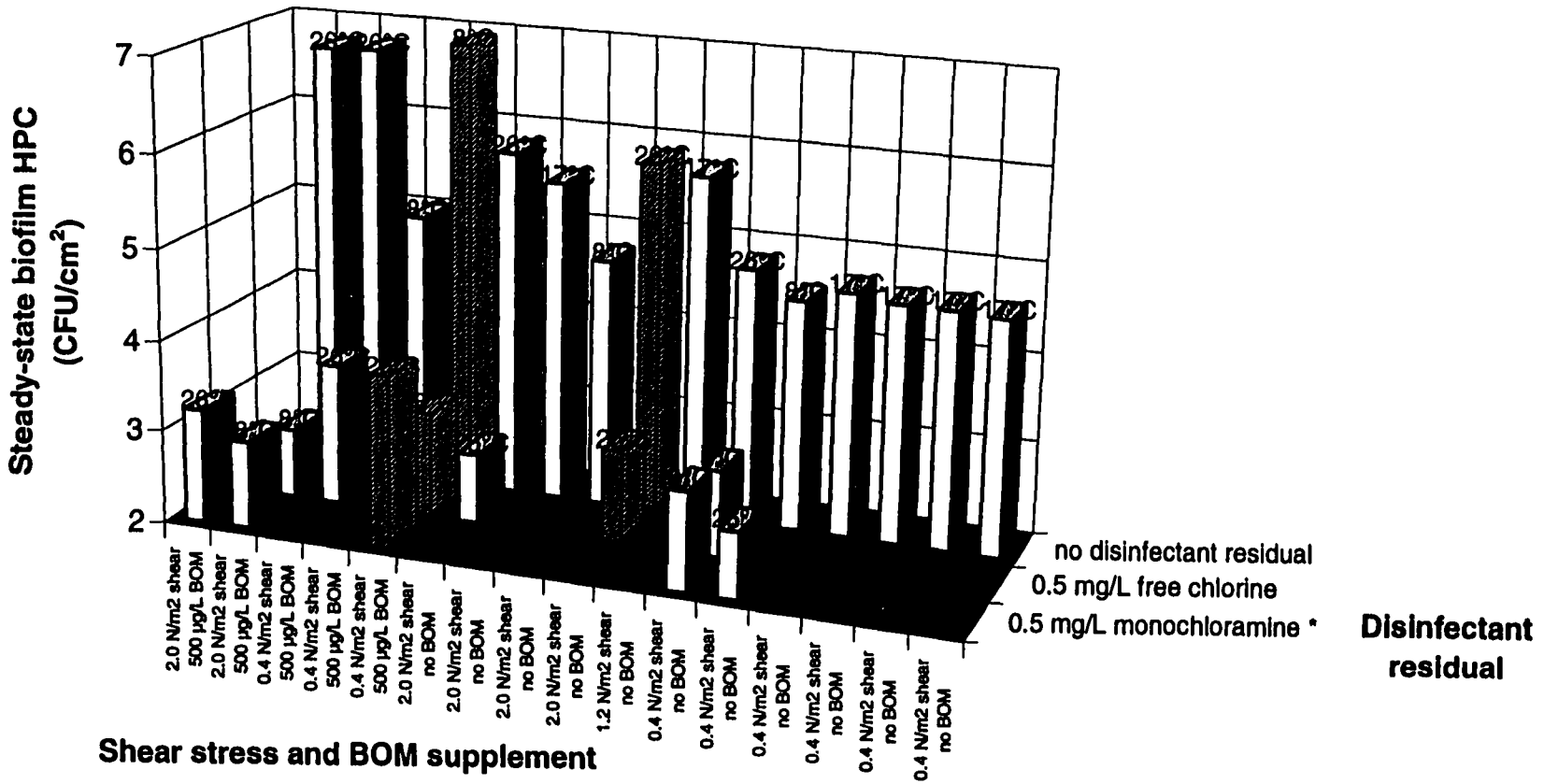
7.1 OVERVIEW

Design factors and system responses of the 26 experiments with synthetic water are introduced in Table 7.1. A bar graph summary of the 26 synthetic water experiments are presented in Figure 7.1. Shear conditions and supplemental BOM concentrations are designated to one of the two horizontal axes. Type and residual of the applied disinfectant are introduced on the second abscissa. Steady-state biofilm HPC numbers are shown on the ordinate in a logarithmic scale. Applied temperatures are shown on top of the bars. Bars without shading represent polycarbonate supporting surfaces. Ductile iron substrata are shaded.

There were 15 experiments conducted in the absence and 11 experiments in the presence of a disinfectant. The comparison of these experiments, in Figure 7.1, clearly shows that disinfectant is an effect. The presence of 0.5 mg/L free chlorine or monochloramine residual resulted in up to four orders of magnitude reduction of steady-state HPC numbers. For similar suppression of HPC numbers on ductile iron surfaces, 2.0 mg/L monochloramine residuals must be maintained.

Table 7.1: Design Matrix and System Responses - Synthetic Water Experiments

Design segment	Experiment #	Variable: BOM supplement (µg/L)	Disinfectant type	Disinfectant residual (mg/L)	Shear stress (N/m^2)	Temperature (°C)	Substratum	Steady-state biofilm HPC (CFU/cm^2) arithmetic	log
#1	S1	zero	chlorine	zero	0.4	26	polycarbonate	6.3×10^4	4.799
	S2	zero	monochloramine	0.5	0.4	8	polycarbonate	1.1×10^3	3.041
	S3	500	monochloramine	2.0	0.4	26	ductile iron	6.9×10^3	3.839
	S4	zero	chlorine	0.5	2.0	26	ductile iron	9.4×10^2	2.973
	S5	500	monochloramine	zero	2.0	26	polycarbonate	5.5×10^6	6.740
	S6	500	chlorine	0.5	2.0	8	polycarbonate	5.3×10^2	2.724
	S7	500	chlorine	zero	0.4	8	ductile iron	8.0×10^6	6.903
#2	S8	500	monochloramine	0.5	2.0	26	polycarbonate	1.6×10^3	3.204
	S9	500	chlorine	zero	0.4	8	polycarbonate	9.7×10^4	4.987
	S10	500	chlorine	0.5	0.4	26	polycarbonate	3.3×10^3	3.519
	S11	zero	chlorine	0.5	2.0	26	polycarbonate	5.3×10^2	2.724
	S12	zero	monochloramine	zero	0.4	8	polycarbonate	3.3×10^4	4.519
	S13	zero	chlorine	zero	2.0	8	polycarbonate	5.3×10^4	4.724
#3	S14	zero	monochloramine	zero	2.0	26	ductile iron	6.7×10^5	5.826
	S15	zero	monochloramine	0.5	0.4	26	polycarbonate	5.0×10^2	2.699
	S16	500	chlorine	0.5	0.4	8	ductile iron	1.3×10^3	3.114
	S17	zero	chlorine	0.5	0.4	8	polycarbonate	8.0×10^2	2.903
#4	S18	500	monochloramine	0.5	2.0	8	polycarbonate	8.3×10^2	2.919
	S19	500	chlorine	zero	0.4	26	polycarbonate	5.8×10^6	6.763
	S20	zero	chlorine	zero	2.0	26	polycarbonate	6.1×10^5	5.785
#5	S21 (P10)	zero	chlorine	zero	0.4	17	polycarbonate	4.6×10^4	4.663
	S22 (P12/2)	zero	chlorine	zero	0.4	17	polycarbonate	4.0×10^4	4.602
	S23 (P13/2)	zero	chlorine	zero	0.4	17	polycarbonate	4.0×10^4	4.602
	S24 (P15)	zero	chlorine	zero	0.4	17	polycarbonate	3.7×10^4	4.568
	S25 (P17)	zero	chlorine	zero	1.2	17	polycarbonate	5.5×10^5	5.740
	S26 (P20)	zero	chlorine	zero	2.0	17	polycarbonate	3.3×10^5	5.519



Note:

* 2.0 mg/L monochloramine on ductile iron substratum

■ ductile iron substratum

Figure 7.1: Summary of synthetic water experiments

As a result of an 18°C decrease in temperature, biofilm HPCs were suppressed by about one order of magnitude. In the absence of both a BOM supplement and a disinfectant, shear appears to be an important factor suggesting that under the conditions tested mass transfer may limit the overall biofilm accumulation process. BOM levels and HPC accumulation appears to correlate. Ductile iron surfaces appear to support higher HPC numbers than polycarbonate substrata.

7.2 EFFECT OF BOM

Figure 7.2 shows the individual effects of BOM supplement, temperature, and shear stress on steady-state HPC numbers on polycarbonate substrata in the absence of a disinfectant. At 0.4 N/m^2 shear stress and 8°C, steady-state bacterial numbers were about half an order of magnitude higher in the presence of 500 µg/L BOM supplement (S9 and S12; Table 7.1). At an elevated temperature (26°C), the corresponding increase was about two orders of magnitude (S1 and S19).

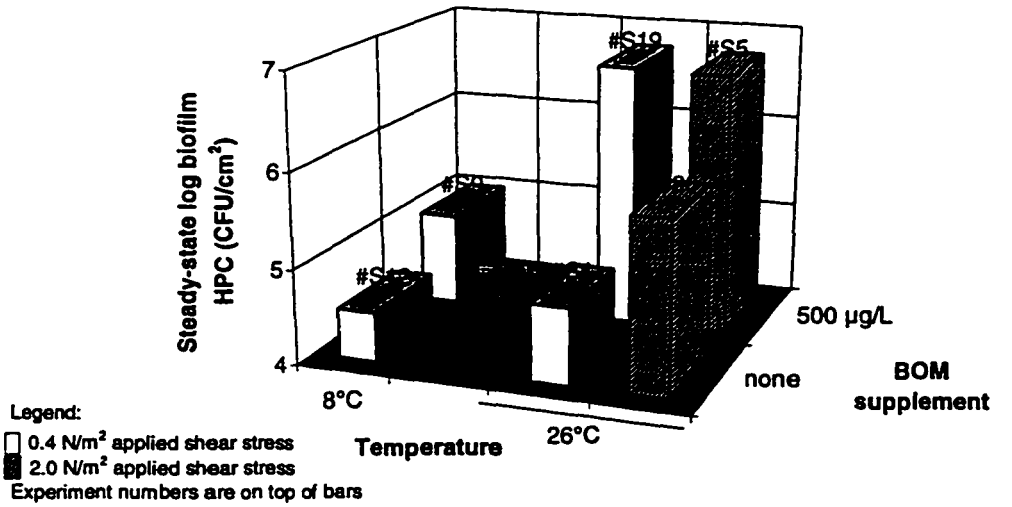


Figure 7.2: Steady-state HPCs on polycarbonate substrata in the absence of a disinfectant (ref. Figure C/1.1, Figure C/1.2 and Figure C/1.3; Appendix C/1)

Comparison of the data obtained with 500 µg/L BOM supplement at 0.4 N/m^2 shear stress (S9 and S19) shows (Figure 7.2) that steady-state HPC numbers were almost two orders of magnitude higher at 26°C. In the absence of a BOM supplement (S1 and S12), temperature

appears to have essentially no effect on accumulation. This suggests that temperature-induced deterioration of bacteriological drinking water quality is less likely to occur in biologically treated surface or low organic content ground waters.

Comparison of experiments S5 and S20 in Figure 7.2 shows that at 2.0 N/m^2 shear stress and 26°C, steady-state HPC numbers were about one order of magnitude higher in the presence of 500 µg/L BOM supplement. Comparison of data obtained with 500 µg/L BOM supplement at 26°C shows (S5 and S19) that steady-state HPCs were little affected by the increase of shear stress from 0.4 to 2.0 N/m^2. Without a BOM supplement, the increase of shear stress from 0.4 to 2.0 N/m^2 resulted in about one order of magnitude increase of steady-state biofilm HPCs. This suggests that while biofilm accumulation may be bioreaction limited at higher BOM conditions, mass transfer is likely the rate limiting process at lower BOM levels.

de Beer *et al.* (1994a) speculated that larger size biofilm particles had a thicker diffusion layer and therefore were mass transfer limited. According to their theory, bioreaction limitation is likely to occur in biofilms with smaller cell clusters. If a direct relationship between BOM and cell size is assumed, the author's findings contradict the theory of de Beer *et al.* (1994a).

LeChevallier *et al.* (1991) traced the concentration of several water quality parameters (nitrate, nitrite, ammonia, phosphorus, TOC, and AOC) and found that only AOC was reduced significantly in concentration along a portion of a full scale drinking water distribution system. van der Kooij *et al.* (1992) found a significant correlation between the AOC concentration in plant final effluent and the number of suspended cells in distributed waters. Servais *et al.* (1995b) reported a significant correlation between BDOC and bacterial biomass in a distribution system. Since bacterial growth was intended to be carbon limited (Section 4.4) in the author's experiments with synthetic waters, these results and the author's finding are in agreement.

Steady-state biofilm HPC numbers of six synthetic water experiments (S1, S5, S9, S12, S19 and S20) were introduced in this section. The corresponding six net bacterial accumulation curves are introduced in Appendix C/1 (Figures C/1.1, C/1.2 and C/1.3).

7.3 EFFECT OF DISINFECTANT TYPE

Figure 7.3 shows the individual effects of disinfectant type and concentration, BOM supplement, and temperature on steady-state biofilm HPC numbers on polycarbonate substrata at 0.4 N/m^2 shear stress. Experiments S2 and S17 in Figure 7.3 shows results obtained for free chlorine and monochloramine. The residual for either disinfectant was 0.5 mg/L, there was no BOM supplement, and the temperature was 8°C. Steady-state biofilm HPC numbers for both substrata were essentially the same (10^3 CFU/cm^2). Therefore under these conditions, and potentially for other non-corrosive supporting surfaces under similar conditions, the bactericidal efficiency of free chlorine and monochloramine are about the same.

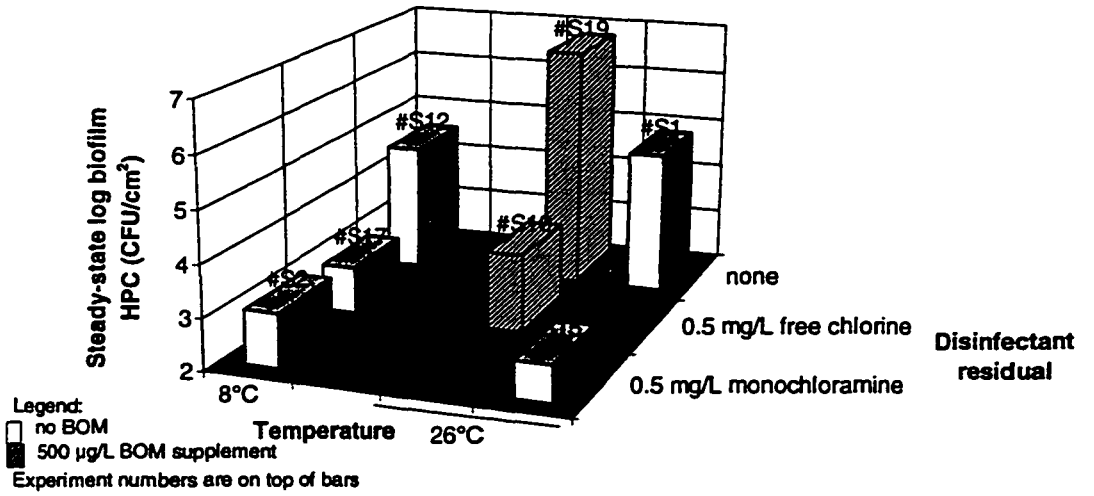


Figure 7.3: Steady-state HPCs on polycarbonate substrata at 0.4 N/m^2 shear stress (ref. Figures C/1.4, C/1.5, C/1.6 and C/1.7; Appendix C/1)

Because of the essentially fractional factorial nature of the experimental design, no other direct comparison of chlorine and monochloramine (with all other factors being the same) could be made. It is reasonable to suspect that at higher BOM levels and/or temperature and with corroding substrata, the effect of the two disinfectants might be different. For example, LeChevallier *et al.* (1993) postulated that free chlorine reacted rapidly with electron donors of lower redox potential, such as corrosion products, and may be exhausted at an early stage of

diffusing into cell clusters. In a pilot-scale network of mild steel pipe material, Camper *et al.*, (1997) found monochloramine to be a more effective biocide.

Comparison of steady-state biofilm HPCs in experiments S2 and S17 (Figure 7.3) using data obtained with gradually increasing free chlorine (Figure 6.7) and monochloramine residuals (Figure 6.8) shows that steady-state HPC numbers are half to one order of magnitude lower in trials with the constant dosage (0.5 mg/L residual) of the applied disinfectant. Caution should be used in interpreting these differences since the experimental conditions were different: 250 µg/L BOM supplement, 18°C temperature, and 1.2 N/m^2 shear stress were maintained in experiments with gradually increasing disinfectant dosage; each of these experimental conditions are assumed to support higher HPCs than their lower level counterparts in experiments S2 and S17.

Although the previous reasoning appears to have solid basis, it may be speculated that in experiments with gradually increasing concentration of a disinfectant (Section 6.6), biofilm HPCs are less susceptible to the disinfectant due to the time allowed for their acclimation to the gradually changing environment. Potentially, dormant endospores may convert back into vegetative activity upon adaptation to the biocide treatment. Since bacteriological results established by gradually increasing disinfectant studies likely represent the worst case scenario, they may be considered as a conservative approach for the design of actual systems.

Steady-state biofilm HPC numbers of experiments S2 and S17 were introduced in this section. Corresponding net bacterial accumulation curves are shown in Appendix C/1 (Figure C/1.4 through Figure C/1.7).

7.4 EFFECT OF DISINFECTANT RESIDUAL

Figure 7.3 shows the individual effects of disinfectant type and concentration, BOM supplement, and temperature on steady-state biofilm HPC numbers on polycarbonate substrata at 0.4 N/m^2 shear stress. In the presence of 500 µg/L BOM supplement and 26°C temperature,

the 0.5 mg/L free chlorine residual decreased steady-state HPC numbers about three orders of magnitude (S10 and S19). At a lower temperature (8°C) on ductile iron substrata, steady-state HPC numbers were almost four orders of magnitude lower in the presence of a 0.5 mg/L free chlorine residual (S7 and S16 in Figure 7.4). These results show a significant disinfectant effect.

The combined effect of temperature and substratum can also be evaluated in Figure 7.3 and Figure 7.4. In the absence of a disinfectant and at the applied experimental conditions, steady-state HPC numbers at 8°C on ductile iron (S7) and 26°C on polycarbonate (S19) were about the same. This suggests that in the absence of a disinfectant, the reported higher HPC supporting characteristics of ductile iron surfaces (Section 6.1) may be offset by a temperature decrease of about 18°C. In the presence of a 0.5 mg/L free chlorine residual (S10 and S16), it appeared that the higher temperature for the polycarbonate experiment more than compensated for the lower HPC supporting characteristics of that substratum.

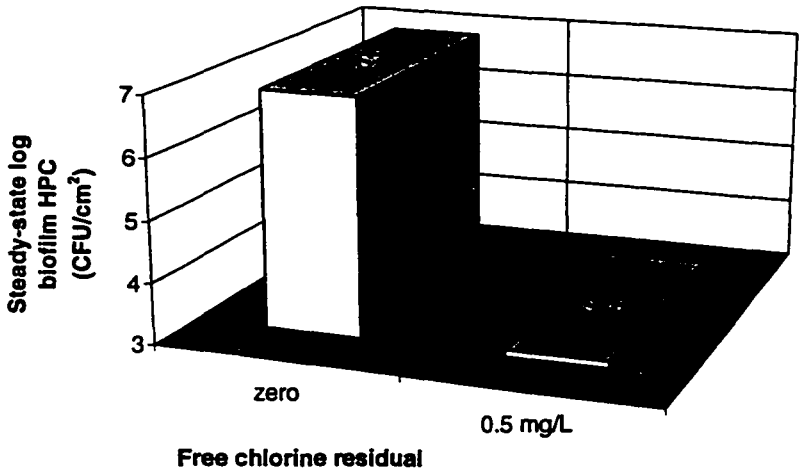


Figure 7.4: Steady-state HPCs on ductile iron substrata in the presence of a 500 mg/L BOM supplement at 0.4 N/m^2 shear stress and 8 °C

Comparison of experiments S1, S2, S12, and S15 in Figure 7.3 shows the effects of temperature and monochloramine residual on steady-state biofilm HPC numbers on polycarbonate substrata in the absence of a BOM supplement. At 8°C, steady-state bacterial numbers were about one and a half orders of magnitude lower (10^3 CFU/cm^2) in the presence of a 0.5 mg/L

monochloramine residual (S2) than with no residual (S12). At an elevated temperature (26°C), the corresponding decrease was about two orders of magnitude (S1 and S15). Comparison of the data obtained with no disinfectant (S1 and S12) shows that steady-state HPC numbers were about half an order of magnitude lower at the lower temperature. In the presence of 0.5 mg/L monochloramine residual (S2 and S15), temperature appears to have essentially no effect on accumulation. The effect of a 0.5 mg/L monochloramine residual appeared to be greater at the higher temperature (S1, S15 vs. S2, S12).

Donlan *et al.* (1994) reported that a biofilm was established on cast iron substrata within 30 days after continued exposure to a monochloramine residual of 0.1 to 0.9 mg/L. In that study higher temperatures resulted in an increased number of biofilm HPCs. Although experimental conditions were not identical, the establishment of a biofilm in the presence of a disinfectant residual is supported by the findings of the author's research. The temperature effect, however, appears to disagree with the author's findings (S2 versus S15 data in Figure 7.3).

Steady-state biofilm HPC numbers of nine synthetic water experiments (S1, S2, S7, S10, S12, S15, S16, S17, and S19) were introduced in this section. Corresponding net bacterial accumulation curves are introduced in Appendix C/1 (Figures C/1.4 through Figure C/1.7).

7.5 EFFECT OF SHEAR STRESS

Figure 7.5 shows the effects of shear stress and BOM concentration on steady-state biofilm HPC numbers on polycarbonate substrata in the absence of a disinfectant at 26°C. In the absence of a BOM supplement, steady-state bacterial numbers were about one order of magnitude higher at 2.0 N/m^2 shear stress (S20) than at 0.4 N/m^2 (S1). In the presence of a 500 µg/L BOM supplement, steady-state biofilm HPCs were higher, but shear appeared to have essentially no effect on accumulation (S5 and S19). Comparison of the data obtained at 0.4 N/m^2 (S1 and S19) shows that steady-state HPC numbers were about two orders of magnitude

higher in the presence of a 500 µg/L BOM supplement. At elevated shear (2.0 N/m^2), the corresponding increase was about one order of magnitude (S5 and S20).

Figure 7.5 shows that the effect of a 500 µg/L BOM supplement appears to be greater at the lower shear stress. The effect of shear stress appears to be more important at lower BOM levels. This suggests that while biofilm accumulation may be bioreaction limited at higher BOM conditions, mass transfer is likely the rate limiting process at lower BOM levels.

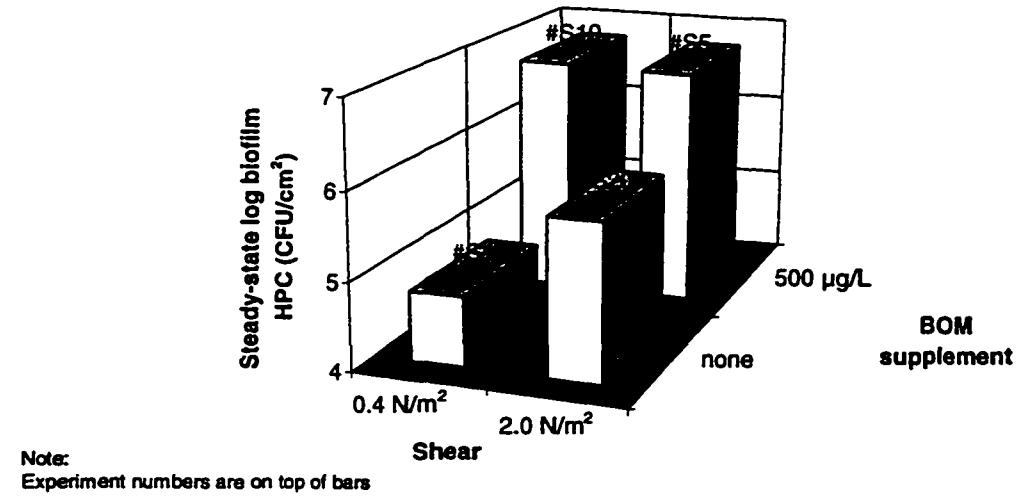


Figure 7.5: Steady-state HPCs on polycarbonate substrata in the absence of a disinfectant at 26°C (ref. Figures C/1.8 and C/1.9; Appendix C/1)

de Beer *et al.* (1994a) measured the diffusion coefficients of chlorine and glucose and found that, under equal conditions, the maximum possible glucose flux was two orders of magnitude below the lowest measured chlorine flux. This suggests that in the presence of both a BOM supplement and a disinfectant residual, the increase of flow velocity decreases biofilm HPC numbers due to the reported higher diffusion coefficient of the disinfectant.

Figure 7.6 shows steady-state HPC numbers of three synthetic water trials (S6, S10, and S16). These experiments were conducted in the presence of both a 500 µg/L BOM supplement and a 0.5 mg/L free chlorine residuals. Shear stress, substratum, and temperature were investigated at two levels. Experiment S6 can be compared to experiments S10 and S16 to indicate whether

there is a shear effect. Comparison of data obtained at 8°C (S6 and S16) shows that HPC numbers were about half an order of magnitude higher on ductile iron substratum at lower shear. Comparison of data obtained with polycarbonate (S6 and S10) suggests that steady-state HPCs were almost one order of magnitude higher at lower shear and higher temperature. Although direct comparison of the data is not possible (due to the fractional factorial nature of the design), the increase of shear stress from 0.4 to 2.0 N/m^2, in the presence of a 0.5 mg/L free chlorine residual, clearly decreased steady-state HPCs. This agrees with Rittmann's (1981) hypothesis.

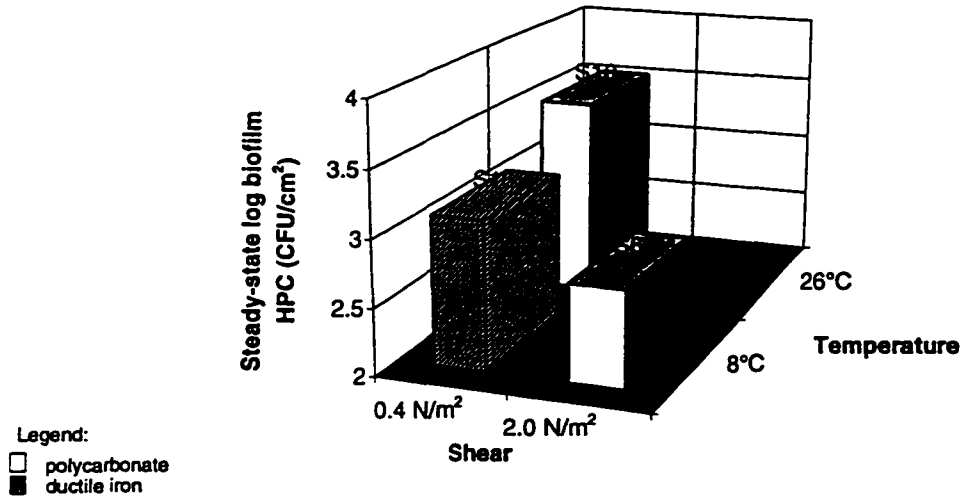


Figure 7.6: Steady-state biofilm HPCs in the presence of both a 500 µg/L BOM supplement and a 0.5 mg/L free chlorine residual

Steady-state biofilm HPC numbers of four synthetic water experiments (S1, S5, S19 and S20) were introduced in this section. These experiments were also included in Figure 7.2. Corresponding net bacterial accumulation curves are introduced in Appendix C/1 (Figures C/1.8 and C/1.9). Lu *et al.* (1995) showed that sloughing events occured more frequently at higher flow velocities which necessitated longer time to establish a biofilm. Figure C/1.9 shows that establishment of a biofilm appears to take longer in experiments utilizing the higher shear condition. This agrees with Lu *et al.*'s (1995) findings.

7.6 EFFECT OF TEMPERATURE

Figure 7.7 shows net accumulation of HPC numbers on polycarbonate substrata in the absence of both a BOM supplement and a disinfectant at 2.0 N/m^2 shear stress. Comparison of data obtained with 8°C and 26°C temperatures shows that pseudo-steady state biofilm HPC numbers were one order of magnitude lower at the lower temperature (S13 and S20). Steady-state HPCs with 8°C and 26°C appear to bracket the result with 17°C (P20 in Table 6.1). This suggests that, on polycarbonate surfaces at 2.0 N/m^2, steady-state bacterial numbers are essentially proportional to temperature in the investigated range of the variable.

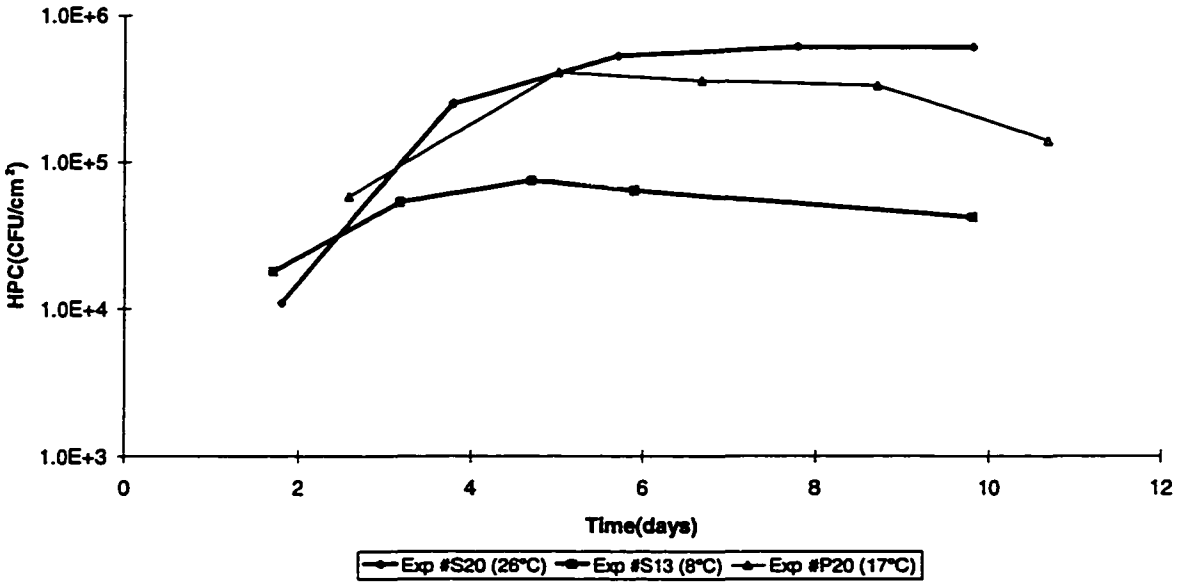


Figure 7.7: Net accumulation of HPCs on polycarbonate substrata in the absence of both a BOM supplement and a disinfectant at 2.0 N/m^2 shear stress

Camper *et al.* (1991a) reported that in their laboratory experiments the growth rate (μ) of the investigated microorganisms increased in a near-linear fashion with increasing temperature. Steady-state preliminary experimental results on mild steel substrata in Figure 7.8 (P8, P14/2, and P16) are in agreement with Camper *et al.*'s (1991a) findings. Because of the essentially fractional factorial nature of the design, direct comparison of three temperature levels in the synthetic water trials could not be made.

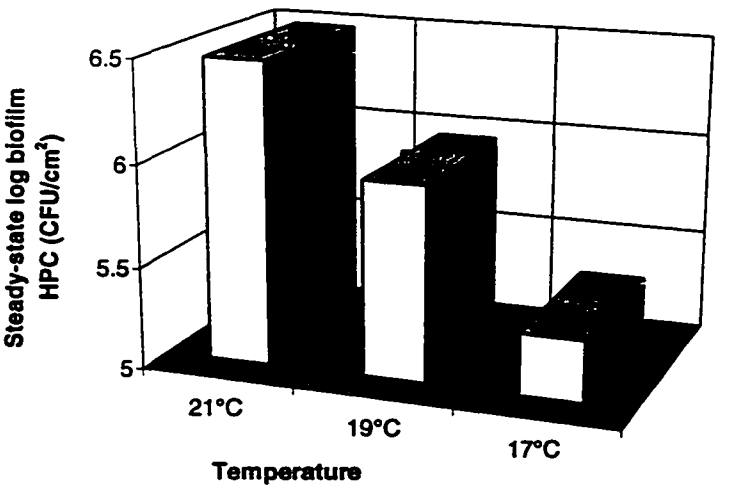


Figure 7.8: Steady-state HPCs on mild steel substrata in the absence of both a BOM supplement and a disinfectant residual at 0.4 N/m^2 shear stress (ref. Table 6.1);

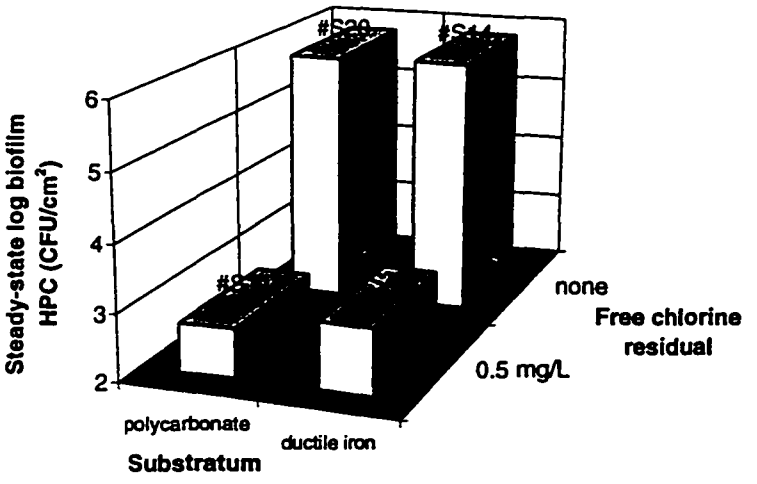
Figure C/1.10 (in Appendix C) shows net accumulation of HPCs on polycarbonate substrata in the absence of both a BOM supplement and a disinfectant at 0.4 N/m^2. Temperature levels varied between 8°C and 26°C in the introduced six experiments (S1, S12, P10, P12/2, P13/2, and P15). Under these conditions, an 18°C increase in temperature yielded only a modest increase in HPC numbers. This suggests that the effect of temperature on accumulation is less important at lower shear.

Camper *et al.* (1996) found that temperature had no significant effect on bulk or biofilm coliforms. In their pipe loop system, the increase of temperature even decreased the number of both bulk and biofilm HPC bacteria. In industrial size ARs, increased number of bulk biofilm HPCs corresponded to higher temperatures. Camper *et al.* (1996) results suggest an uncertainty about the actual effect of temperature on bacterial accumulation. The author's results partially support this.

Comparison of data in Figure 7.7 and Figure C/1.10 shows that, at 0.4 N/m^2, steady-state biofilm HPCs are about half to one order of magnitude lower. This suggests mass transfer limitation.

7.7 EFFECT OF SUBSTRATUM

Figure 7.9 shows the effects of free chlorine residual and substratum on steady-state HPC numbers in the absence of BOM supplement at 2.0 N/m^2 shear stress and 26°C. Steady-state bacterial numbers were about three orders of magnitude lower on either polycarbonate or ductile iron substrata in the presence of 0.5 mg/L free chlorine residual (S4, S11, S14, and S20). Comparison of data obtained with or without a free chlorine residual shows that, in the absence of a BOM supplement, substratum appears to have essentially no effect on steady-state biofilm HPCs.



Note:
Experiment numbers are on top of bars

Figure 7.9: Steady-state biofilm HPCs in the absence of a BOM supplement at 2.0 N/m^2 shear stress and 26°C (ref Figures C/1.11 and C/1.12; Appendix C/1)

Figure 7.10 shows net accumulation of biofilm HPCs in the presence of a 500 µg/L BOM supplement but in the absence of a disinfectant at 0.4 N/m^2 shear stress and 8°C. Under these conditions, steady-state biofilm HPCs on ductile iron substratum was not established within 10 days of AR operation. Therefore under these conditions, ductile iron substrata appears to support at least two orders of magnitude higher steady-state HPC numbers than polycarbonate, and potentially other non-corrosive surfaces.

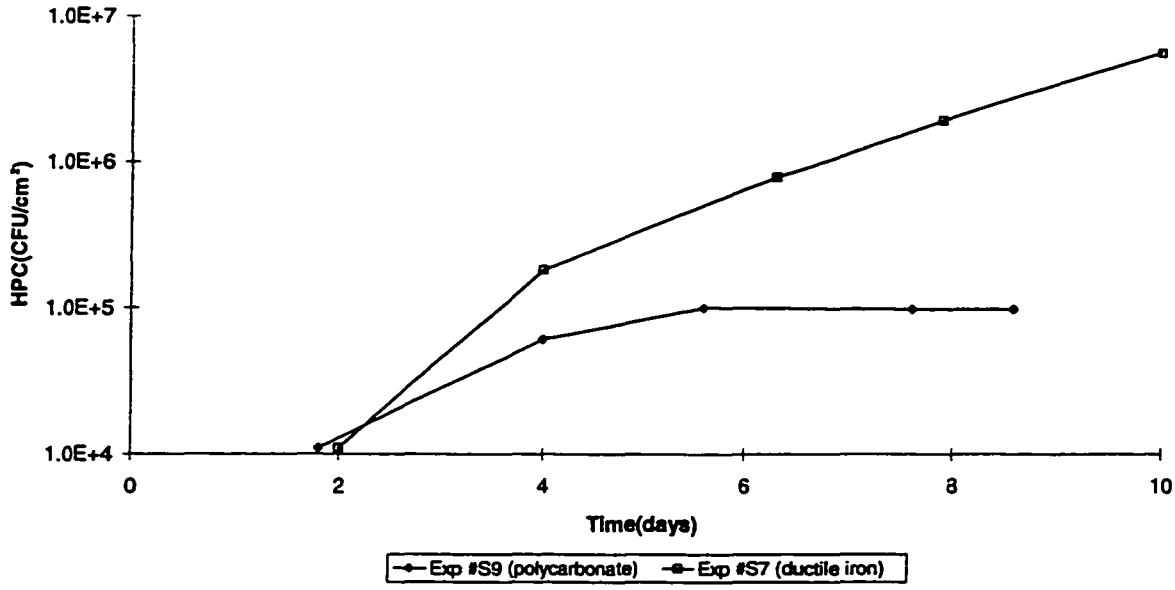


Figure 7.10: Net accumulation of biofilm HPCs in the presence of 500 µg/L BOM supplement but in the absence of a disinfectant at 0.4 N/m^2 shear stress and 8°C

Steady-state biofilm HPC numbers of four synthetic water experiments (S4, S11, S14, and S20) were introduced in this section. The corresponding four net bacterial accumulation curves are introduced in Appendix C/1 (Figures C/1.11, and C/1.12).

7.8 SUMMARY

- In the absence of a BOM supplement, temperature appears to have essentially no effect on net accumulation of HPCs.
- In the presence of a 500 µg/L BOM supplement, steady-state biofilm HPCs were little affected by shear conditions. Without a BOM supplement, shear appeared to be an important factor. This suggests that (1) biofilm accumulation is bioreaction limited at higher BOM conditions, (2) mass transfer is likely the rate limiting process at lower BOM levels.
- Bactericidal efficiency of free chlorine and monochloramine are about the same on non-corrosive surfaces in the absence of a BOM supplement and at 8°C. On ductile iron

(corrosive) surfaces, approximately a 1 to 4 chlorine to monochloramine ratio must be maintained for equal bactericidal effects.

- The provision of 0.5 mg/L free chlorine or monochloramine residual on polycarbonate supporting surface suppressed steady-state HPC numbers by 3 to 4 orders of magnitude.
- A 0.5 mg/L monochloramine residual appears to have a greater effect on steady-state HPCs at higher temperatures.
- The increase of shear stress appears to enhance disinfection efficiency.
- Biofilm HPC numbers increased with increasing BOM concentration. The effect of a 500 μg/L BOM supplement on HPCs appeared to be greater at higher temperature and lower shear stress. This supports the hypothesis of bioreaction process limitation.
- Net accumulation of biofilm HPCs increased in a near-linear fashion with increasing temperature.
- HPC supporting characteristics of polycarbonate and ductile iron substrata were about the same in the absence of a BOM supplement. With 500 μg/L BOM supplement, ductile iron substrata appears to support at least two orders of magnitude higher steady-state HPC numbers than polycarbonate surface.
- The experiments completed in this chapter investigated net accumulation of HPC numbers in 'young' biofilms with less diversified habitats. Therefore, some caution should be exercised at extrapolating 'young' biofilm data to established 'older' biofilms in distribution systems.

CHAPTER 8: RESULTS OF TRIALS WITH REAL WATERS

The primary objective of experimentation with real waters was to generate biofilm accumulation data for model testing. The coded design matrix of the 14 real water experiments was introduced in Table 5.7. Operation principles and the design of experiments with real waters were discussed in Section 4.1.2 and Section 5.3. Design factors and the system response are the same as those in the synthetic water trials. For convenience they are repeated here. The design factors are: (1) BOM concentration, (2) disinfectant type, (3) disinfectant residual, (4) shear stress, (5) temperature, and (6) substratum. The system response was the pseudo-steady state number of HPCs calculated as an average of four replicates. Similarly to the synthetic water discussion in Chapter 7, design factors will be evaluated by the one-variable-at-a-time strategy. Combined effects of the variables on the response will be shown in Chapter 9. Since actual distribution systems operate for an extended period with established biofilms, system responses will generally be introduced in terms of steady-state biofilm HPCs. Net accumulation of HPCs will also be shown for experiments R1, R11, R12, and R13. It was concluded in Section 6.8 that real water samples from two actual WTPs (WTP 'C' and WTP 'E') would be included in the further investigation. Decoding of coded real water sources is introduced in Appendix B/4.

Experiments R1 to R8 (Table 8.1) are part of a 2^3 factorial experimental design investigating the effect of (1) water source, (2) substratum and (3) free chlorine residual on the response. Experiments R9 and R10 can be compared to experiments R1 and R2 to indicate whether there is a temperature effect. Experiments R11 and R12 investigated the effect of a combined chlorine residual in an untreated groundwater. Experiments R13 and R14 investigated systems which were disinfected right from the start of the experiment and which therefore had low initial HPC

numbers. This chapter discusses major trends from the real water results. The fit of these data to the later developed models will be introduced in Chapter 9.

Table 8.1: Design Matrix and System Responses - Real Water Experiments

Water source	Exp #	BOM (µg/L)	Disinfectant type	Disinfectant residual (mg/L)	Shear stress (N/m^2)	Temp (°C)	Substratum	Steady-state biofilm HPC (CFU/cm^2)	
								arithmetic	log
filter effluent	R1	150	chlorine	0.1	1.2	16	polycarbonate	6.1×10^4	4.785
filter effluent	R2	150	chlorine	0.1	1.2	16	ductile iron	1.7×10^5	5.230
filter effluent	R3	150	chlorine	0.3	1.2	20	polycarbonate	5.4×10^3	3.732
filter effluent	R4	150	chlorine	0.3	1.2	20	ductile iron	8.3×10^3	3.919
filter influent	R5	600	chlorine	0.1	1.2	22	polycarbonate	1.7×10^5	5.230
filter influent	R6	600	chlorine	0.1	1.2	22	ductile iron	4.2×10^5	5.623
filter influent	R7	600	chlorine	0.3	1.2	22	polycarbonate	1.0×10^4	4.000
filter influent	R8	600	chlorine	0.3	1.2	22	ductile iron	3.8×10^4	4.578
filter effluent	R9	150	chlorine	0.1	1.2	24	polycarbonate	6.1×10^4	4.785
filter effluent	R10	150	chlorine	0.1	1.2	24	ductile iron	2.7×10^5	5.431
ground water	R11	50	monochloramine	0.8	1.2	16	polycarbonate	1.2×10^4	4.079
ground water	R12	50	monochloramine	0.8	1.2	16	ductile iron	3.9×10^4	4.591
filter effluent	R13 *	150	chlorine	0.1	1.2	12	polycarbonate	1.0×10^4	4.000
filter effluent	R14 *	150	chlorine	0.1	1.2	12	ductile iron	3.0×10^4	4.477

Note: Disinfectant is dosed after 96 hours of system start-up unless noted otherwise
* Disinfectant is doced from time zero

8.1 OVERVIEW

An overview of the 14 experiments with real water is presented in Figure 8.1. BOM concentrations and examined substrata are designated to one of the two horizontal axes. Type and concentration of the applied disinfectant are introduced on the other abscissa. Steady-state biofilm HPC numbers are shown on the ordinate in a logarithmic scale. Liquid phase temperatures are shown on top of the bars. Bars without shading represent conditions where a disinfectant was dosed from day #4. Disinfectant dosage from the beginning of an experiment is indicated by shading.

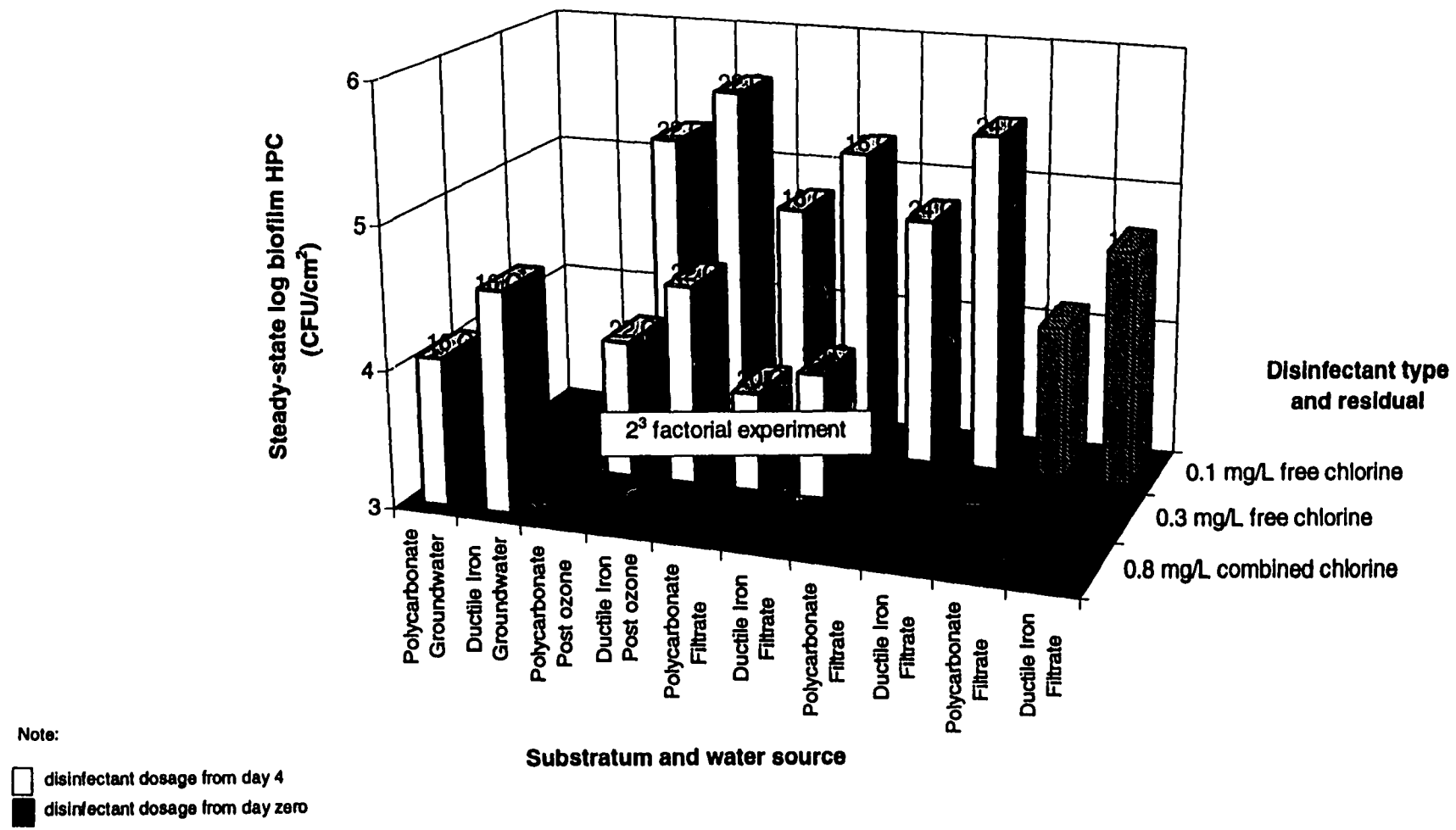


Figure 8.1: Experiments with real waters - summary (ref. Table 8.1) Note: all experiments were conducted at 1.2 N/m^2 shear stress

There were twelve experiments conducted in the presence of either 0.1 or 0.3 mg/L free chlorine residuals and two experiments in the presence of 0.8 mg/L monochloramine residual (Figure 8.1). AR experiments with free chlorine residuals were supplied with filter influents or filtrates of a BAC filter in WTP 'E'. The water source for trials R11 and R12 was the raw ground water of WTP 'C'. The temperature ranges from 12°C to 24°C in these experiments.

The comparison of these experiments, in Figure 8.1, clearly shows that steady-state biofilm HPC numbers are affected by both the concentration of the applied disinfectant and substratum. Biofilm accumulation is about one and a half orders of magnitude lower with the higher free chlorine residuals. Ductile iron appears to support about half an order of magnitude higher steady-state HPCs than polycarbonate. Steady-state biofilm bacterial numbers for the surface water with 0.1 mg/L and 0.3 mg/L free chlorine residuals appear to bracket the results for the ground water with 0.8 mg/L combined chlorine residual, when all disinfectants were applied on an established biofilm.

8.2 2^3 FACTORIAL EXPERIMENT

The objective of this factorial experiment was to determine which variable or variables had a significant effect on the system response. Three variables were investigated at two levels. The two qualitative variables were (1) BOM concentration (sampling location within the surface WTP) and (2) supporting surface (substratum). The single quantitative variable was (3) the concentration of a free chlorine residual. Therefore, these experiments studied the importance of biological filtration following ozonation. Polycarbonate and ductile iron were the two substrata utilized in the ARs. Free chlorine residual of either 0.1 or 0.3 mg/L were applied from day 4 in each of these experiments. Due to seasonal variation, the temperature of the surface water at the collection points increased from 16°C to 22°C throughout the duration of the 8 experiments.

A graphical representation of the results from the factorial design is given in Figure 8.2. The circled numbers in the figure stand for the logarithm of steady-state biofilm HPC numbers. In

the presence of 0.1 mg/L free chlorine residual and at the applied experimental conditions, steady-state biofilm HPC numbers with the filter influent on polycarbonate and with the filter effluent on ductile iron were about the same. This suggests that under the conditions tested, the reported higher HPC supporting characteristics of ductile iron surfaces (Section 6.1) may be offset by a BOM decrease of about 450 mg/L. Comparison of polycarbonate and ductile iron data shows that steady-state HPC numbers are 0.2 to 0.6 order of magnitude higher on ductile iron. This supports the hypothesis that biofilm accumulation is closely associated with corrosion (LeChevallier *et al.*, 1993). The combined effect of filter influents exposed to ductile iron substratum with 0.1 mg/L free chlorine residual supported almost two orders of magnitude higher steady-state biofilm HPC numbers than the conditions determined by their alternative design level. The effect of free chlorine residual appears to be the most important factor. This has important practical implications for control of biofilms in distribution systems suggesting the need for a well defined level of chlorine-based residual in an ozonated surface water before discharging into the distribution network. The effect of temperature is discussed later.

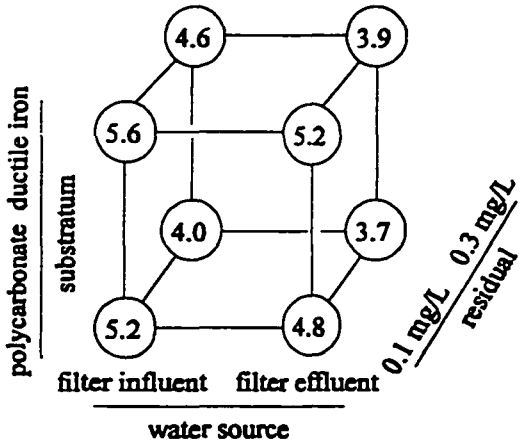


Figure 8.2: 2^3 factorial experiment with real surface waters (Experiments R1 to R8)
Note: the effect of temperature is not considered (ref Table 8.1)

Prévost *et al.* (1997) investigated the effects of BOM, residence time and a free chlorine residual on microbiological water quality in a full scale service line. By comparing suspended HPC data obtained with 1 hour and 11 hour residence times, Prévost *et al.* (1997) found that the number of suspended HPCs increased at the longer detention time. The free chlorine

residual at the longer detention time was lower. This and the author's research results are in agreement.

8.3 SUPPLEMENTARY EXPERIMENTS

8.3.1 EFFECT OF CHLORINE IN SYSTEMS WITHOUT AN ESTABLISHED BIOFILM

The objective of these experiments (R1, R2, R13 and R14) was to quantify steady state biofilm HPCs in the presence of a chlorine residual for conditions when initially a biofilm is either present or absent. Experiments R13 and R14 utilized polycarbonate and mild steel substrata at a liquid phase temperature of 12°C (chronologically these experiments were conducted before the factorial trials). From the beginning of each these two runs, a free chlorine residual of about 0.1 mg/L in the AR was maintained throughout the trials (Table 8.1). Steady-state HPC numbers for these trials are shown without shading in Figure 8.1. Experiments R1 and R2 were performed under similar conditions with the exception that the disinfectant dosage was initiated after 96 hours of the commencement of the trials, as was the normal practice, and the temperature was higher (16°C). Comparison of polycarbonate data in experiments R1 and R13 clearly shows (Figure 8.3) that pseudo steady-state HPC numbers were about half an order of magnitude lower when the disinfectant was dosed from time zero. A similar result was obtained in experiments with ductile iron substrata (Figure 8.1). Prévost and co-workers (Koudjonou *et al.* 1997) found that chlorine was a less effective biocide on an established biofilm in their bench scale bioreactors. These results suggest that for systems which rely on a disinfectant residual to control HPC accumulation, even temporary operation without a residual could lead to higher levels of accumulation. Temperature is considered to play a minor role in these observed differences.

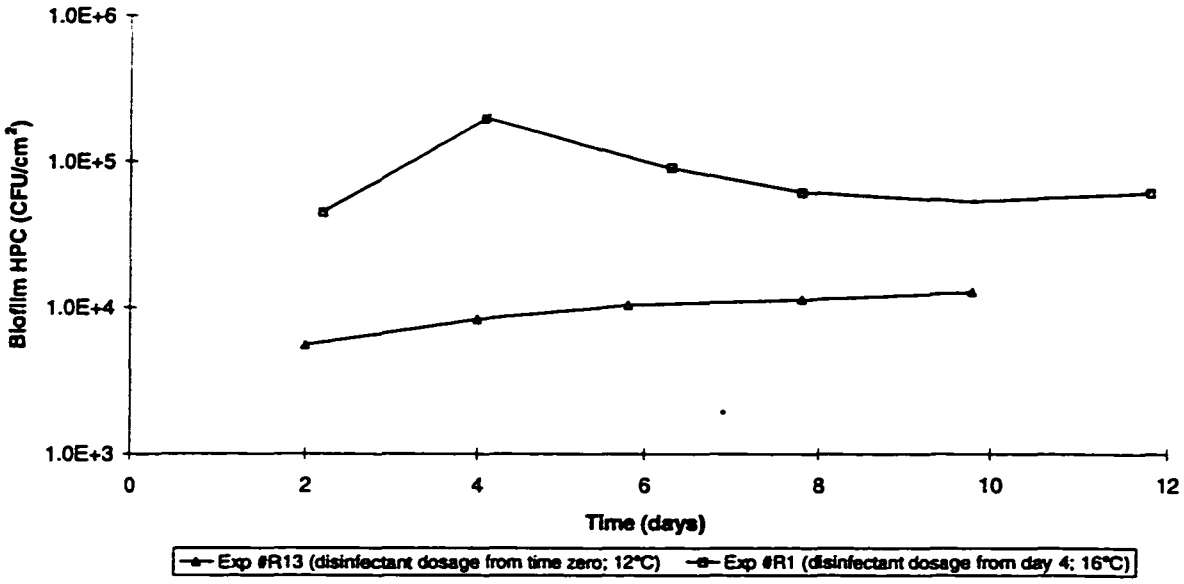


Figure 8.3: Net accumulation of HPCs on polycarbonate substrata in the presence of 0.1 mg/L free chlorine residual in WTP 'E' filtrate (ref. R1 and R13)

8.3.2 TEMPERATURE EFFECT

Accumulation on polycarbonate and mild steel substrata in the presence of 0.1 mg/L free chlorine residual at a temperature of 24°C was investigated in experiments R9 and R10 (Table 8.1). The comparison of these experimental data to results of experiments R1 and R2 (16°C) shows that in the presence of a 0.1 mg/L free chlorine residual, pseudo-steady state biofilm HPC numbers were about half an order of magnitude lower at the lower temperature on both substrata (Figure 8.1). Therefore under these conditions, an 8°C increase in temperature yielded only a modest increase in HPC numbers. This also means that the unavoidable changes in temperature during the 2^3 factorial experiment did not have a large impact on results.

A potential factor that may affect bacterial accumulation in distribution systems is the temperature. In a pilot-scale distribution system with mild steel pipe material, Camper (1995) found that, in the presence of an AOC supplement, the temperature had no effect on accumulation. This essentially agrees with the author's findings.

8.3.3 HPC SUPPORTING CHARACTERISTICS OF AN UNTREATED GROUNDWATER

Experiments R11 and R12 investigated biofilm supporting characteristics of a groundwater supply in the presence of a combined chlorine residual of 0.8 mg/L at 16°C (Table 8.1). Figure 8.4 indicates that, in the presence of a combined chlorine residual on an established biofilm, steady-state biofilm HPC numbers were about half an order of magnitude higher on a ductile iron surface than on a polycarbonate substratum. Comparison of these experiments with the surface water experiments (Figure 8.1) shows that groundwater steady-state HPC numbers were about one order of magnitude lower than the surface water experiments with a 0.1 mg/L free chlorine residual, but somewhat higher than the surface water trials utilizing a 0.3 mg/L free chlorine residual (Figure 8.1).

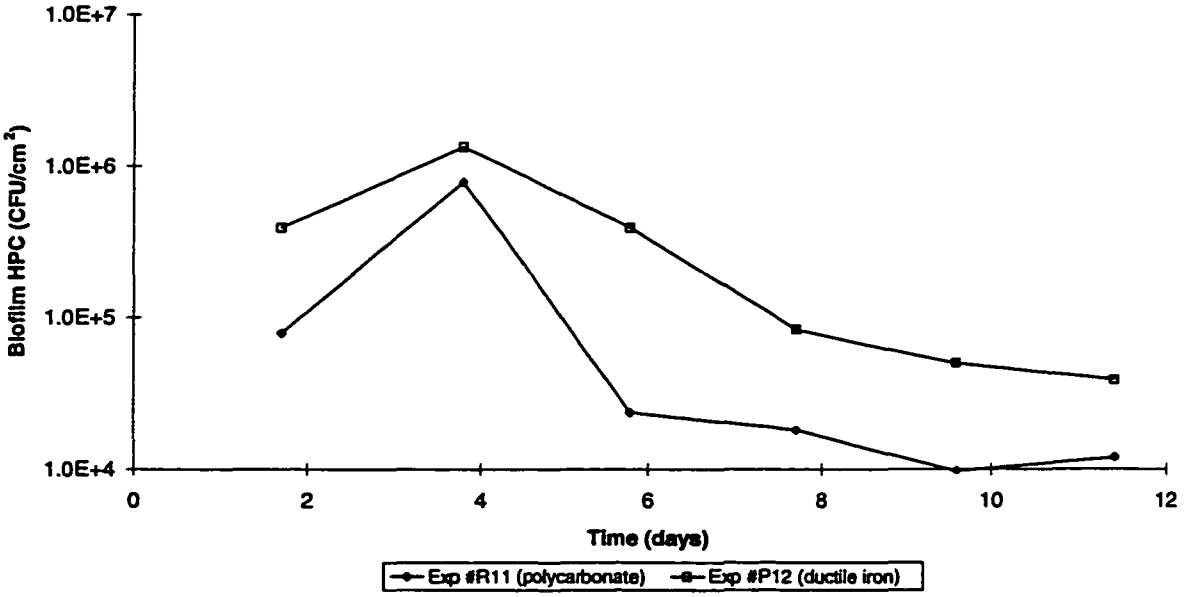


Figure 8.4: Net accumulation of biofilm HPCs in the presence of 0.8 mg/L monochloramine residual (from day 4) at 1.2 N/m^2 shear stress at 16 °C in WTP 'C' influent (ref. R11, and R12)

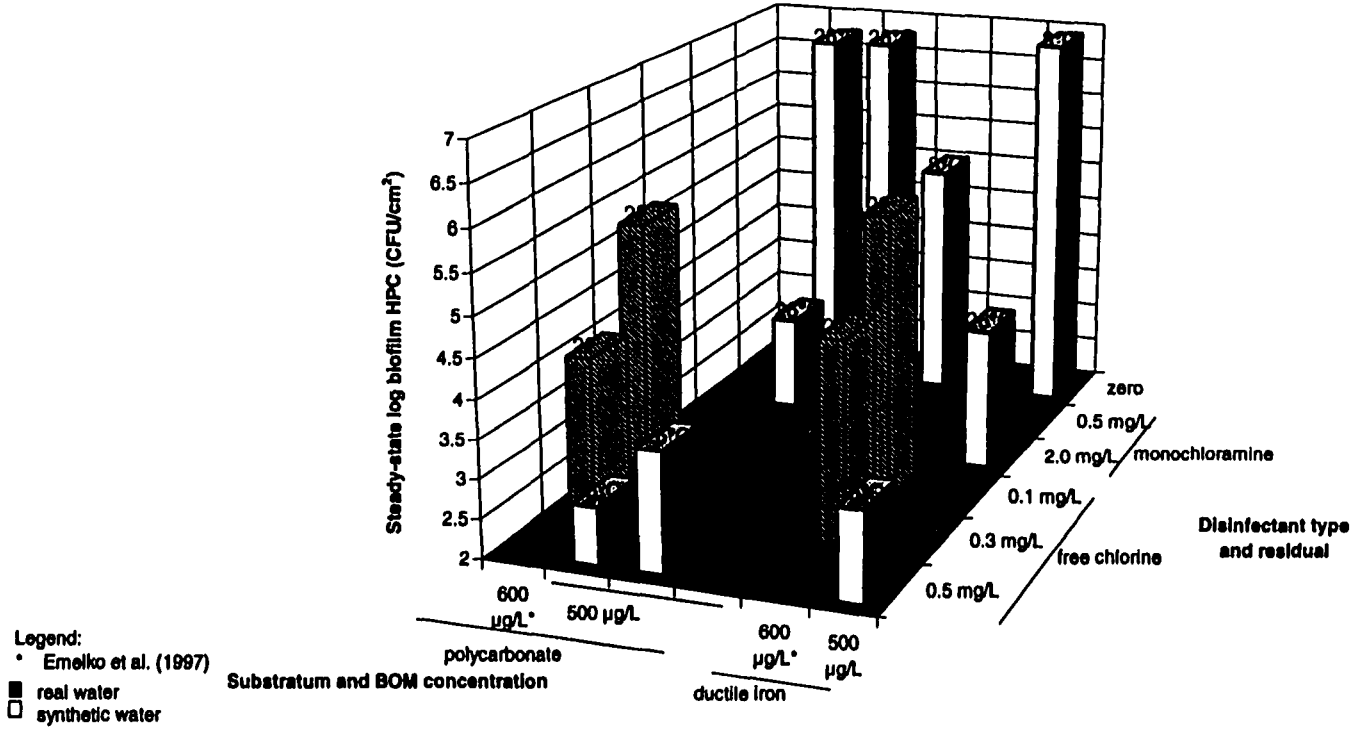
For both substrata steady-state biofilm bacterial numbers in the groundwater experiments were about the same magnitude as those from surface water trials using a 0.1 mg/L free chlorine from time zero. Although there was some difference in BOM levels between the groundwater and surface waters, these results suggest that, for the conditions examined, the effect of a 0.8 mg/L chloramine residual lies somewhere between that of a 0.1 and 0.3 mg/L free chlorine residual.

Therefore, under these conditions a given free chlorine residual was about 4 times as effective as a monochloramine residual. In comparing the effectiveness of free chlorine and chloramine residuals, pH is probably important because it affects both dissociation and speciation. In a full scale survey in North America, Shull (1981) found that bactericidal efficiencies of a free chlorine and a chloramine residuals were the highest at pH values of 6.5 and 8, respectively.

8.4 COMPARISON OF EXPERIMENTS WITH SYNTHETIC AND REAL WATERS

Although the design factors were the same, their investigated levels were different in the synthetic (Table 7.1) and real water (Table 8.1) experiments. Because of the differences, no direct comparison of synthetic and real water trials could be made. Synthetic water experimental results suggested that the effect of shear was more important at lower BOM levels (Section 7.5). In the presence of 500 µg/L BOM supplement and under the conditions tested, shear had essentially no effect on net accumulation of HPCs (Figure 7.5) suggesting a bioreaction limitation. Therefore, experiments with higher BOM concentrations may be compared regardless of the applied shear conditions.

Experiments with 500 µg/L BOM supplement were selected from among the 26 synthetic water trials (Figure 7.1). With respect to the real water trials, experiments using the BAC filter influent were chosen. The reported BOM concentration of this water source was about 600 µg/L (Emelko *et al.*, 1997). Figure 8.5 shows all the selected 14 trials. Synthetic water experiments are shown without shading. Real water trials are shaded. Although shear levels ranged from 0.4 to 2.0 N/m^2 in these trials, they were not considered. While BOM concentration and substratum are shown on one of the abscissas, disinfectant type and concentration is denoted on the other horizontal axis. As usual, steady-state log biofilm HPC numbers are shown on the ordinate. Temperatures are shown on top of the bars. Experiment numbers and corresponding HPC values (two decimal accuracy) are tabulated below the figure.



	S19 / 6.76	S5 / 6.74	S9 / 4.987		S7 / 6.90
	S8 / 3.20	S18 / 2.92			
					S3 / 3.84
R5 / 5.23				R6 / 5.62	
R7 / 4.00				R8 / 4.58	
	S6 / 2.72	S10 / 3.52			S16 / 3.11

Figure 8.5: Comparison of synthetic and real water experiments
Note: Experiment numbers and steady-state log biofilm HPCs, corresponding to each bar, are tabulated

Experiments with and without a free chlorine residual in Figure 8.5, show that the residual of the applied disinfectant has an effect. In terms of the context of this section, data obtained with monochloramine are not evaluated. It may be said that the 500 µg/L and 600 µg/L (fillter influent) BOM levels in Figure 8.5 are close enough to assume no difference at all. After averaging S6 and S10, as well as S5, S9 and S19 tabulated HPC values (Figure 8.5), data with and without a free chlorine residual may be graphed. Figure 8.6 shows steady-state log biofilm HPC vs. free chlorine residual relationships for both the polycarbonate and ductile iron. Synthetic water is indicated without shading. Real water data are shaded. Figure 8.6 shows that data obtained with synthetic waters with 0.5 mg/L free chlorine residual and without a residual bracket the HPCs of real water experiments with 0.1 and 0.3 mg/L free chlorine residuals. The essentially linear relationship shows an excellent agreement of synthetic and real water data. It may be concluded that experimentation with synthetic waters is a good substitute for the often more difficult real water analysis.

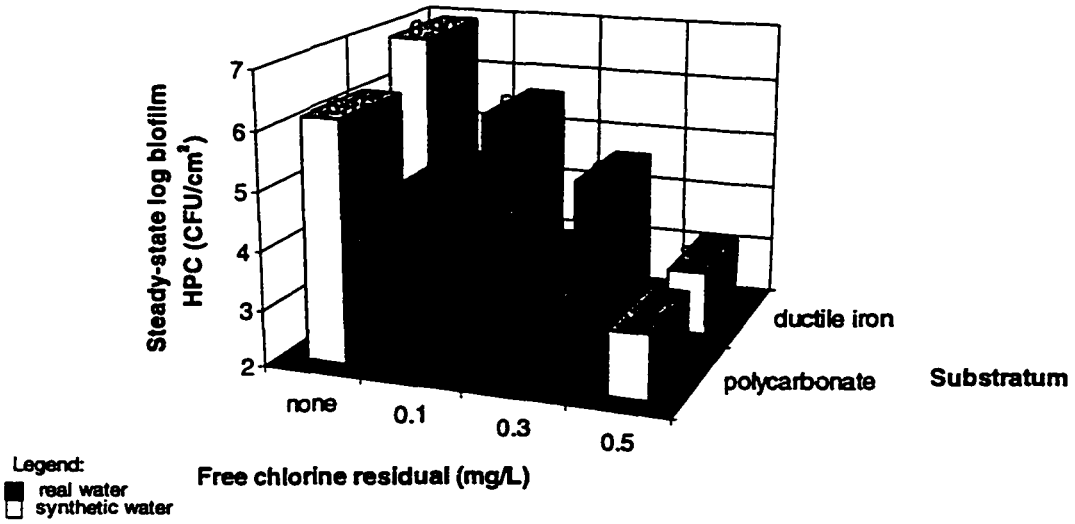


Figure 8.6: Comparison of synthetic and real water experiments - a simplified approach

8.5 SUMMARY

Three distinct real water sources originating from two WTPs were examined. A 2^3 factorial experiment was the basis for the investigation. Supporting surfaces, water sources, and free chlorine residuals were investigated at two design levels. Under the conditions tested, ductile iron supported almost half an order of magnitude higher steady-state biofilm HPCs than polycarbonate. In terms of steady-state biofilm HPCs, the BAC filtration process showed about half an order of magnitude efficiency. In excess of one log reduction in HPC numbers was achieved by the increase of free chlorine residuals from 0.1 mg/L to 0.3 mg/L. Polycarbonate surface exposed to filter influent and ductile iron substratum in contact with filtrate supported about the same biofilm HPCs. The combined effect of filter effluent exposed to polycarbonate surface in the presence of 0.3 mg/L free chlorine residual resulted in the least amount of biofilm HPCs (log HPC number of 3.7, i.e HPC=$10^{3.7}$/cm^2).

Comparison of data obtained with free chlorine residual showed about half an order of magnitude lower HPCs in experiments where the disinfectant was applied from the beginning of the experiment (in contrast to ones where free chlorine was dosed from day #4). These results suggest that for systems which rely on a disinfectant residual to control HPC accumulation, even temporary operation without a residual could lead to higher levels of accumulation. Experimental results suggested a 1 to 4 ratio of free chlorine and monochloramine residuals on ductile iron for similar suppression of biofilm HPC numbers. Under the conditions tested, synthetic and real water experimental results showed good agreement.

CAPTER 9: MODELING

The water industry has become increasingly interested in developing models which predict bacterial activity and/or microbial water quality within drinking water distribution systems. Modeling basic water quality parameters in distribution systems has been studied extensively since the early 1980's (Section 3.6). One problem is that the models developed for analyzing water quality characteristics and/or biofilm accumulation tend to be extremely complex making their use difficult, if not impossible, in actual applications. Therefore, a main objective of this research was the development of a less complex, steady-state biofilm accumulation model which can be readily used either as a drinking water distribution system operation or design tool by operator staff or practitioner engineers. The application range and accuracy of the developed empirical model maybe somewhat compromised, when compared to some more sophisticated mechanistic models, but its user-friendly nature and simplicity may easily overcome its shortcomings, making it a real asset for the water industry.

Experiments with both synthetic (Table 7.1) and real waters (Table 8.1) were conducted. Effects of individual change of design variables on the system response were introduced in Chapters 7 and Chapter 8. As said, the real life value of 'one-variable-at-a-time' approach was limited since it tacitly assumed that the effect of one variable is independent of the level of the other variables, which is usually not true. Joint functional dependence of the system response on design variables (i.e. combined effects of the variables on the response) is the basis of regression modeling and the subject of Chapter 9. Besides the main effects of the parameters, interactions are also considered in the regression models. Because higher interactions are less likely to occur, four-factor and higher interactions are not considered in the modeling approach. A linear regression model, for example, could have as many as 42 terms (constant term + 6 main effects + 15 two-factor interactions + 20 three-factor interactions). The optimization of the number of

parameters is generally considered to be a good modeling practice, since well constructed models can be reasonable well described by the main effects and some of the important lower order interactions. The supporting philosophical principle behind this is that 'nature is simple'.

Three simple but powerful tests, based on Bayesian principles (Section 3.9), were developed (P.M. Reilly, 1998) which were the basis for parameter selection:

- test #1:(prior mean)/(prior standard deviation)
- test #2:(posterior mean)/(posterior standard deviation)
- test #3:(posterior mean - prior mean)/(posterior standard deviation)

Test #1 provides information about parameters based on prior knowledge alone. Test #2 is the most realistic (posterior data) and therefore it is the primary decision making test. The actual values of test #2 are essentially standardized variables of a normal probability distribution; consequently the direct indication of the level of significance of a parameter. Test #3 shows the level of agreement of the experimental results with the original opinion, or in other words, it indicates how much new information was revealed by the experiment. Similarly to test #2, the values of both tests #1 and #3 are standardized variables and show directly the level of significance of the test.

Modeling efforts can be divided into two distinct segments. First, a so called 'synthetic water model' was developed based on data generated by experiments with synthetic water (Chapter 7.0). Section 9.1 introduces the development of both linear and quadratic synthetic water models, as well the fit of the models to both synthetic and real water data. To maximize utilization of experimental efforts, an innovative modeling approach was adapted. Based on Bayesian design principles, so called 'real water models' were developed utilizing data of both synthetic and real water trials for model development. Section 9.2 describes the development of both linear and quadratic real water models, as well as the fit of these models to real water data. Synthetic and real water models are compared in Section 9.3. Not only selection criteria and analysis of the most informative model, but also the process of parameter reestimation, and the fitting of the model to synthetic and real water data are also introduced in Section 9.4. A user-

friendly interface was developed to make the usage of the most informative model extremely easy to virtually anyone (including the little green guys from Mars). The interface and its Visual Basic© code, as well as modeling results are introduced in the second part of Section 9.4. A summary of the most informative model is provided in Section 9.5.

9.1 SYNTHETIC WATER MODELS

9.1.1 MODEL DEVELOPMENT

A linear regression model including all possible interactions, as introduced in equation (3-1) of Section 3.9, is the basis of the empirical modeling. The multivariate normal distribution of the posterior distribution of the true value of the parameters was introduced in equation (3-4) of Section 3.9. As described, $\left\{\left[\underline{U}^{-1}+\left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}\left[\underline{U}^{-1}\underline{\alpha}+\left(1/\sigma^2\right)\underline{X}'\underline{y}\right]\right\}$ is the posterior parameter estimates, and $\left[\underline{U}^{-1}+\left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}$ is the posterior covariance matrix of the multivariate normal distribution. In order to define these posterior values, the knowledge of (1) the vector of prior point estimates ($\underline{\alpha}$), (2) observation vector ($\underline{y}$), (3) prior covariance matrix ($\underline{U}$), (4) system variance (σ^2), and (5) regression matrix ($\underline{X}$) is necessary. It was emphasized in Section 3.9 that the generalized variance, $|\underline{V}|$, could be designed before the commencement of actual experimentation, since the posterior covariance matrix did not contain the observation vector. At the development of synthetic water models, two distinct approaches were considered: (1) the linear approach, i.e. only linear effects of all the variables on the response are assumed and (2) the quadratic approach, i.e. besides linear effects, the response is potentially impacted by defined quadratic effects.

Linear synthetic water models

Prior information for the linear synthetic water models is shown in Appendix D/1. For convenience, the prior point estimates and the diagonal elements of the prior covariance matrix

($\underline{U}$) are repeated in Table 9.1. With the exception of the mean and the six main effects, all interactions are zero in the 42 x 1 vector of prior point estimates. As described in Section 3.9, an effect is numerically twice the parameter. The numerical values of the 26 x 1 observation vector are those of the actual steady-state biofilm HPC numbers (in logarithmic form) of the synthetic water experiments (Table 7.1). Model related matrix operation is based on logarithmic values (Section 5.2). The numerical values of the diagonal elements of the 42 x 42 diagonal prior covariance matrix are listed in vector U1 in Appendix C/1. Two factor interactions and their assigned importances are shown in Table 5.5. Main effects were considered to have high importance. Three factor interactions were assigned with a low importance.

Table 9.1: Prior Point Estimates and Diagonal Elements of Prior Covariance Matrix - Linear Synthetic Water Models (ref. Appendix D/1)

Actual parameter	Coded parameter	Prior parameter estimates (α)	Diagonal elements of prior covariance matrix (U1)
mean	l	5.0	0.6990
BOM	a	0.8	1
disinfectant type	b	0.7	1
disinfectant residual	c	-1.5	1
shear	d	0.4	1
temperature	e	0.7	1
substratum	f	1.0	1
BOM - disinfectant type	ab	0	0.0016
BOM - disinfectant residual	ac	0	0.6021
BOM - shear	ad	0	0.3010
BOM - temperature	ae	0	0.4771
BOM - substratum	af	0	0.0016
disinfectant type - disinfectant residual	bc	0	0.6990
disinfectant type - shear	bd	0	0.0016
disinfectant type - temperature	be	0	0.3010
disinfectant type - substratum	bf	0	0.6990
disinfectant residual - shear	cd	0	0.3010
disinfectant residual - temperature	ce	0	0.3010
disinfectant residual - substratum	cf	0	0.6990
shear - temperature	de	0	0.3010
shear - substratum	df	0	0.3010
temperature - substratum	ef	0	0.3010

Notes: Parameters are listed in natural order
Prior parameter estimates (α) of all 3 factor interactions are 0 (zero)
Diagonal elements of the prior covariance matrix (U1) of all 3 factor interactions are 0.0016

Because smaller initial variance for a parameter indicates more knowledge about that parameter before the experiment, the above variances indicate that it is desired to find out the most about the main effects and the least about some two and all the three factor interactions. Supporting data for the 0.0189 system variance is shown in Table 5.6. The 26 x 42 regression matrix ($\underline{X}$) consists of rows corresponding to the 26 synthetic water trials in an order identical to the one in Table 5.3. The columns of ($\underline{X}$) are the constant term, main effects, two and three factor interactions in natural order.

The posterior parameter estimate of the linear synthetic water model, the 42 x 42 posterior covariance matrix, the correlation matrix (evolved from the covariance matrix by dividing all the rows and all the columns of the covariance matrix by the square root of the diagonal elements of the covariance matrix) are shown in Appendix D/1. For convenience, posterior parameter estimates are repeated in Table 9.2.

Table 9.2: Posterior Parameter Estimates - Linear Synthetic Water Model (ref. Appendix D/1)

Actual parameter	Coded parameter	Posterior parameter estimates
mean	1	4.57
BOM	a	0.37
disinfectant type	b	-0.24
disinfectant residual	c	-1.58
shear	d	-0.06
temperature	e	0.40
substratum	f	0.35
BOM - disinfectant type	ab	-0.01
BOM - disinfectant residual	ac	-0.22
BOM - shear	ad	0.04
BOM - temperature	ae	0.24
BOM - substratum	af	0.00
disinfectant type - disinfectant residual	bc	0.09
disinfectant type - shear	bd	-0.02
disinfectant type - temperature	be	-0.14
disinfectant type - substratum	bf	-0.15
disinfectant residual - shear	cd	-0.18
disinfectant residual - temperature	ce	-0.16
disinfectant residual - substratum	cf	-0.29
shear - temperature	de	0.03
shear - substratum	df	-0.23
temperature - substratum	ef	0.11

Notes: Parameters are listed in natural order
Values for 3 factor interactions are shown in Appendix D/1

The correlation matrix suggests a certain degree of correlation of the parameters. Values of off-diagonal elements in excess of 0.5 (underlined for easy detection) suggest relatively high correlation of the corresponding parameters (e.g. the third row fifth column value of 0.6508 indicates that factors 'b' [disinfectant type] and 'd' [shear stress] are highly correlated). A total of 86 such off-diagonal elements were found.

Parameter selection test principles were introduced earlier in this chapter. Results of test #1, test #2, and test #3 for the linear synthetic water models are introduced in Appendix D/1. Selection of parameters at given significance level is based on test #2 [(posterior mean) / (posterior standard deviation)]. For convinience, test #2 results are introduced in Table 9.3.

Table 9.3: Test #2 Results - Linear Synthetic Water Models (ref. Appendix D/1)

Actual parameter	Coded parameter	test #2 (posterior mean / posterior standard deviation)
mean	1	23.77
BOM	a	3.31
disinfectant type	b	-1.37
disinfectant residual	c	-15.18
shear	d	-0.25
temperature	e	4.98
substratum	f	1.47
BOM - disinfectant type	ab	-0.23
BOM - disinfectant residual	ac	-2.38
BOM - shear	ad	0.19
BOM - temperature	ae	3.27
BOM - substratum	af	0.07
disinfectant type - disinfectant residual	bc	1.25
disinfectant type - shear	bd	-0.44
disinfectant type - temperature	be	-1.12
disinfectant type - substratum	bf	-0.58
disinfectant residual - shear	cd	-1.60
disinfectant residual - temperature	ce	-1.31
disinfectant residual - substratum	cf	-2.41
shear - temperature	de	0.34
shear - substratum	df	-1.24
temperature - substratum	ef	0.41

Notes: Parameters are listed in natural order
Test #2 values for 3 factor interactions are shown only in Appendix D/1
Highest absolute value of 3 factor interaction is 0.80 (BOM - shear -temperature)
Test #2 values above: 1.96 corresponds to 5% significance level
1.65 corresponds to 10% significance level
1.04 sorresponds to 30% significance level

Significant parameters were determined at 5%, 10%, and 30% significance levels. In addition each of these levels was evaluated with the significant parameters plus 'd' (shear stress) which turned out to be of special interest at a later stage of the analysis (Reilly, 1998). 5% significance level corresponds to a test #2 value of equal or greater than 1.96. Similarly 10% significance level is related to 1.65 and 30% significance level is related to 1.04. The selected and only the selected parameters are the building blocks of a regression equation (model). Naturally, the higher the significance level, the more parameters the model will have. Table 9.4 shows the number of parameters along with the actual coded parameter designation for each of the six linear synthetic water models. Covariance and correlation matrices are also qualified in Table 9.4 (e.g. excellent, good, etc.) based on the earlier introduced evaluation criteria.

Table 9.4: Linear and Quadratic Synthetic Water Models - Summary

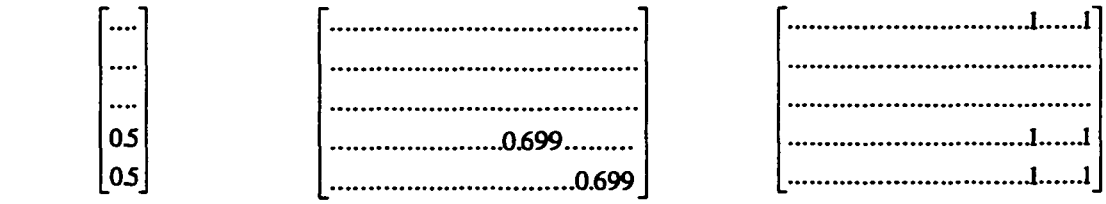
Water source	Model type	Parameter selection	Significance level*	# of parameters	Actual parameters	Covariance/ correlation matrix
synthetic	linear	test #2	5%	7	1,a,c,e, ac,ae,cf	excellent/ excellent
"	"	"	5% + d	8	1,a,c,d,e, ac,ae,cf	excellent/ excellent
"	"	"	30%	14	1,a,b,c,e,f, ac,ae,bc,be, cd,ce,cf,df	excellent/ excellent
"	"	"	30% + d	15	1,a,b,c,d,e,f, ac,ae,bc,be, cd,ce,cf,df	excellent/ excellent
synthetic	quadratic	test #2	5%	7	1,a,c, ac,ae,cf,d2	excellent/ good
"	"	"	5% + d	8	1,a,c,d, ac,ae,cf,d2	excellent/ good
"	"	"	10%	9	1,a,c,e, ac,ae,ce,cf,d2	excellent/ good
"	"	"	10% + d	10	1,a,c,d,e, ac,ae,ce,cf,d2	excellent/ good
"	"	"	30%	14	1,a,b,c,e, ac,ae,bf,cd, ce,cf,df,d2,e2	excellent/ good
"	"	"	30% + d	15	1,a,b,c,d,e, ac,ae,bf,cd, ce,cf,df,d2,e2	excellent/ excellent

* linear models based on 5% and 10% significance levels are identical (ref. Table 9.3)

Quadratic Synthetic Water Models

The process of determining significant parameters for the quadratic synthetic water model is quite similar to the one for the previously described linear synthetic water model. Therefore, only differences will be discussed in detail. The investigated quadratic effects are shear stress (d) and temperature (e), since these two variables were investigated at three levels in the synthetic water trials (Table 5.3).

Prior information for the quadratic synthetic water models is shown in Appendix D/2. The vector of prior point estimates is now 44 x 1 and accomodates two new values corresponding to the quadratic effects. The added values (0.5) anticipate half an order of magnitude increase of steady-state log biofilm HPCs due to the quadratic terms of either shear (d) or temperature (e). The size of the prior covariance matrix has increased to 44 x 44 by the addition of two new terms to its diagonal. The added values are 0.699, suggesting a moderate knowledge or interest about the quadratic effects of shear and temperature. The 26 x 44 regression matrix has two new columns corresponding to the quadratic terms of shear (d^2) and temperature (e^2). Naturally, all values in these two columns are +1. The unchanged system variance is 0.0189. The vector of prior point (parameter) estimates, as well as the prior covariance and regression matrices are shown schematically below:



prior point estimates (44 x 1) | prior covariance matrix (44 x 44) | regression matrix (26 x 44)

The posterior information (parameter estimates, correlation and covariance matrices) of the quadratic synthetic water models is introduced in Appendic D/2. For convenience, the posterior point estimates are repeated in Table 9.5. Both the covariance and correlation matrices increased to a size of 44 x 44. The magnitude of the off-diagonal elements of the correlation matrix indicates the strength of correlation between corresponding parameters. There are 87

off-diagonal values in excess of 0.5 in the correlation matrix. Similarly to the linear model, these values are underlined in Appendix D/2.

Table 9.5: Posterior Parameter Estimates - Quadratic Synthetic Water Model (ref. Appendix D/2)

Actual parameter	Coded parameter	Posterior parameter estimates
mean	1	5.32
BOM	a	0.36
disinfectant type	b	-0.23
disinfectant residual	c	-1.60
shear	d	-0.18
temperature	e	0.52
substratum	f	0.23
BOM - disinfectant type	ab	-0.01
BOM - disinfectant residual	ac	-0.23
BOM - shear	ad	-0.08
BOM - temperature	ae	0.21
BOM - substratum	af	0.00
disinfectant type - disinfectant residual	bc	0.02
disinfectant type - shear	bd	-0.02
disinfectant type - temperature	be	-0.11
disinfectant type - substratum	bf	-0.26
disinfectant residual - shear	cd	-0.14
disinfectant residual - temperature	ce	-0.22
disinfectant residual - substratum	cf	-0.34
shear - temperature	de	-0.01
shear - substratum	df	-0.30
temperature - substratum	ef	0.23
$(\text{shear})^2$	d^2	-0.66
$(\text{temperature})^2$	e^2	-0.20

Notes: Parameters are listed in natural order
Values for 3 factor interactions are shown in Appendix D/2

Parameter selection procedure is identical to the one described for the linear model, and based on the evaluation of the numerical values of test #2. Test results are introduced in Appendix D/2. For convenience, test #2 results are introduced in Table 9.6. Based on test #2 results, six quadratic synthetic water models were analysed. These models along with their significance levels, number of involved parameters, designation of actual parameters, and the qualification of covariance/correlation matrices are introduced in Table 9.4. Models were analysed at the 5%, 10%, and 30% significance levels. The quadratic term of the shear stress (d) was found to be significant at all the investigated levels. On the other hand, linear shear effect was shown to be

not significant at either of the investigated levels. It is generally accepted modeling practice that linear terms are investigated along with their quadratic counterparts even if the linear terms were not originally found significant at an investigated level. This was the justification for duplicating modeling efforts and include the additional linear shear (d) effect in all the linear as well as in the quadratic models.

Table 9.6: Test #2 Results - Quadratic Synthetic Water Model (ref. Appendix D/2)

Actual parameter	Coded parameter	test #2 (posterior mean / posterior standard deviation)
mean	1	89.16
BOM	a	3.24
disinfectant type	b	-1.33
disinfectant residual	c	-15.28
shear	d	-0.75
temperature	e	1.81
substratum	f	0.94
BOM - disinfectant type	ab	-0.17
BOM - disinfectant residual	ac	-2.48
BOM - shear	ad	-0.39
BOM - temperature	ae	2.80
BOM - substratum	af	0.08
disinfectant type - disinfectant residual	bc	0.22
disinfectant type - shear	bd	-0.42
disinfectant type - temperature	be	-0.83
disinfectant type - substratum	bf	-1.04
disinfectant residual - shear	cd	-1.26
disinfectant residual - temperature	ce	-1.75
disinfectant residual - substratum	cf	-2.82
shear - temperature	de	-0.12
shear - substratum	df	-1.61
temperature - substratum	ef	0.84
$(\text{shear})^2$	d^2	-4.34
$(\text{temperature})^2$	e^2	-1.53

Notes: Parameters are listed in natural order
Test #2 values for 3 factor interactions are shown in Appendix D/2
Highest absolute value of 3 factor interaction is 0.90
(disinfectant type - disinfectant residual - temperature)
Test #2 values above: 1.96 corresponds to 5% significance level
1.65 corresponds to 10% significance level
1.04 sorresponds to 30% significance level

9.1.2 RESIDUALS

Similarly to the posterior parameter estimation method described in Section 9.1.1, significant parameters (selected by test #2) of each synthetic water model were reestimated before model fitting. Reestimation method of the significant parameters will be introduced in detail for the most informative model in Section 9.4.1.

Having obtained the reestimates of the unknown parameters, an estimated response ($\underline{\hat{y}}$) can be calculated by substituting the significant parameter reestimates ($\underline{\hat{\theta}}$) into the general regression model

$$\underline{y} = \underline{X\theta^*} + \underline{\varepsilon} \qquad (3\text{-}1)$$

with $\underline{y} = \underline{\hat{y}}$ and $\underline{\theta^*} = \underline{\hat{\theta}}$ substitutions. The estimated response may be compared with true observation values ($\underline{y}$). The quantities $\underline{y} - \underline{\hat{y}}$ are the residuals.

There were a total of 12 synthetic water models obtained with the formerly introduced parameter selection criteria (Table 9.4). All these (linear and quadratic) synthetic water models were fitted to both the generating synthetic water and real water data. The 26 data points in the synthetic water fitted plots correspond to the same number of synthetic water trials (Table 5.3). The 12 data points in the real water fitted plots correspond to 12 data points (R1 through R12) of the real water data set (Table 5.7). Experiments R13 and R14 were not consistent with the other trials (disinfectant was dosed from time zero), therefore could not be considered for the fitting procedure.

The residual plots show the data points in the residual (ordinate) vs. estimated response (abscissa) log-log relationship. Variances (σ^2), standard deviations (σ), and 2σ values for each

fit were calculated. These statistical values are denoted in the footnote of the residual plots in Figures D/3.1 to D/3.20 in Appendix D/3. The same values are reproduced in the summary tables (Table 9.7 and Table 9.8). Since 5% significance level (or 95% confidence interval) of a normal probability distribution is 1.96σ, the 2σ values are close approximation of a 95% confidence region. Residuals situated outside of the 95% confidence boundaries (i.e. not significant at the 5% level) are called outliers and identified by their experiment numbers on both the plots and the summary tables.

Linear Synthetic Water Model Fit to Synthetic Water Data

Figure D/3.1 (Appendix D/3) shows the fit of a linear synthetic water model (parameters selected at 5% significance level) to the generating synthetic water data. The calculated variance of the residuals is 0.188 and the 2σ value is 0.866. The residual plot is a random 'gun-shot blast' type with a single outlier (S9; Table 7.1). The overall subjective rating of the plot is 'excellent'.

Figures D/3.2 through D/3.4 show the fits of other linear synthetic water models to synthetic water data. Parameters were selected at 5% + 'd', 30%, and 30% + 'd' levels in these models (Table 9.4). According to the selection criteria (test #2 in Appendix D/1), the number of parameters are the same at 5% or 10% levels. Calculated variances, standard deviations (σ), and 2σ values for each fit are tabulated in Table 9.7. Each plot is qualified, the number of outliers are reported and reference figure numbers are shown also in Table 9.7.

Quadratic Synthetic Water Model Fit to Synthetic Water Data

Figures D/3.5 through D/3.10 (Appendix D/3) show the fits of quadratic synthetic water models to synthetic water data. Parameters were selected at 5%, 5% + 'd', 10%, 10% + 'd', 30%, and 30% + 'd' significance levels in these models (Table 9.4). The corresponding number of reesimated parameters are 7, 8, 9, 10, 14, and 15 respectively. Calculated variances, standard deviations (σ), 2σ values, plot qualification, number of outliers, and reference figure numbers are tabulated in Table 9.7.

Table 9.7: Fit of Synthetic Water Models to Synthetic Water Data

Water source	Model type	Parameter selection	Significance level*	# of parameters	Actual parameters	Covariance/ correlation matrix	Variance	Std.	2 x Std.	# of outliers	Residual plot	Reference Appendix D/3
synthetic	linear	test #2	5%	7	1,a,c,e, ac,ae,cf	excellent/ excellent	0.188	0.433	0.866	1	excellent	Fig D/3.1
"	"	"	5% + d	8	1,a,c,d,e, ac,ae,cf	excellent/ excellent	0.18	0.424	0.848	1	excellent	Fig D/3.2
"	"	"	30%	14	1,a,b,c,e,f, ac,ae,bc,be, cd,ce,cf,df	excellent/ excellent	0.036	0.19	0.38	1	good	Fig D/3.3
"	"	"	30% + d	15	1,a,b,c,d,e,f, ac,ae,bc,be, cd,ce,cf,df	excellent/ excellent	0.036	0.19	0.38	"	good	Fig D/3.4
synthetic	quadratic	test #2	5%	7	1,a,c, ac,ae,cf,d2	excellent/ good	0.223	0.472	0.944	1	good	Fig D/3.5
"	"	"	5% + d	8	1,a,c,d, ac,ae,cf,d2	excellent/ good	0.205	0.452	0.904	1	excellent	Fig D/3.6
"	"	"	10%	9	1,a,c,e, ac,ae,ce,cf,d2	excellent/ good	0.145	0.381	0.762	1	good	Fig D/3.7
"	"	"	10% + d	10	1,a,c,d,e, ac,ae,ce,cf,d2	excellent/ good	0.14	0.374	0.748	"	excellent	Fig D/3.8
"	"	"	30%	14	1,a,b,c,e, ac,ae,bf,cd, ce,cf,df,d2,e2	excellent/ good	0.077	0.277	0.554	2	good	Fig D/3.9
"	"	"	30% + d	15	1,a,b,c,d,e, ac,ae,bf,cd, ce,cf,df,d2,e2	excellent/ excellent	0.075	0.273	0.546	"	excellent	Fig D/3.10

* linear models based on 5% and 10% significance levels are identical (ref. Table 9.3)

Std standard deviation

Linear Synthetic Water Model Fit to Real Water Data

Figures D/3.11 through D/3.14 (Appendix D/3) show the fits of linear synthetic water models to real water data. Parameters were selected at 5%, 5% + 'd', 10%, 10% + 'd', 30%, and 30% + 'd' levels in these models (Table 9.8). The corresponding number of reestimated parameters are 7, 8, 7, 8, 14, and 15 respectively. Calculated variances, standard deviations (σ), 2σ values, plot qualification, number of outliers, and reference figure numbers are tabulated in Table 9.8.

Quadratic Synthetic Water Model Fit to Real Water Data

Figures D/3.15 through D/3.20 (Appendix D/3) show the fits of quadratic synthetic water models to real water data. Parameters were selected at 5%, 5% + 'd', 10%, 10% + 'd', 30%, and 30% + 'd' levels in these models (Table 9.8). The corresponding number of reesimated parameters are 7, 8, 9, 10, 14, and 15 respectively. Calculated variances, standard deviations (σ), 2σ values, plot qualification, number of outliers, and reference figure numbers are tabulated in Table 9.8.

Comparison of Fits of Synthetic Water Models

Figure 9.1 clearly shows that fits of synthetic water models to generating synthetic water data is better (i.e. smaller residual variances) than their fits to real water data points.

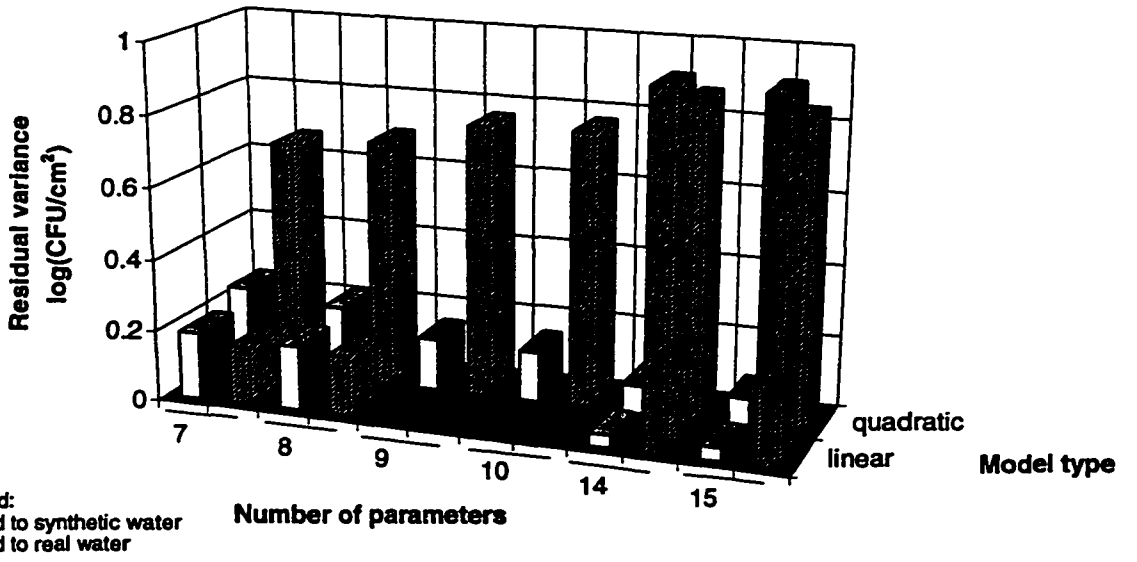


Figure 9.1: Variances - fit of synthetic water model to synthetic and real water data (ref. Table 9.7 and Table 9.8)

Table 9.8: Fit of Synthetic Water Models to Real Water Data

Water source	Model type	Parameter selection	Significance level *	# of parameters	Actual parameters	Variance	Standard deviation (Std)	2 x Std	# of outliers	Residual plot	Reference Appendix D/3
real	linear	test #2	5%	7	1,a,c,e, ac,ae,cf	0.16	0.4	0.8	2	good	Fig D/3.11
real	"	"	5% + d	8	1,a,c,d,e, ac,ae,cf	0.168	0.41	0.82	2	good	Fig D/3.12
real	"	"	30%	14	1,a,b,c,e,f, ac,ae,bc,be, cd,ce,cf,df	0.964	0.982	1.964	"	medium	Fig D/3.13
real	"	"	30% + d	15	1,a,b,c,d,e,f, ac,ae,bc,be, cd,ce,cf,df	0.964	0.982	1.964	"	medium	Fig D/3.14
real	quadratic	test #2	5%	7	1,a,c, ac,ae,cf,d2	0.665	0.815	1.63	0	good	Fig D/3.15
real	"	"	5% + d	8	1,a,c,d, ac,ae,cf,d2	0.691	0.831	1.662	0	good	Fig D/3.16
real	"	"	10%	9	1,a,c,e, ac,ae,ce,cf,d2	0.76	0.871	1.742	"	good	Fig D/3.17
real	"	"	10% + d	10	1,a,c,d,e, ac,ae,ce,cf,d2	0.766	0.875	1.75	"	good	Fig D/3.18
real	"	"	30%	14	1,a,b,c,e, ac,ae,bf,cd, ce,cf,df,d2,e2	0.869	0.932	1.864	0	good	Fig D/3.19
real	"	"	30% + d	15	1,a,b,c,d,e, ac,ae,bf,cd, ce,cf,df,d2,e2	0.839	0.916	1.832	"	good	Fig D/3.20

* linear models based on 5% and 10% significance levels are identical (ref. test #2 of Appendix C/1)

Std standard deviation

Comparison of linear and quadratic model fits in Figure 9.1 suggests that the fit of linear models is better to both synthetic and real water data. The increase of parameters appeared to improve the fits to synthetic water data but made the fits to the real water data less attractive. This suggests that as a result of increasing number of parameters, the model describes more and more precisely its generating synthetic water data. Since errors of different sources are always involved in the synthetic data, the model describes with increasing accuracy this erronous data series. At the same time, it deviates from the actual or real water data points.

The fit of the 7 and 8 parameter linear models to synthetic and real water data was essentially the same. Comparison of fits of models with and without the shear term (d) parameter shows that the involvement of the 'd' parameter improves the fit of synthetic water models to synthetic water data but has a small negative effect on fit to real water data points (Figure 9.1). The fits of 14 and 15 parameter quadratic models to real water data are exceptions to this (Table 9.8).

Comparison of residual plots in Appendix D/3 suggests the superiority of synthetic water fitted plots (Figures D/3.1 through D/3.10). These synthetic water fitted plots show no discernible pattern and the number of outliers is low (typically 1). An example for an 'excellent' rated residual plot is shown in Figure D/3.8 (Appendix D/3). One of the problem with the real water fits is that the residuals are typically negative values which indicates that the model 'overshot' the real data. This could be related to BOM composition and/or slower reaction kinetics. Another problem with the real water fits is that the residuals tend to have a negative correlation with the estimated response. This pattern suggests an increasing variance and reduced modeling accuracy in regions of higher system response (typically waters without a disinfectant residual). An example for a 'medium' rated plot with overshoting tendency and with a negative correlation could be Figure D/3.19 (Appendix D/3).

9.1.3 PREFERRED SYNTHETIC WATER MODEL

There are no black and white rules for selecting a preferred model; subjectivity and personal judgement are involved. It appears that the 7 and 8 parameter linear synthetic water models describe reasonably well the generating synthetic water data and their fit to real water data is the best (Figure 9.1). Since the added 'd' parameter improves the synthetic fit to a minor extent and worsen the fit to real water data (Table 9.7 and Table 9.8), the 7 parameter linear synthetic model is recommended for further consideration. The synthetic water fitted residual plot of the preferred synthetic water model is shown in Figure 9.2 and reproduced in Figure D/3.1 (Appendix D/3).

Figure 9.3 shows the real water fitted residual plot of the preferred synthetic water model. This plot is reproduced in Figure D/3.11. Selected parameters of the preferred synthetic water model are (coded parameters are in bracket):

- constant term (1)
- BOM (a)
- disinfectant residual (c)
- temperature (e)
- BOM - disinfectant type (ac)
- BOM - temperature (ae)
- Disinfectant residual - substratum (cf)

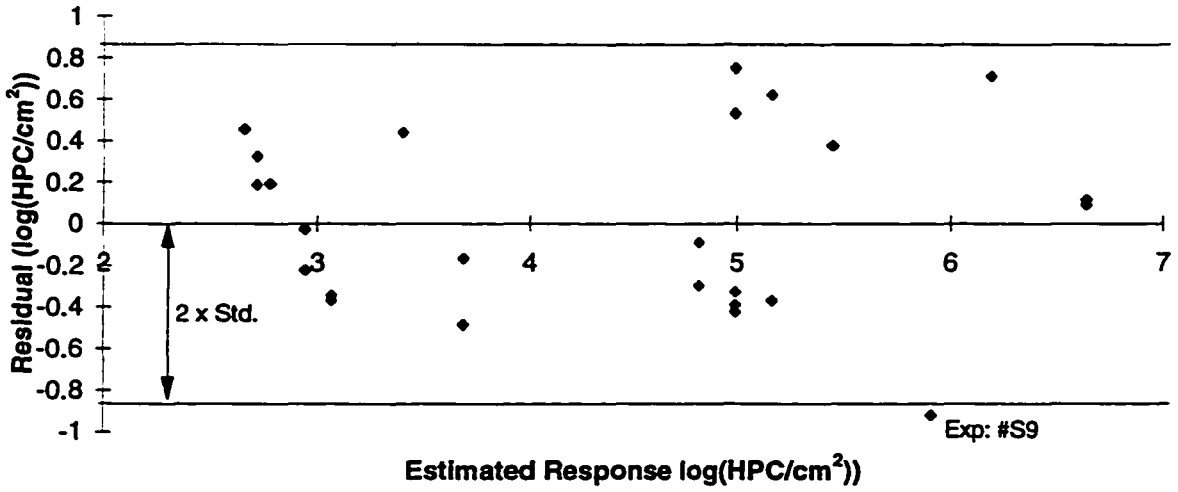


Figure 9.2: Residual plot - preferred synthetic water model fitted to synthetic water data
5% confidence, Var: 0.188, Std.: 0.433
7 reestimated parameters: l=4.369; a=0.428; c=-1.408; e=0.273; ac=-0.216; ae=0.098; cf=-0.143
(ref. Figure D/3.1);

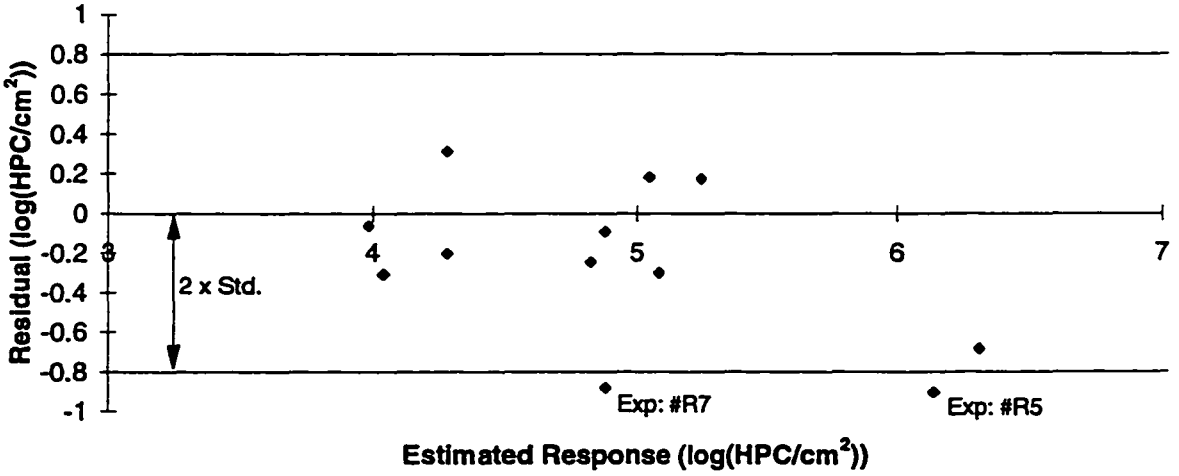


Figure 9.3: Residual plot - preferred synthetic water model fitted to real water data
5% confidence, Var: 0.160, Std.: 0.400
7 reestimated parameters: l=4.369; a=0.428; c=-1.408; e=0.273; ac=-0.216; ae=0.098; cf=-0.143
(ref. Figure D/3.11)

9.2 REAL WATER MODELS

At the time of conceptual design of this project, modeling concepts of Section 9.2 was not considered. It was thought that the 'end product' would be the preferred synthetic water model (Section 9.1.3). P.M. Reilly (1998) suggested a 'second generation' modeling concept. Based on an alternative usage of the available data set, but still with the Bayesian approach (Section 3.8), so called 'real water models' were developed utilizing the synthetic water data for prior information, and the experimental results with real waters for actual model building. This approach 'guaranteed' the most effective utilization of experimental results by comprising the most information into the models. Of course, no data remained for testing the real water models, but meaningful comparison of real and synthetic water models will suggest presumed model performance. Real water modeling procedures are similar to those of synthetic waters, consequently only differences will be discussed in detail in this section. At least a glance through Section 9.1 is recommended before 'diving' into the subject matter of Section 9.2.

9.2.1 MODEL DEVELOPMENT

The design protocol is similar, in concept, to the one introduced in Section 9.1.1. According to Bayesian principles, posterior parameter estimates and posterior covariances are the function of prior data input. While mainly published research results served as prior knowledge for the development of synthetic water models, the basis for prior information of the real water models was the posterior data of the synthetic water models. Since the output of synthetic water modeling is, essentially, improved published data, real water models are based on both valuable research results of reputable scientists and results of the author's laboratory and modeling work.

The vector of posterior parameter estimates and the posterior covariance matrix are described by $\left\{\left[\underline{U}^{-1}+\left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}\left[\underline{U}^{-1}\underline{\alpha}+\left(1/\sigma^2\right)\underline{X}'\underline{y}\right]\right\}$ and $\left[\underline{U}^{-1}+\left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}$, respectively (Section 3.9). The required prior information includes (1) vector of prior point estimates ($\underline{\alpha}$), (2) observation vector ($\underline{y}$), (3) prior covariance matrix ($\underline{U}$), (4) system variance (σ^2), and (5)

regression matrix ($\underline{X}$). Similarly to synthetic water modeling, a series of both linear and quadratic real water models were developed. Each series was supplied with two distinct sets of prior covariances. Each element of the posterior covariance matrix of the linear synthetic water model was multiplied by a factor of either two or three to be utilized as the prior covariance matrix for the linear real water models. Similarly, each element of the posterior covariance matrix of the quadratic synthetic water model was multiplied by a factor of either two or three to be utilized as the prior covariance matrix for the quadratic real water models. Unlike the prior covariance matrices of synthetic water models, the prior covariance matrices of real water models are non-diagonal. Although these four distinct prior covariance matrices are not shown, the generating posterior covariance matrices of the linear and quadratic synthetic water models are introduced in Appendices D/1 and D/2 respectively. The factor of three multiplied prior covariance matrix is to provide a more flexible or 'loosened up' parameter estimation which is to be less controlled by the prior point estimates.

The numerical values of the 12 x 1 observation vector of real water models (both linear and quadratic) are those of the actual steady-state log biofilm HPC numbers of experiments R#1 to #R12 (Table 8.1). The system variance is 0.0189 (Table 5.6). The regression matrix of real water models (both linear and quadratic) consists of rows corresponding to the 12 real water trials. The columns of these regression matrices are the constant term, main effects, two and three factor interactions (and quadratic terms) in natural order. The generating coded design matrix of real water experiments is shown in Table 5.7. The regression matrix of linear real water experiments is shown schematically below:

$$\begin{bmatrix} 1 \ldots -0.40 \ldots\ldots -0.07 \ldots 0 \\ 1 \ldots -0.40 \ldots\ldots 0.07 \ldots 0 \\ \ldots\ldots\ldots\ldots \\ \ldots\ldots\ldots\ldots \\ 1 \ldots -0.80 \ldots 1 \ldots\ldots 0 \ldots 0.02 \ldots 0 \end{bmatrix}$$

regression matrix
(12 x 42)

Linear Real Water Models

Two distinct sets of posterior parameter estimates of the linear real water models are included in Table 9.9. The two sets of parameter estimates were calculated by the two distinct prior covariance matrices of linear real water models, as discussed in the paragraph above. The non-zero coefficients of the 42 x 1 vector of prior point estimates of the linear real water model are identical to the posterior parameter estimates of linear synthetic water models (Appendix D/1).

Two posterior covariance matrices, corresponding to the two prior covariance inputs, were calculated (data not shown) and used, along with the posterior parameter estimates to the parameter selection tests for the linear real water models. Since the procedure is identical to the one used for the linear synthetic modeling (Section 9.1.1), only the test results are shown in Appendix E/1. For convenience, a summary of these test results are included in Table 9.10. Parameter selection was based on results of test #2. Significant parameters were determined at 5% and 30% significance levels. Table 9.11 shows the number of parameters along with the actual coded parameter designation for each of the four linear synthetic water model sets. Covariance and correlation matrices are also qualified in Table 9.11 (e.g. excellent, good, etc.).

Table 9.9: Real Water Models - Parameter Estimates

Parameter	Linear model		Quadratic model	
	Prior Covariance Matrix =			
	2 x posterior covariance matrix	3 x posterior covariance matrix	2 x posterior covariance matrix	3 x posterior covariance matrix
1	4.294	4.284	4.458	4.440
a	0.173	0.179	0.315	0.318
b	0.084	0.082	0.099	0.102
c	-1.342	-1.343	-1.376	-1.381
d	-0.178	-0.180	-0.067	-0.074
e	0.245	0.248	0.224	0.228
f	0.138	0.144	0.181	0.181
ab	0.051	0.058	0.004	0.003
ac	-0.100	-0.082	-0.128	-0.107
ad	-0.319	-0.315	-0.235	-0.234
ae	0.197	0.196	0.225	0.226
af	-0.010	-0.008	0.023	0.021
bc	0.021	0.025	0.013	0.011
bd	-0.009	-0.006	-0.019	-0.019
be	0.107	0.107	0.002	0.000
bf	-0.052	-0.042	-0.007	-0.005
cd	0.095	0.104	0.042	0.054
ce	-0.249	-0.241	-0.193	-0.186
cf	-0.168	-0.168	-0.148	-0.150
de	0.023	0.025	0.053	0.060
df	-0.149	-0.148	-0.109	-0.114
ef	-0.052	-0.043	-0.065	-0.054
abc	-0.035	-0.044	-0.038	-0.044
abd	0.069	0.079	0.030	0.035
abe	0.020	0.020	-0.017	-0.020
abf	-0.034	-0.042	-0.026	-0.030
acd	0.043	0.040	0.038	0.035
ace	0.000	0.001	0.007	0.010
acf	-0.003	0.014	0.010	0.025
ade	-0.065	-0.074	-0.046	-0.049
adf	-0.021	-0.020	-0.022	-0.021
aef	-0.032	-0.035	-0.022	-0.023
bcd	-0.012	-0.016	-0.017	-0.020
bce	-0.015	-0.010	0.000	0.000
bcf	0.017	0.013	0.010	0.009
bde	0.004	0.009	-0.025	-0.029
bdf	-0.006	-0.007	0.009	0.012
bef	-0.015	-0.020	-0.018	-0.025
cde	-0.064	-0.061	-0.030	-0.029
cdf	0.078	0.081	0.079	0.083
cef	0.090	0.092	0.087	0.089
def	0.022	0.023	-0.064	0.030
a^2	-	-	-0.288	-0.278
c^2	-	-	0.034	0.037
e^2	-	-	-0.047	-0.050

a - BOM
b - disinfectant type
c - disinfectant residual
d - shear stress
e - temperature
f - substratum

Table 9.10: Test #2 Results - Linear and Quadratic Real Water Models (ref. Appendix E/1 and Appendix E/2)

Actual parameter	Coded parameter	Test #2 results			
		Linear real water model		Quadratic real water model	
		Prior covariance matrix =			
		2 x posterior covariance matrix	3 x posterior covariance matrix	2 x posterior covariance matrix	3 x posterior covariance matrix
mean	1	71.57	62.49	74.30	38.36
BOM	a	2.33	2.04	4.25	2.82
disinfectant type	b	-1.51	1.30	1.78	1.56
disinfectant residual	c	-15.93	-13.23	-16.33	-13.05
shear	d	-1.45	-1.27	-0.55	-0.48
temperature	e	2.51	2.22	2.30	1.96
substratum	f	2.33	2.20	3.06	2.56
BOM - disinfectant type	ab	1.09	1.01	0.09	0.05
BOM - disinfectant residual	ac	-1.34	-0.94	-1.71	-1.20
BOM - shear	ad	-3.26	-2.70	-2.40	-1.89
BOM - temperature	ae	2.18	1.79	2.48	1.96
BOM - substratum	af	-0.22	-0.15	0.51	0.38
disinfectant type - disinfectant residual	bc	0.27	0.27	0.17	0.11
disinfectant type - shear	bd	-0.18	-0.10	-0.37	-0.30
disinfectant type - temperature	be	1.30	1.09	0.02	0.00
disinfectant type - substratum	bf	-0.95	-0.70	-0.13	-0.08
disinfectant residual - shear	cd	0.95	0.86	0.42	0.42
disinfectant residual - temperature	ce	-2.67	-2.15	-2.07	-1.60
disinfectant residual - substratum	cf	-2.23	-1.90	-1.96	-1.67
shear - temperature	de	0.26	0.24	0.61	0.56
shear - substratum	df	-1.19	-1.02	-0.87	-0.77
temperature - substratum	ef	-0.59	-0.43	-0.74	-0.51
$(BOM)^2$	a^2	N/A	N/A	-5.44	-2.43
$(disinfectant\ residual)^2$	c^2	N/A	N/A	0.64	0.19
$(temperature)^2$	e^2	N/A	N/A	-0.89	-0.29
Notes: Highest absolute value of 3 factor interaction:	cef	1.88	1.58	1.81	1.53

Tabulated values 1.96 correspond to 5% significance level
Tabulated values 1.65 correspond to 10% significance level
Tabulated values 1.04 correspond to 30% significance level
3 factor interactions and values for 3 decimal points are shown in Appendix E/1 and E/2
Parameters are listed in natural order
N/A not applicable

Table 9.11: Real Water Models - Summary

Model type	Prior covariance matrix	Significance level	# of parameters	Actual parameters	Covariance/ correlation matrix	Variance	Standard deviation (Std)	2 x Std	# of outliers	Residual plot	Reference Appendix E/3
linear	2 x posterior	5%	9	1,a,c,e,f, ad,ae,ce,cf	good/ excellent	0.0089	0.0944	0.1888	1	excellent	Fig E/3.1
"	"	30%	20	1,a,b,c,d,e,f, ab,ac,ad,ae,be,ce,cf, df,abd,ade,cde,cdf,cef	excellent	0.0113	0.1061	0.2122	0	"	Fig E/3.2
"	3 x posterior	5%	7	1,a,c,e,f,ad,ce	excellent/ excellent	0.0083	0.0909	0.1818	1	"	Fig E/3.3
"	"	30%	19	1,a,b,c,d,e,f, ab,ad,ae,be,ce,cf,df, abd,ade,cde,cdf,cef	ecxcellent/ good	0.0081	0.0900	0.1800	0	"	Fig E/3.4
quadratic	2 x posterior	5%	10	1,a,c,e,f, ad,ae,ce,cf,a2	good/ good	0.0081	0.0901	0.1802	1	"	Fig E/3.5
"	"	30%	15	1,a,b,c,e,f, ac,ad,ae,ce,cf, cdf,cef,def,a2	excellent/ excellent	0.0098	0.0990	0.1980	0	"	Fig E/3.6
"	3 x posterior	5%	7	1,a,c,e,f, ae,a2	good/ good	0.0082	0.0908	0.1816	1	"	Fig E/3.7
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
"	"	30%	14	1,a,b,c,e,f, ac,ad,ae,ce,cf, cdf,cef,a2	excellent/ good	0.0096	0.0978	0.1956	1	"	Fig E/3.9

Note: most informative model is indicated with shading

Quadratic Real Water Models

Two distinct sets of posterior parameter estimates of the quadratic real water models are included in Table 9.9. The two sets of parameter estimates were calculated by the two distinct prior covariance matrices of quadratic real water models, as discussed earlier in this section. The size of the non-diagonal prior covariance matrices of quadratic real water models was chaged from 44 x 44 to 45 x 45 by replacing the last but one column (d^2) by two new columns (a^2 and c^2). Similarly, the last but one row was replaced by two new rows as well. The fact that real water trials investigated BOM supplement (*a*), disinfectant concentration (*c*), and temperature (*e*) at more than two levels (Table 5.7), necessitated the increase of matrix size. Shear (*d*) was investigated at a single level in the real water trials (Table 5.7), therefore its quadratic effect could not be considered.

The non-zero coefficients of the 45 x 1 vector of prior point estimates of the quadratic real water models are identical to the 44 x 1 posterior parameter estimates of quadratic synthetic water models (Appendix D/2) with the exception that the last but one parameter estimate was replaced by two new ones. The numerical values of the two new prior estimates were set to be identical to the value of the last parameter estimate (-0.199). The prior point estimates and prior covariance matrix of the quadratic real water models are shown schematically below. Although prior data for the real water models are not shown, the utilized posterior data for the quadratic synthetic water models are shown in Appendix D/2.

$$
\begin{bmatrix} \ldots 5.323 \\ \ldots 0.364 \\ \ldots\ldots\ldots \\ \ldots\ldots\ldots \\ \ldots -0.004 \\ -0.199 \\ -0.199 \\ -0.199 \end{bmatrix}
\qquad
\begin{bmatrix}
0.1791 \ldots 0.0311 \ldots\ldots\ldots 0 \ldots\ldots 0 \ldots -0.0197 \\
0.0311 \ldots 0.0379 \ldots\ldots\ldots 0 \ldots\ldots 0 \ldots\ \ldots 0.0011 \\
\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \\
\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \\
\ldots\ldots\ldots\ldots\ldots\ldots 0.0047 \ldots 0 \ldots 0 \ldots 0.0001 \\
\ldots 0 \ldots\ \ldots 0 \ldots\ldots\ldots 0.0511 \ldots 0 \ldots 0 \\
\ldots 0 \ldots\ldots 0 \ldots\ldots\ldots\ldots 0 \ldots 0.0511 \ldots 0 \\
-0.0197 \ldots 0.0011 \ldots\ldots\ldots 0 \ldots\ldots 0 \ldots 0.0511
\end{bmatrix}
$$

prior point estimates (45 x 1) | prior covariance matrix (45 x 45)

The 12 x 45 regression matrix of quadratic real water models consists of 12 rows in the same order as presented in Table 8.1. The columns of the regression matrix are the constant term, main effects, two factor interactions, three factor interactions, and the three quadratic terms in natural order.

The two 45 x 45 posterior covariance matrices of quadratic real water models, corresponding to the two distinct prior covariance matrices, were calculated and used, along with the corresponding two posterior parameter estimates to the parameter selection tests for the quadratic real water models. As a result, two distinct test sets were obtained. Since matrix layouts and operation are similar to the ones introduced in Section 9.1.1, posterior covariance and correlation matrices of quadratic real water models are not shown. Test procedures were introduced earlier in this chapter. Since the procedure is identical to the one used for the linear synthetic modeling (Section 9.1.1), only the two distinct test results are shown in Appendix E/2. For convenience, a summary of these test results are also included in Table 9.10. Parameter selection was based on results of test #2. Significant parameters were determined at 5% and 30% significance levels for both test #2 series. This resulted four models with 10, 15, 7, and 14 parameters as shown in Table 9.11. An additional parameter, disinfectant type (b) was also evaluated for the 7 parameter model. Table 9.11 shows the number of parameters along with the actual coded parameter designation for each of the five quadratic synthetic water models. Covariance and correlation matrices are also qualified in Table 9.11.

A comprehensive study by Camper (1995) looked at several important factors and their impact on both coliform and HPC counts in a pilot as well as in a bench-scale experimental systems. The factors examined in that study were AOC, temperature, and chlorine dose. Camper (1995) found a positive correlation between AOC concentration and bacterial accumulation regardless of the temperature. The lack of a significant interaction between AOC and temperature cannot be confirmed by the author's reseach. Table 9.10, a summary of real water model test results, shows a statistically significant interaction of these two factors. Synthetic water data in Appendix D/1 and Appendix D/2 also suggest that the BOM-temperature interaction is significant at the 5% level. In the presence of an AOC supplement, Camper (1995) also found

an increase of coliforms when chlorine was added. The positive effect of BOM - free chlorine interaction on HPC accumulation could not be demonstrated in the author's research (Table 9.10, Appendix D/1, and Appendix D/2). In another bench scale study, Camper *et al.* (1994c) found that the interaction between chlorine, AOC, and the substratum is important on the growth of coliforms. This is not supported by the author's research results.

9.2.2 RESIDUALS

Similarly to the posterior parameter estimation method described in Section 9.1.1, significant parameters (selected by test #2) of each real water model were reestimated before model fitting. Reestimation method of the significant parameters will be introduced in detail for the most informative model in Section 9.4.

Having obtained the reestimates of the unknown parameters, an estimated response ($\underline{\hat{y}}$) can be calculated by substituting the significant parameter reestimates ($\underline{\hat{\theta}}$) into the general regression model

$$\underline{y} = \underline{X\theta}^{*} + \underline{\varepsilon} \qquad (3\text{-}1)$$

with $\underline{y}=\underline{\hat{y}}$ and $\underline{\theta}^{*}=\underline{\hat{\theta}}$ substitutions. The estimated response may be compared with true observation values ($\underline{y}$). The quantities $\underline{y}-\underline{\hat{y}}$ are the residuals.

There were a total of 9 real water models obtained with the formerly introduced parameter selection criteria (Section 9.2.1). The real water models (both linear and quadratic) were fitted to 12 data points (R1 through R12) of the real water data set (Table 5.7). Experiments R13 and R14 were not consistent with the other trials (disinfectant was dosed from time zero), therefore could not be considered for the fitting procedure.

The residual plots (Figures E/3.1 to E/3.9 in Appendix E/3) show the data points in the residual (ordinate) vs. estimated response (absicca) log-log relationship. Variances (σ^2), standard deviations (σ), and 2 x σ values for each fit were calculated. These statistical values are included in Table 9.11 and also denoted in the footnote of the residual plots. Since 95% confidence interval of a normal probability distribution is 1.96σ, the 2σ values are close approximation of a 95% confidence region. Residuals situated outside of the 95% confidence boundaries are called outliers and identified by their experiment numbers in both Figures E/3.1 through E/3.9 and Table 9.11.

Linear Real Water Model Fit to Real Water Data

Figures E/3.1 and E/3.2 (Appendix E/3) show the fits of linear real water models to the real water data. Parameters were selected at 5% and 30% levels based on a prior covariance matrix obtained by multiplying the posterir covariance matrix of the linear synthetic water models by a factor of two. The calculated variance, standard deviation (σ), and 2σ value were 0.0089, 0.0944, and 0.1888 respectively for the 9 parameter model (Table 9.11). Corresponding values for the 20 parameter model (Table 9.11) were 0.0113, 0.1061, and 0.2122 respectively. The residual plots (Figure E/3.1 and Figure E/3.2) are random 'gun-shot blast' types. In the 9 parameter model, a single outlier (R7) was recorded. The overall subjective rating of both residual plots is 'excellent'.

Figures E/3.3 and E/3.4 (Appendix E/3) show the fits of linear real water models to the real water data. Parameters were selected at 5% and 30% levels based on a prior covariance matrix obtained by multiplying the posterior covariance matrix of the linear synthetic water models by a factor of three. Calculated variances, standard deviations (σ), and 2σ values, for each of these fits are tabulated in Table 9.11. Each plot is qualified, the number of outliers are reported and reference figure numbers are shown also in Table 9.11.

Quadratic Real Water Model Fit to Real Water Data

Figures E/3.5 and E/3.6 (Appendix E/3) show the fits of quadratic real water models to the real water data. Parameters were selected at 5% and 30% significance levels based on a prior covariance matrix obtained by multiplying the posterir covariance matrix of the quadratic synthetic water models by a factor of two. The residual plots are random 'gun-shot blast' types. The overall subjective rating of both residual plots is 'excellent'. Calculated variances, standard deviations (σ), and 2σ values, for each of these fits are tabulated in Table 9.11. Each plot is qualified, the number of outliers are reported and reference figure numbers are shown also in Table 9.11.

Figure E/3.7, Figure E/3.8 and Figure E/3.9 (Appendix E/3) show the fits of quadratic real water models to the real water data. Parameters were selected at 5%, 5% + 'b', and 30% significance levels based on a prior covariance matrix obtained by multiplying the posterior covariance matrix of the quadratic synthetic water models by a factor of three. Calculated variances, standard deviations (σ), and 2σ values, for each of these fits are tabulated in Table 9.11. Each plot is qualified, the number of outliers are reported and reference figure numbers are shown also in Table 9.11.

Comparison of Fits of Real Water Models

Figure 9.4 shows residual variances of the real water data fitted real water models as a function of both the number of parameters included in the model and the model type (i.e. linear or quadratic). The bar graph shows that the residual variances of the nine investigated models are about the same which suggests that the number of parameters included in a model has little effect on the fit. This suggests an excellent fit of all the real water models to the real water data. In contrast, the variance behaviour of the synthetic water models (Figure 9.1) showed that the synthetic water data fitted residual variances typically decreased, and variances corresponding to real water fits increased as the number of parameters increased.

Comparison of residual plots (Figures E/3.1 through E/3.9) in Appendix E/3 suggests a generally excellent fit of the real water models. These real water fitted plots show a 'gun-shot

blast' type of distribution, no discernible pattern about the experimental error (ε), and a low number of outliers (typically none or 1). The number of outliers and the rating of these residual plots are included in Table 9.11.

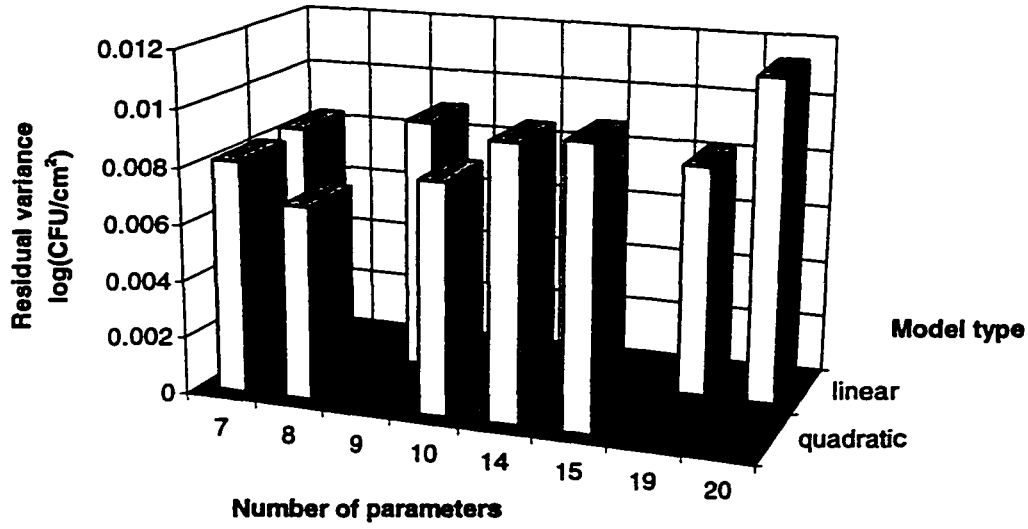


Figure 9.4: Variances - real water model fitted to real water data (ref. Table 9.11)

9.3 COMPARISON OF SYNTHETIC AND REAL WATER MODEL

Since real water models were built by utilizing both published research results of reputable scientists and the author's synthetic water research results (Section 9.1), the most informative model is likely to be one of the real water models. Comparison of the (residual) variance data in Figure 9.1 and Figure 9.4 clearly shows that the variances of the real water models are much smaller than those of the synthetic water models. Specifically, residual variances of the real water models (0.0068 to 0.0098) were about one order of magnitude lower than those of the synthetic water fitted synthetic water models (0.036 to 0.223), and about one and a half orders of magnitude lower than those of the real water fitted synthetic water models (0.1600 to 0.9640). The different prior information of the synthetic and real water models as well as the narrower experimental range of the real water trials are likely to be the explanation for this great improvement of fit. Consequently, selection of the ultimate model will be restricted for the real water models.

From among the real water models (Figure 9.4), the 8 parameter quadratic model with higher prior covariances (posterior covariance matrix of quadratic synthetic water model multiplied by a factor of three) have the lowest residual variance (σ^2=0.0068). Figure 9.5 shows the real water fitted residual plot of the preferred quadratic real water model (repeated in Figure E/3.8 in Appendix E/3). This residual plot is a random 'gun-shot blast' type without a discernible pattern, and with a single outlier (R4).

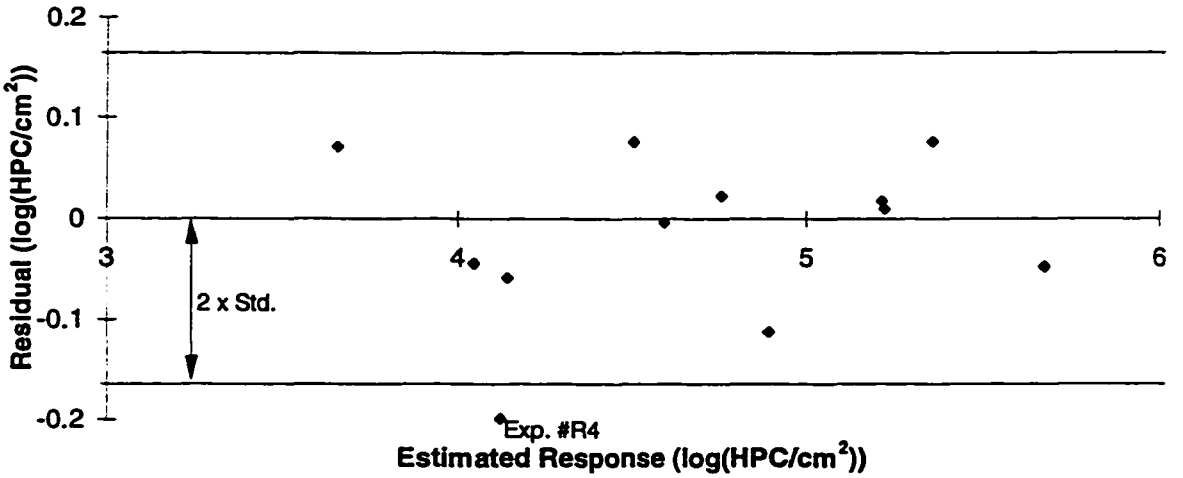


Figure 9.5: Residual plot - most informative model fitted to real water data
prior covariance = 3 x posterior covariance of quadratic synthetic water models
5% confidence + 'd' (type), Var: 0.006765, Std.: 0.082252
8 reestimated parameters:1=4.490725; a=0.415003; b=0.138714; c=-1.460490; e=0.236191; f=0.228892; ae=0.212368; a^2=-0.337130 (ref. Figure E/3.8)

The magnitude of the off-diagonal elements of a correlation matrix indicates the strength of correlation between corresponding parameters. There are 12 off-diagonal values in excess of 0.5 in the 8 x 8 correlation matrix of the 8 parameter quadratic real water model (Appendix F/1). This is compatible with the correlation characteristics of the other real water models. The overall performance of the 8 parameter quadratic real water model (shaded row in Table 9.11) appears to be the most favoured, therefore this model is considered for further discussion and will represent the author's research results. Selected parameters of the most informative model are listed below (coded parameters are in brackets):

- constant term (1)
- BOM (a)
- disinfectant type (b)
- disinfectant residual (c)
- temperature (e)
- substratum (f)
- BOM - temperature (ae)
- $(BOM)^2$ (a^2)

9.4 THE MOST INFORMATIVE MODEL

It was concluded in Section 9.3 that the most informative model is the 8 parameter quadratic real water model. The parameter estimation procedures of this model are discussed below.

9.4.1 PARAMETER REESTIMATION

Quadratic real water model posterior data generation procedures were introduced in Section 9.2.1. Table 9.9 includes the two distinct sets of posterior parameter estimates for the quadratic real water models. Each vector of posterior parameter estimates is 45 x 1 (Table 9.9). The 8 parameters of the 'most informative model' (1, a, b, c, e, f, ae, a^2) were selected at the 5% + 'b' confidence level by test #2 (Section 9.2.1). The rightmost column in Table 9.10 shows test #2 results of the most preferred model. Absolute values above 1.96 indicate 5% significance level. Test #2 result of the shear is -0.48 suggesting that shear at the 5% level is not significant therefore excluded from the model. Peyton *et al.* (1993) also reported that shear stress was minor significance for biofilm accumulation. Since the number of parameters was reduced from 45 to 8 by test #2, the formerly estimated paremeter values (Table 9.9) could not be used in the most preferred model. They must be reestimated.

The vector of prior point estimates of the most informative model is 8 x 1. The elements of this vector were obtained from the posterior parameter estimates of the quadratic synthetic models which was a 44 x 1 vector (Appendix D/2). The 8 selected elements correspond to the 95% + 'b' parameter selection. The prior point estimates (a) of the most informative model is:

$$a = [5.323; 0.364; -0.299; -1.595; 0.515; 0.230; 0.211; -0.199]$$

The 12 x 1 vector of observations contains steady-state biofilm HPCs of the real water experiments (R1 to R12). These values were introduced in Table 8.1. Experiments R13 and R14 are excluded because the disinfectant was dosed from day zero in these two trials. The observation vector (y) of the most informative model is:

y = [4.785; 5.230; 3.732; 3.919; 5.230; 5.623; 4.000; 4.578; 4.785; 5.431; 4.079; 4.591]

The 8 x 8 prior covariance matrix of the most informative model contains a factor of three multiplied elements of the 44 x 44 posterior covariance matrix of the quadratic synthetic water models (Appendix D/2). Rows and columns were selected according the 95% + 'b' parameter selection, i.e. 1, a, b, c, e, f, ae, a^2. The prior covariance matrix (u) of the most informative model is:

u = [0.1791 0.0311 0.0693 0.0249 -0.1360 0.1258 0.0035 0
0.0311 0.0379 0.0070 -0.0025 -0.0325 0.0428 -0.0061 0
0.0693 0.0070 0.0886 0.3080 -0.1208 0.0727 0.0006 0
0.0249 -0.0025 0.0380 0.0327 -0.0588 0.0319 0.0043 0
-0.1360 -0.0325 -0.1208 -0.0588 0.2431 -0.1754 -0.0105 0
0.1258 0.0428 0.0727 0.0319 -0.1754 0.1802 0.0086 0
0.0035 -0.0061 0.0006 0.0043 -0.0105 0.0086 0.0170 0
-0.0027 0.0005 0.0016 0.0008 0.0015 -0.0023 0.0002 0.0511]

The system variance (σ^2) is 0.0189 (Table 5.6). The 12 x 8 regression matrix of the most informative model is essentially an extended form of the real water design matrix (Table 5.7), containing the coded values of interaction 'ae' and the quadratic term 'a^2' in its rightmost two columns. The regression matrix (x) of the most informative model is:

x = [1 -0.4 -1 -0.6 -0.11 -1 0.04 0.16
1 -0.4 -1 -0.6 -0.11 1 0.04 0.16
1 -0.4 -1 0.2 0.33 -1 -0.13 0.16
1 -0.4 -1 0.2 0.33 1 -0.13 0.16
1 1.4 -1 -0.6 0.55 -1 0.77 1.96
1 1.4 -1 -0.6 0.55 1 0.77 1.96
1 1.4 -1 0.2 0.55 -1 0.77 1.96
1 1.4 -1 0.2 0.55 1 0.77 1.96
1 -0.4 -1 -0.6 0.78 -1 -0.31 0.16
1 -0.4 -1 -0.6 0.78 1 -0.31 0.16
1 -0.8 1 -0.2 -0.11 -1 0.09 0.64
1 -0.8 1 -0.2 -0.11 1 0.09 0.64]

The posterior parameter reestimates and posterior covariance matrix of the most informative model were calculated by $\left\{\left[\underline{U}^{-1}+\left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}\left[\underline{U}^{-1}\underline{\alpha}+\left(1/\sigma^2\right)\underline{X}'\underline{y}\right]\right\}$ and $\left[\underline{U}^{-1}+\left(1/\sigma^2\right)\underline{X}'\underline{X}\right]^{-1}$, respectively (Section 3.9). Table 9.12 shows the reestimated parameter values, as well as the corresponding prior parameter estimates.

Table 9.12: Prior and Posterior Parameter Estimates - Most Informative Model

Actual parameter	Parameter	Parameter estimates	
		prior	posterior
mean	θ_0	5.32	4.49
BOM	θ_1	0.36	0.42
disinfectant type	θ_2	-0.30	0.14
disinfectant residual	θ_3	-1.60	-1.46
temperature	θ_5	0.52	0.24
substratum	θ_6	0.23	0.23
BOM - temperature	θ_{15}	0.21	0.21
$(BOM)^2$	θ_1^2	-0.20	-0.34

The regression equation (Bayesian) of the most infromative model is introduced below:

$$\log(HPC) = 4.490725198 + 0.415002937 * X_1 + 0.13871358 * X_2 - 1.46049132 * X_3 + 0.236190789 * X_5 + 0.22889209 * X_6 + 0.21236774 * X_{15} - 0.33712887 * X_1^2$$

The posterior correlation matrix of the most informative model is:

1	0.88	0.73	-0.53	-0.62	0.08	-0.23	-0.90
0.68	1	0.66	-0.76	-0.54	0.07	-0.41	-0.76
-0.06	0.38	1	-1.92	-0.34	-0.01	-0.07	-0.12
0.34	0.01	-0.08	1	0.10	0.01	-0.04	-0.10
-1.49	-0.73	0.11	-2.40	1	-0.19	0.54	0.64
0.06	0.07	0.02	-0.07	-0.15	1	-0.00	-0.06
-0.55	-0.59	-0.32	-0.52	0.08	-0.03	1	0.11
-0.49	-0.72	-0.53	1.29	0.42	-0.05	-0.17	1

Estimated responses ($\hat{y}$), i.e. steady-state biofilm HPCs were obtained by substituting each row of the coded real water design matrix (Table 5.7) into the 'x_i' regressors of the above introduced regression equation. The elements of the real water design matrix were calculated by decoding relationships relating actual values to corresponding coded values. Decoding formulas, for the qualitative variables, are:

BOM Supplement

$X_1 = -1 + (C_1 / 250)$

where C_1 is the actual BOM concentration in μg/L.

Disinfectant Type

$X_2 = -1$ for chlorine

$X_2 = +1$ for monochloramine

Qualitative variable; no decoding is required

Disinfectant Residual

(i) free chlorine residual on both polycarbonate and ductile iron and monochloramine residual on polycarbonate substrata

$X_3 = -1 + (C_3 / 0.25)$

where C_3 is the actual free or monochloramine residual in mg/L

(ii) monochloramine residual on ductile iron substrata

$X_3 = -1 + C_3$

where C_3 is the actual monochloramine residual in mg/L

Shear Stress

$X_4 = -1.5 + (C_4 / 0.8)$

where C_4 is the actual shear stress in N/m^2

Temperature

$X_5 = -1.89 + (C_5 / 9)$

where C_5 is the actual liquid phase temperature in °C

Substratum

$X_6 = -1$ for polycarbonate

$X_6 = +1$ for ductile iron

Qualitative variable; no decoding is required

The quantities $y - \hat{y}$ are the residuals. Residuals of the most informative model are shown in Figure 9.5. Prior and posterior data of the most informative model are also introduced in Appendix F/1.

9.4.2 FIT OF MOST INFORMATIVE MODEL TO SYNTHETIC AND REAL WATER DATA

Figure 9.6 shows the fit of the most informative model to all the available data points in terms of steady-state biofilm HPC numbers. The actual versus estimated system response relationship shows that the 26 synthetic water, and the 12 real water data are described by the model well within one order of magnitude accuracy. The fit is especially good to the real water data which response range is well within the investigated range of HPC numbers of the synthetic water trials. Synthetic and real water system responses, as well as the model estimates are shown in Table 9.13.

An attempt was made to fit the model to the real water data by the ordinary least squares method. The attempt failed because $\underline{X}'\underline{X}$ was shown to be singular (i.e. $|\underline{X}'\underline{X}|=0$), which means that there were insufficient independent data relevant to that model to obtain a fit. By the use of prior information in the Bayesian approach, this obstacle could be overcome.

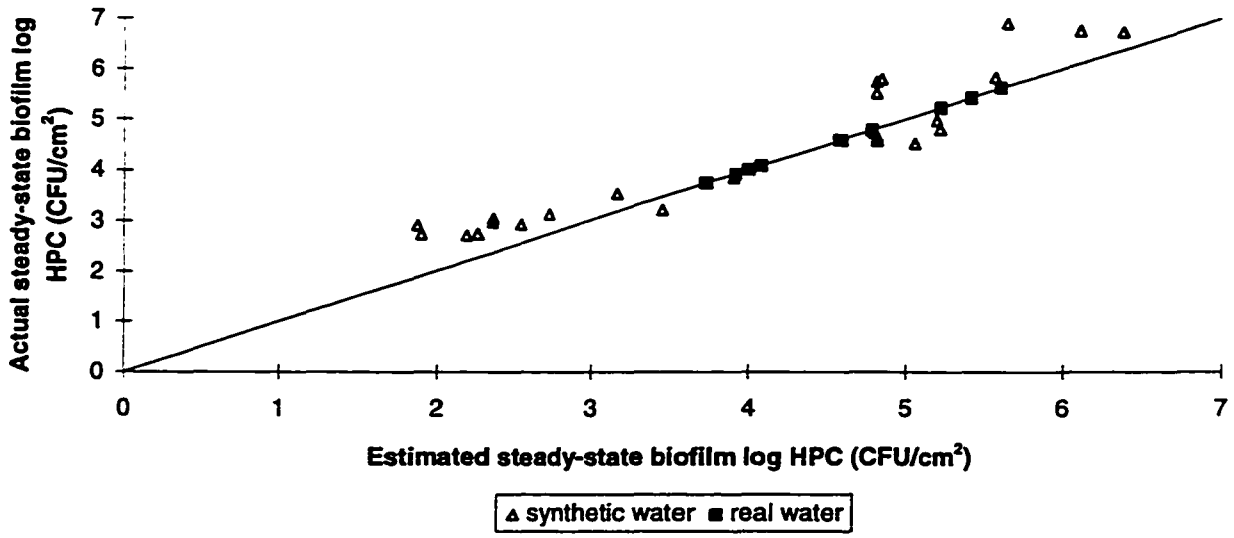


Figure 9.6: Actual versus estimated steady-state biofilm HPC numbers - most preferred model

Table 9.13: Actual and Estimated Steady-State Biofilm HPC Numbers - Most Preferred Model

Experiment number	Synthetic water data	Model estimates	Residual	Experiment number	Real water data	Model estimates	Residual
S1	4.799	5.225	-0.426	R1	4.785	4.763	0.022
S2	3.041	2.360	0.681	R2	5.230	5.204	0.026
S3	3.839	3.904	-0.065	R3	3.732	3.632	0.100
S4	2.973	2.359	0.614	R4	3.919	4.093	-0.174
S5	6.740	6.380	0.360	R5	5.230	5.204	0.026
S6	2.724	2.263	0.461	R6	5.623	5.665	-0.042
S7	6.903	5.665	1.238	R7	4.000	4.030	-0.030
S8	3.204	3.464	-0.260	R8	4.578	4.491	0.087
S9	4.987	5.204	-0.217	R9	4.785	4.889	-0.104
S10	3.519	3.171	0.348	R10	5.431	5.350	0.081
S11	2.724	1.904	0.820	R11	4.079	4.138	-0.059
S12	4.519	5.057	-0.538	R12	4.591	4.596	-0.005
S13	4.724	4.805	-0.081				
S14	5.826	5.581	0.245				
S15	2.699	2.192	0.507				
S16	3.114	2.731	0.383				
S17	2.903	1.877	1.026				
S18	2.919	2.542	0.377				
S19	6.763	6.111	0.652				
S20	5.785	4.847	0.938				
S21	4.663	4.814	-0.151				
S22	4.602	4.814	-0.212				
S23	4.602	4.814	-0.212				
S24	4.568	4.814	-0.246				
S25	5.740	4.814	0.926				
S26	5.519	4.814	0.705				

9.4.3 USER-FRIENDLY INTERFACE OF MOST INFORMATIVE MODEL

This section describes those aspects of the research which focused on making the model input/output user-friendly, offering the usage of the most informative model (called 'the model' in this chapter) to those with a less detailed background in biofilm research. An interface of the model was developed using Visual Basic© version 4 programming language. Two distribution disks (3 1/2") containing the setup files for the interface are attched in Appendix G/2. The user should install and be able to run the program on any computer with an available disk space of at least 4.7 MB. This space demand is only 71.072 KB if the Visual Basic© version 4 software is already installed. The coding of the interface required an approximately 1,000 line program. This code is reproduced, in its full entity, in Appendix G/1. The program must not be reproduced without the consent and written authorization of the author. The author acknowledges with thanks the assistance of Luis Leon in developing the code.

The user-friendly interface is shown in Figure 9.7. The left side of the interface contains frames for managing tasks related mainly to data input. Command buttons, an on-line help window, and the actual data output window are situated on the right hand side of the interface. Before introducing the physical layout of the interface in depth, the author reminds the reader that the model contains 5 out of the 6 system variables, i.e. BOM supplement, disinfectant type, disinfectant concentration, temperature, and substratum. The only excluded variable was the shear stress, as discussed in Section 9.4.1.

The user may specify the type of disinfectant and substratum in the upper two frames on the left of the interface (Figure 9.7). The choices are chlorine or chloramine disinfectants and polycarbonate or ductile iron pipe materials. The default setting is chlorine and polycarbonate.

The remaining three system variables (disinfectant residual, temperature, and BOM) plus the system response (i.e. steady-state log biofilm HPCs) are all potential variables of concern which can be handled by the interface and will, therefore, be called 'interface variables'. The user is urged to select one of these so called 'interface variables', so then the selection will be set as

constant. This is necessary since four variables can not be easily shown in a two dimensional graph. The interface, therefore, handles three constants and three variables. The value of the selected constant can then be specified in the lower subwindow of the frame. The units of the actual constant are shown to the left of the subwindow and updated according to the actual selection of the constant. The default constant setting is the HPC.

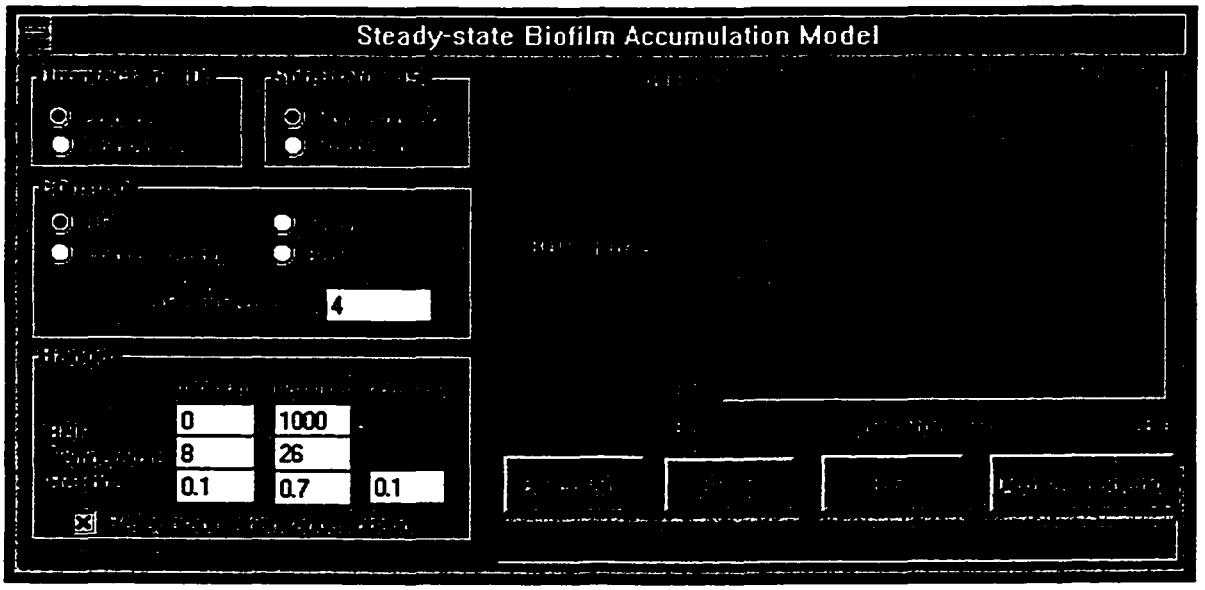


Figure 9.7: Steady-state biofilm accumulation model - interface

Output ranges of nonselected 'interface variables' can be specified in the frame at the bottom left of the interface. Not only the minimum and maximum but also the incremental value of the so called 'third interface variable' can be specified by the user. There are four possible distinct sets of interface variables pending on the designation of the constant. The description of the variables, at the left inside of the frame, is automatically updated upon selection of another constant.

When the 'Calculate' button is pressed on the interface, an output graph will be displayed in the output window at the right hand side of the interface. The output window is essentially a coordinate system. Naturally, the output of the interface is the function of the input parameters. The 'interface variable' listed upmost in the 'Ranges' frame will be indicated on the ordinate of the coordinate system. The second item in the list of the 'Ranges' frame will be designated on

the abscissa. The user set values of the 'third variable' will determine the number of displayed curves in the graphs. The actual values of these color coded curves may be revealed by pressing the 'Display Legend' button on the interface.

Pressing the 'Clear' button on the interface removes the legend from the screen. As a result of pressing the 'Exit' button on the interface the application will quit.

Upon specifying another constant or selecting another quantitative variable, the coordinate system is typically cleared (reset) to accommodate the new setting of the 'interface variables'. This built-in reset feature prevents the display of "mish-mash" output. Should specific circumstances require graphs of different 'interface variable' settings to be superimposed, the user must remove the cross ('X') from the 'Reset graph when ranges change' display at the left bottom corner of the interface. Normal output conditions can be restored by resetting the 'X' in the 'Reset graph when ranges change' display.

Prediction accuracy of empirical models is often quite comparative with that of more complex mechanistic models especially in the investigated range(s) of the variable(s). Extension of empirical models and interpretation of their output beyond the limits (investigated ranges) can lead to compromised prediction accuracy or even results of very limited scientific value. Therefore, the default setting of minimum and maximum values of the 'interface variables', in the 'Ranges' frame, correspond to the investigated actual range of system variables. The user is allowed to change the default setting of all the 'interface variables'. A caution box will, however, pop-up if defaults are changed to values outside the investigated range. The user is prompted with the message 'Proceed with caution', which must be acknowledged to restore normal interface operation. There are a total of 58 pop-up boxes installed in the program to warn the user, ask for confirmation, or deny a not-allowed data input by the user.

The above description provided a brief overview of the major features of the interface without aiming at a global introduction of the software. The information provided should, however, be

of adequate value for the user to get started and gradually fully acquainted with the operation of the system.

9.4.4 MODELING RESULTS

Effects of individual variables on the response were shown, for both synthetic and real waters, in Chapter 7 and Chapter 8. What those results did not establish was what might have happened if the variables had been changed, not individually, but together. Actually, the combined effect of continually changing variables is what really determines the response of a real system. This section is concerned with the data output of the model which was built on a joint functional dependence approach. Based on the introduction of the most informative model (Section 9.4.1 and Section 9.4.2) and its interface (Section 9.4.3), it should be easy, depending on the data input, to graph a virtually infinite number of meaningful results using the interactive model interface. Selected modeling results will be introduced in four groups depending on the selection of the single constant from among the four 'interface variables'. A complex sample nomograph is presented near the end of the section. A summary of these results is provided in Section 9.5.

HPC Held Constant

Figure 9.8 shows the joint effect of temperature and chlorine residual on allowable BOM level for a log(HPC) number of 4 CFU/cm^2 (i.e. HPC=10^4/cm^2) on polycarbonate substratum. Numerically, the graph suggests that to restrict the HPC numbers to a concentration of 10^4 CFU/cm^2 on polycarbonate supporting surface at temperature of 10°C and a chlorine residual of 0.3 mg/L, the BOM concentration must not exceed 400 µg/L in the system.

With identical functional dependence as before, log(HPC) numbers were restricted to 3 in Figure 9.9. For net accumulation not to exceed 10^3 CFU/cm^2 on polycarbonate substratum, the BOM concentration cannot be higher than about 180 µg/L in the presence of 0.3 mg/L free chlorine residual at 10°C.

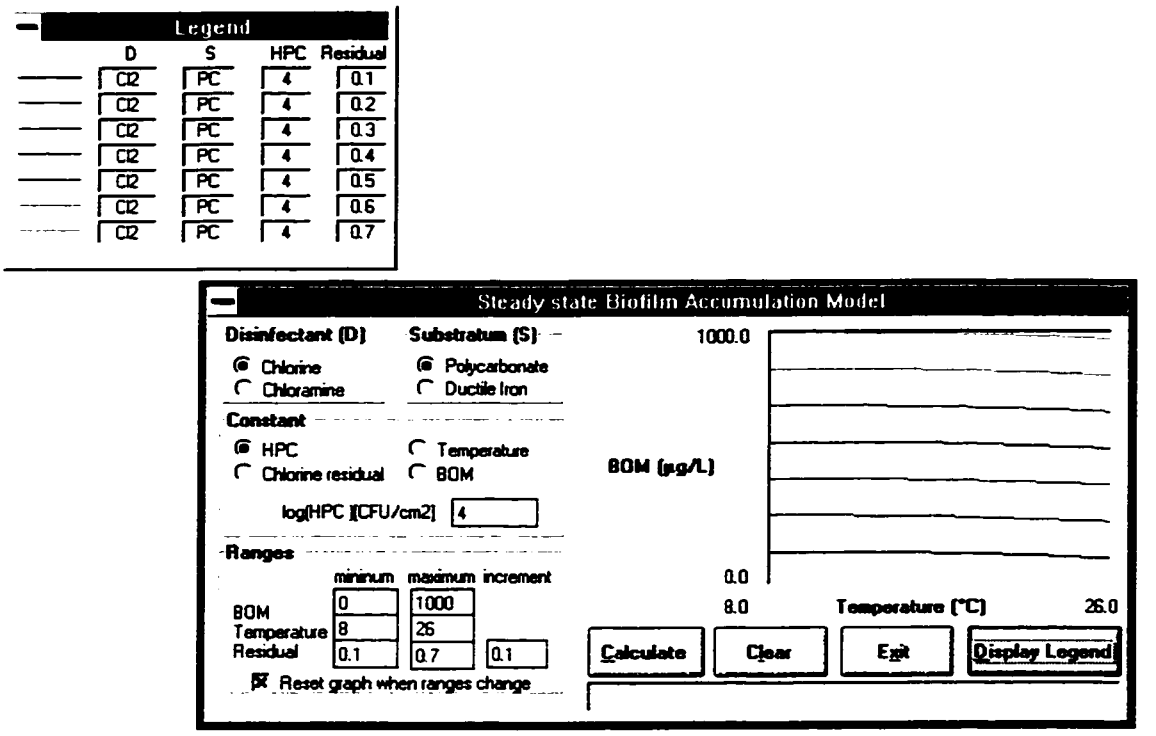


Figure 9.8: Steady-state biofilm accumulation model
Constants: polycarbonate, and HPC (10^4 CFU/cm^2)
Variables: BOM, temperature, and free chlorine residual

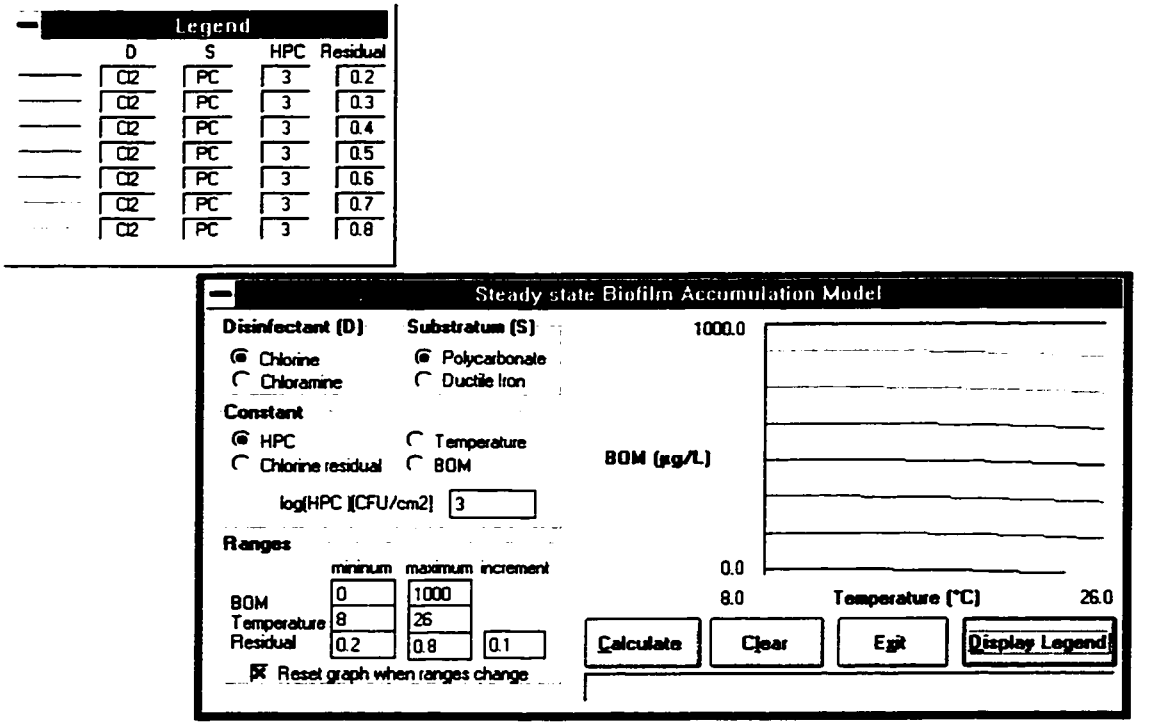


Figure 9.9: Steady-state biofilm accumulation model
Constants: polycarbonate, and HPC (10^3 CFU/cm^2)
Variables: temperature, BOM, and free chlorine residual

The comparison of the two numerical examples suggests a BOM effect. Under the conditions tested, the BOM concentration must be reduced from 400 μg/L to about 180 μg/L in order to reduce biofilm bacterial numbers from 10^4 CFU/cm^2 to 10^3 CFU/cm^2.

Both Figure 9.8 and Figure 9.9 show that, under the conditions tested, the increase of temperature from 8°C to 26°C may be offset by the reduction of BOM concentration of about 30 μg/L. Another interpretation of these data suggests that under these conditions and at a given temperature, the effect of, say, a 500 μg/L increase of BOM concentration can be compensated by an additional 0.35 mg/L free chlorine residual.

Disinfectant Held Constant

Figure 9.10 shows the joint effect of temperature and steady-state biofilm HPC numbers on allowable BOM concentration for a monochloramine residual on polycarbonate substratum. In the presence of 50 μg/L BOM concentration and 0.1 mg/L monochloramine residual, the predicted steady-state log(HPC) net accumulation is about 4.0 CFU/cm^2 (i.e. HPC=10^4/cm^2) on polycarbonate substratum at 22°C.

With identical functional dependence as before, the monochloramine residual concentration was increased from 0.1 mg/L to 0.3 mg/L in the system as indicated in Figure 9.11. As a result of the increased level of the disinfectant residual, the expected HPC numbers on polycarbonate substratum will be reduced from 10^4 CFU/cm^2 to about 10^3 CFU/cm^2 in the presence of 50 μg/L BOM concentration at 22°C temperature.

The comparison of the two numerical examples (Figures 9.10 and 9.11) suggests that the monochloramine residual is an effect. Under the conditions tested, steady-state HPC numbers on polycarbonate substrata decreased from 10^4 CFU/cm^2 to about 10^3 CFU/cm^2 as a result of the increase of monochloramine residual concentration from 0.1 to 0.3 mg/L (50 μg/L BOM concentration, 22°C).

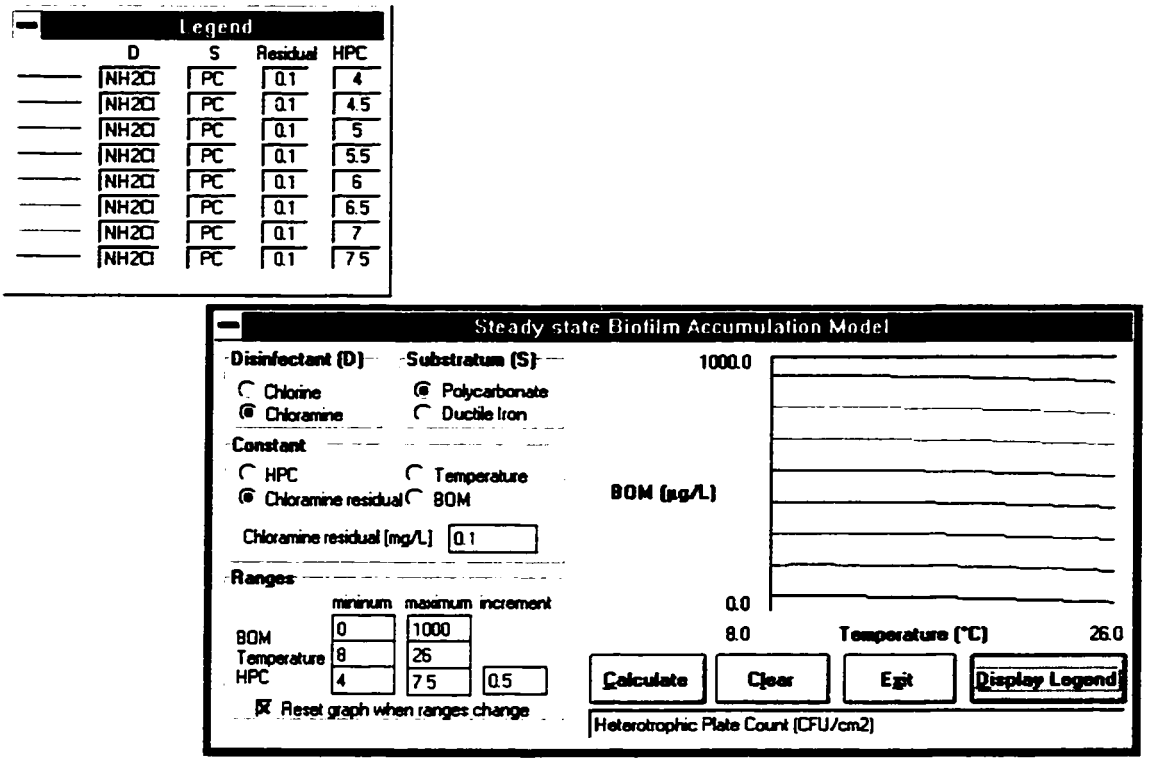


Figure 9.10: Steady-state biofilm accumulation model
Constants: polycarbonate, and monochloramine residual (0.1 mg/L)
Variables: Temperature, BOM, and HPC

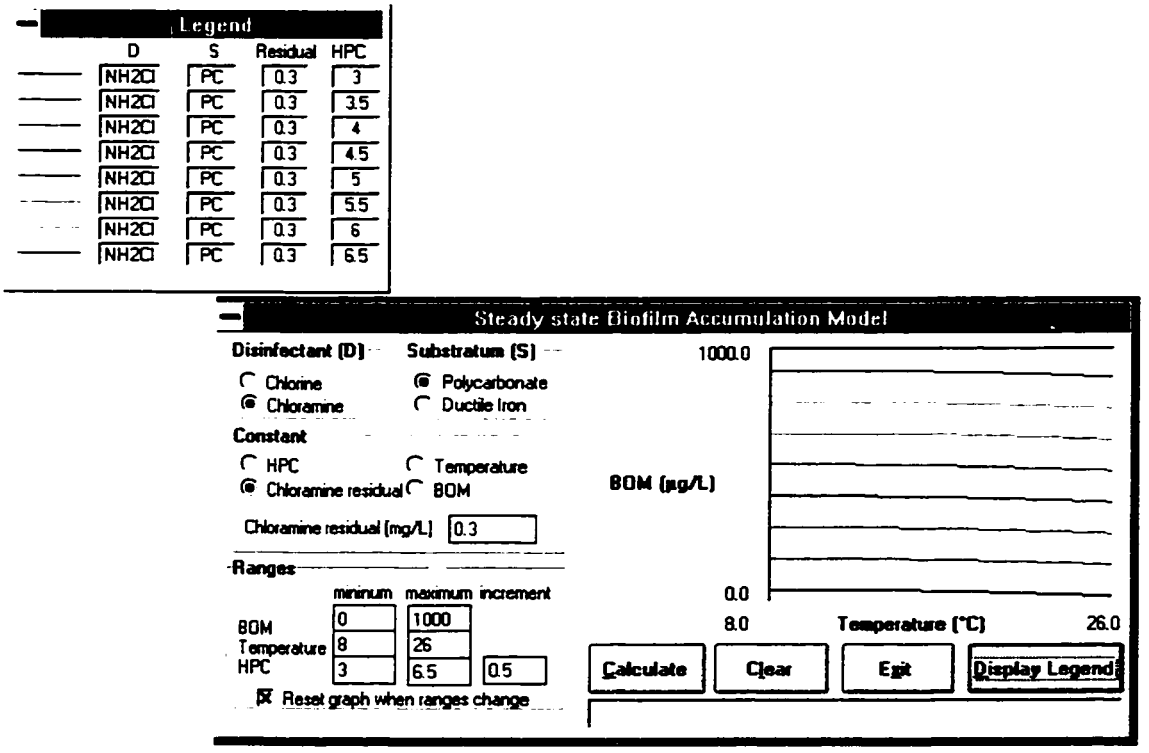


Figure 9.11: Steady-state biofilm accumulation model
Constants: polycarbonate, and monochloramine residual (0.3 mg/L)
Variables: temperature, BOM, and HPC

With identical functional dependence as before (Figure 9.11), 0.3 mg/L free chlorine was used instead of the 0.3 mg/L monochloramine residual in Figure 9.12. As a result of the change of disinfectant type, steady-state HPC numbers on polycarbonate substratum were (further) reduced from 10^3 CFU/cm^2 to about $10^{2.5}$ CFU/cm^2 (50 μg/L BOM concentration at 22°C).

With identical functional dependence as in Figure 9.12, HPC supporting characteristics of the alternative supporting surface (i.e. ductile iron) was tested in Figure 9.13. The model suggests that, under the condition tested, steady-state HPC numbers on ductile iron substratum were about $10^{0.6}$ CFU/cm^2 higher (i.e. $10^{3.1}$ CFU/cm^2) than on a polycarbonate surface (50 μg/L BOM concentration, 0.3 mg/L free chlorine residual, 22°C).

Summary

Predicted steady-state biofilm HPC numbers on polycarbonate surfaces dropped from 10^4 CFU/cm^2 to about 10^3 CFU/cm^2 as a result of the increase of monochloramine residual from 0.1 mg/L to 0.3 mg/L in the presence of 50 μg/L BOM concentration at 22°C. The change of 0.3 mg/L monochloramine to 0.3 mg/L free chlorine residual resulted in a further drop of HPCs to about $10^{2.5}$ CFU/cm^2. Under the conditions tested, ductile iron supported $10^{0.6}$ CFU/cm^2 higher HPCs than polycarbonate. Consequently, in the presence of 50 μg/L BOM concentration at 22°C, 0.3 mg/L monochloramine residual on polycarbonate and 0.3 mg/L free chlorine residual on ductile iron appear to support essentially the same number of HPCs (10^3 and $10^{3.1}$ CFU/cm^2 respectively). It is reasonable to suspect that at different pH, BOM, and/or pipe 'age', results could be different.

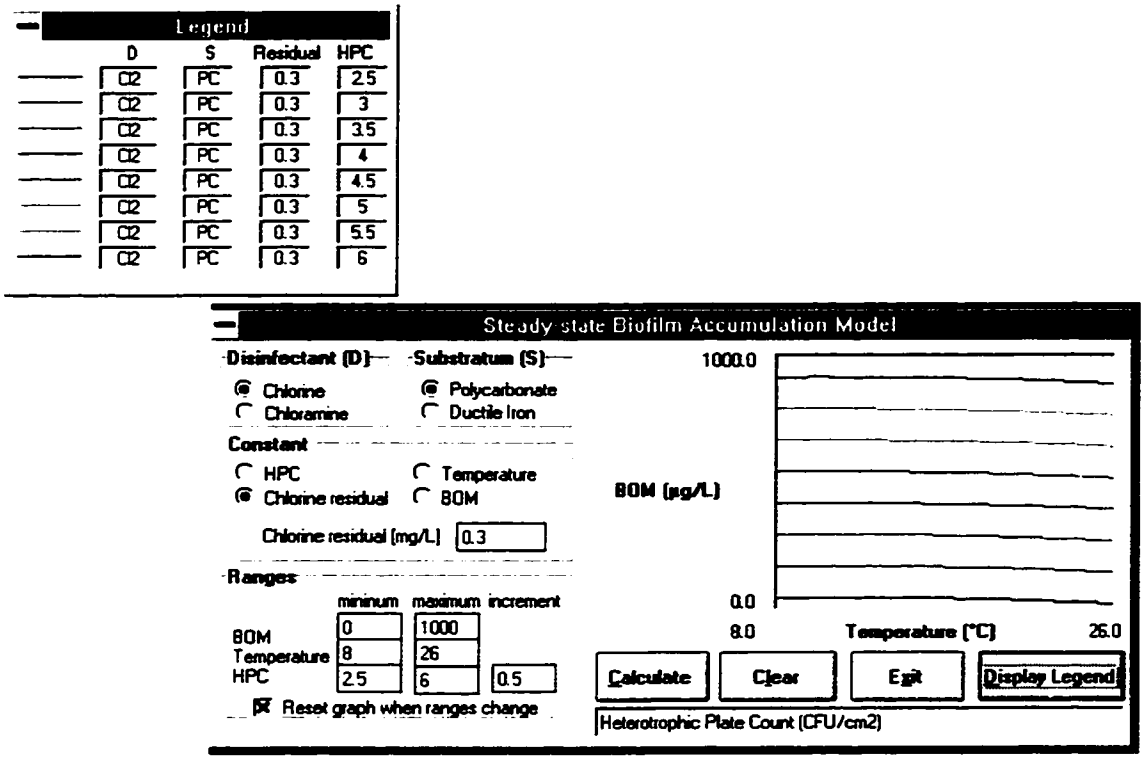


Figure 9.12: Steady-state biofilm accumulation model
Constants: polycarbonate, and free chlorine residual (0.3 mg/L)
Variables: temperature, BOM, and HPC

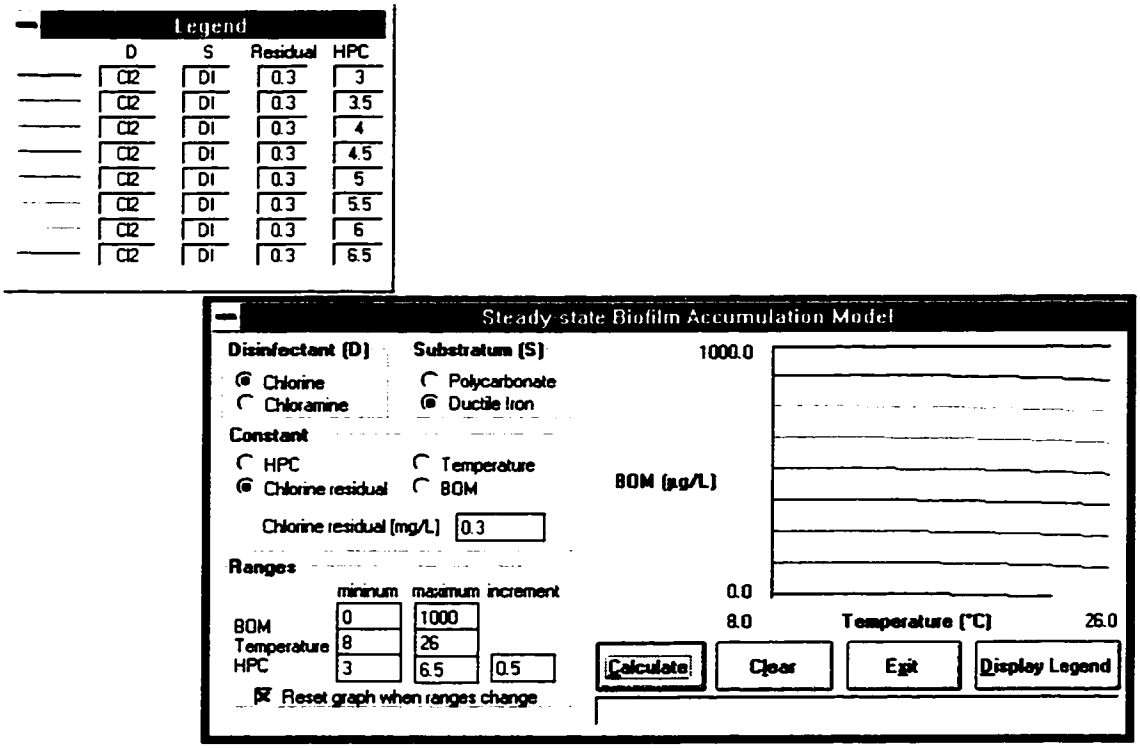


Figure 9.13: Steady-state biofilm accumulation model
Constants: ductile iron, and free chlorine residual (0.3 mg/L)
Variables: temperature, BOM, and HPC

Temperature Held Constant

Figure 9.14 shows the joint effect of BOM and free chlorine residual on steady-state HPC numbers on polycarbonate substratum at 15°C. If steady-state HPCs on polycarbonate must not exceed 10^4 CFU/cm^2 in the presence of 300 µg/L BOM concentration at 15°C, the required concentration of free chlorine residual is about 0.28 mg/L.

With identical functional dependence as before (Figure 9.14), two distinct sets of curves were superimposed in Figure 9.15. One set was obtained with 8°C, the other with a 26°C. For a steady-state log(HPC) number of 4.0 CFU/cm^2 (i.e. HPC=10^4/cm^2) on polycarbonate supporting surface in the presence of 300 µg/L BOM concentration, the required free chlorine residual is about 0.24 mg/L and 0.35 mg/L at 8°C and 26°C, respectively. This model output corresponds to practical experience, that is the increased number of bacteriological water quality concerns throughout the warm weather operation of actual drinking water distribution systems. The dosage of a disinfectant may have to be increased significantly during warm weather operation to meet both the demonstrated increased chlorine demand of a system and the (not demonstrated but well known) fact that disinfectants decay more rapidly at higher temperatures (HDT in the AR was only 2 hours).

With identical functional dependence as in Figure 9.14, monochloramine residual was investigated (instead of free chlorine), in Figure 9.16. If steady-state HPC numbers on polycarbonate surface are restricted to 10^4 CFU/cm^2 in the presence of 300 µg/L BOM concentration at 15°C, the required concentration of monochloramine residual is about 0.32 mg/L (0.04 mg/L higher than free chlorine residual). Comparison of data obtained with free chlorine (Figure 9.14) and monochloramine residuals (Figure 9.16) shows that under the conditions tested, 0.04 mg/L higher monochloramine residual was necessary to suppress steady-state HPC numbers to 10^4 CFU/cm^2. For practical applications this difference in residuals is small.

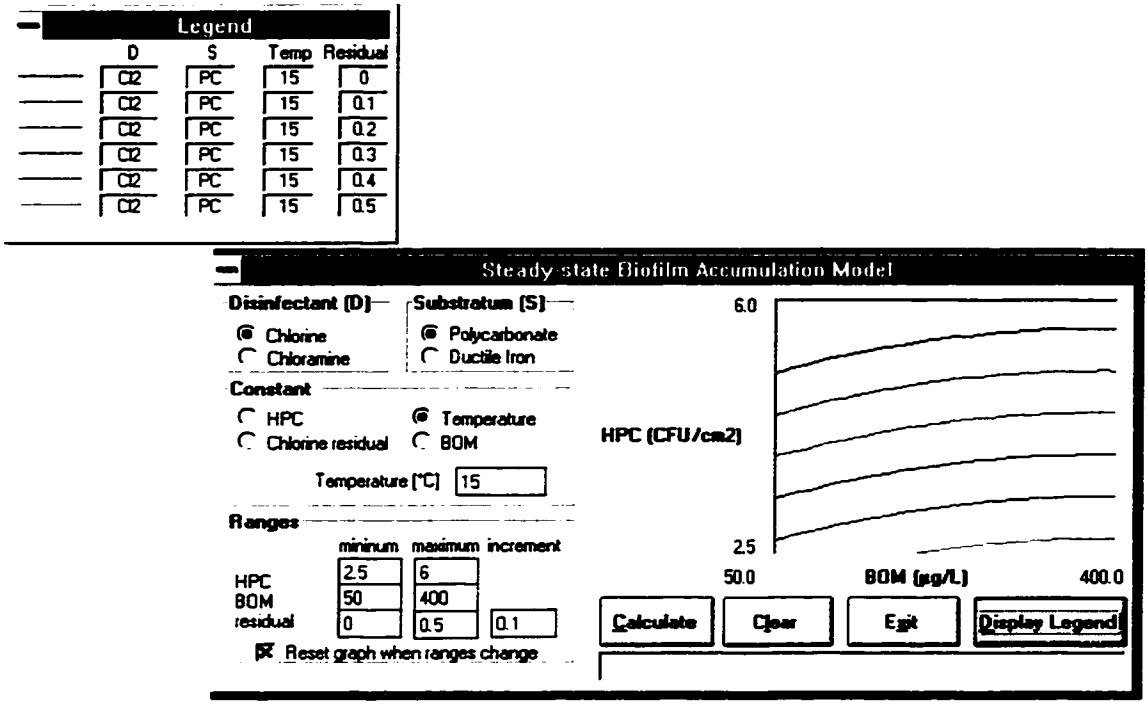


Figure 9.14: Steady-state biofilm accumulation model
Constants: polycarbonate, and temperature (15°C)
Variables: BOM, HPC, and free chlorine residual

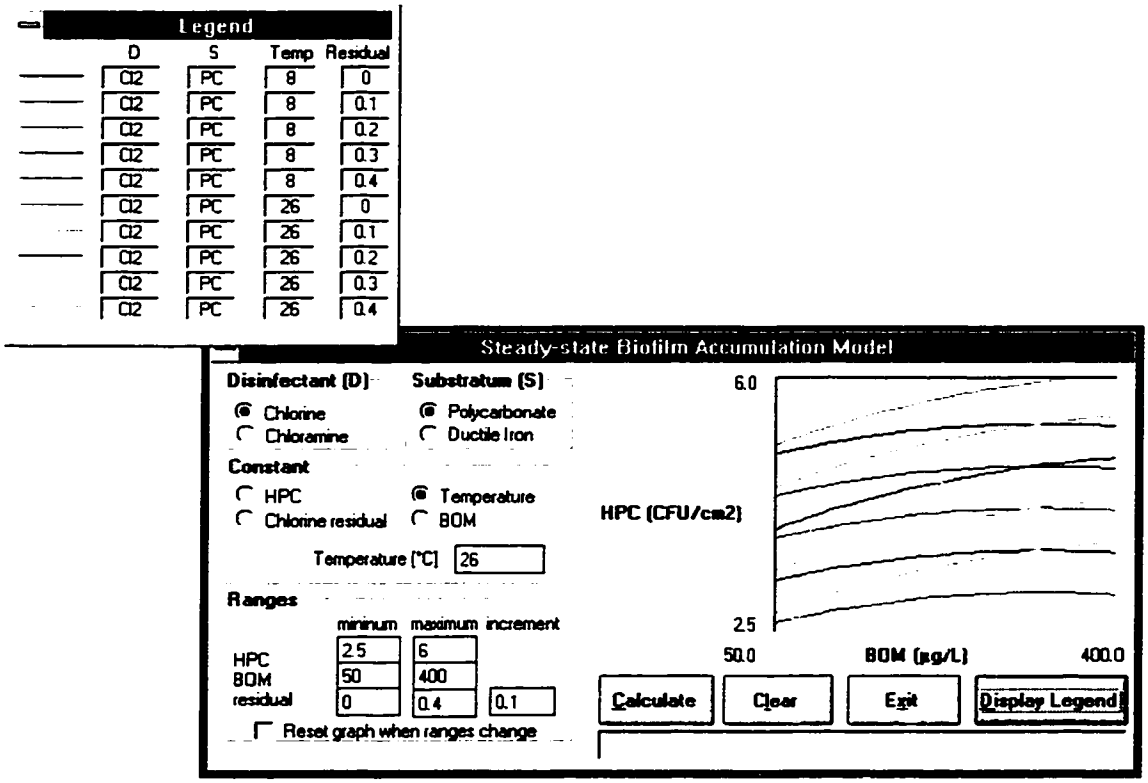


Figure 9.15: Steady-state biofilm accumulation model
Constants: polycarbonate, and temperature (8°C and 26°C)
Variables: BOM, HPC, and free chlorine residual

Naturally, more than one meaningful interpretation of any of these graphs is possible. For example, an alternative evaluation of Figure 9.16 suggests that in the presence of 300 µg/L BOM and 0.5 mg/L monochloramine residual in a polycarbonate supported system at 15°C, the predicted steady-state HPC number are about 10^3 CFU/cm^2.

Summary

Under the conditions tested 0.11 mg/L higher free chlorine residual was required at 26°C than at 8°C to maintain 10^4 CFU/cm^2 steady-state HPC numbers in a given system. If steady-state HPCs on polycarbonate surface are not to exceed 4.0 CFU/cm^2 in the presence of 300 µg/L BOM concentration at 15°C temperature, the required monochloramine residual is about 0.32 mg/L (0.04 mg/L higher than free chlorine residual).

BOM Held Constant

Figure 9.17 shows the joint effect of a free chlorine residual and temperature on steady-state log(HPC) numbers on ductile iron substratum in the presence of 150 µg/L BOM. In the presence of 150 µg/L BOM and a free chlorine residual of 0.3 mg/L on ductile iron substratum at 8°C, the predicted steady-state biofilm HPC numbers are about 10^4 CFU/cm^2. For the same conditions but at 26°C, predicted HPCs are about $10^{4.3}$ CFU/cm^2.

With identical functional dependence as before (Figure 9.17), the BOM concentration was increased from 150µg/L to 600 µg/L. Figure 9.18 shows that predicted HPC numbers were $10^{3.8}$ CFU/cm^2 and $10^{4.8}$ CFU/cm^2 at 8°C and 26°C temperatures, respectively (600 µg/L BOM, 0.3 mg/L free chlorine residual, and ductile iron)

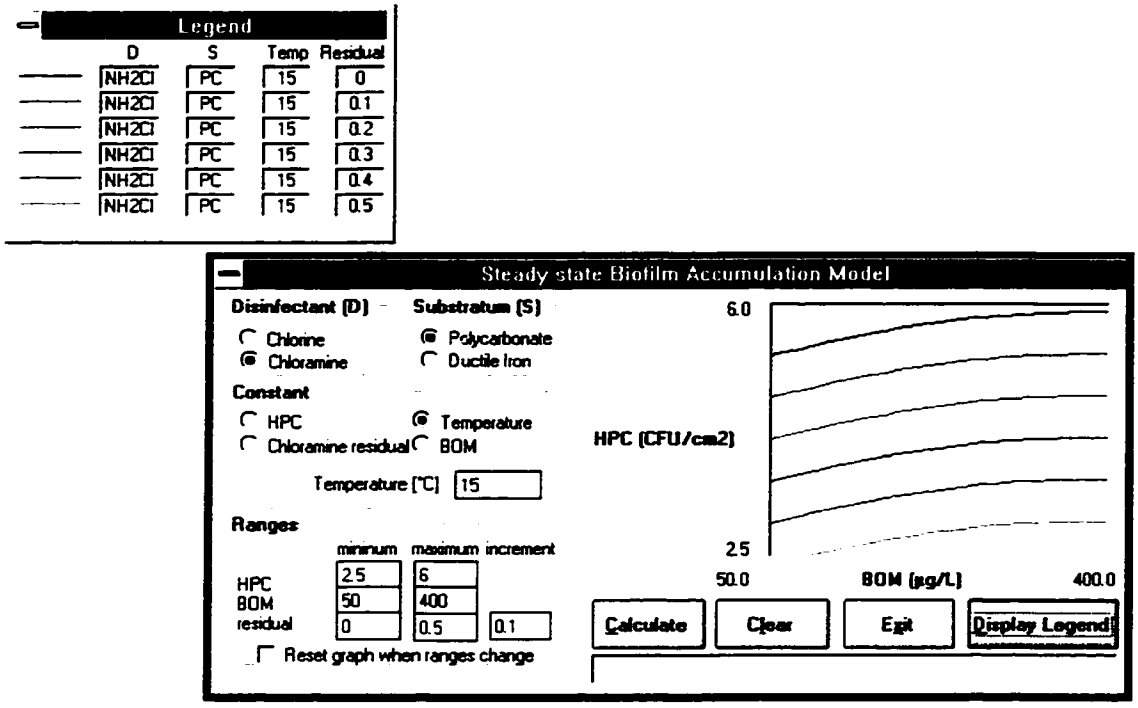


Figure 9.16: Steady-state biofilm accumulation model
Constants: polycarbonate, and temperature (15°C)
Variables: BOM, HPC, and monochloramine residual

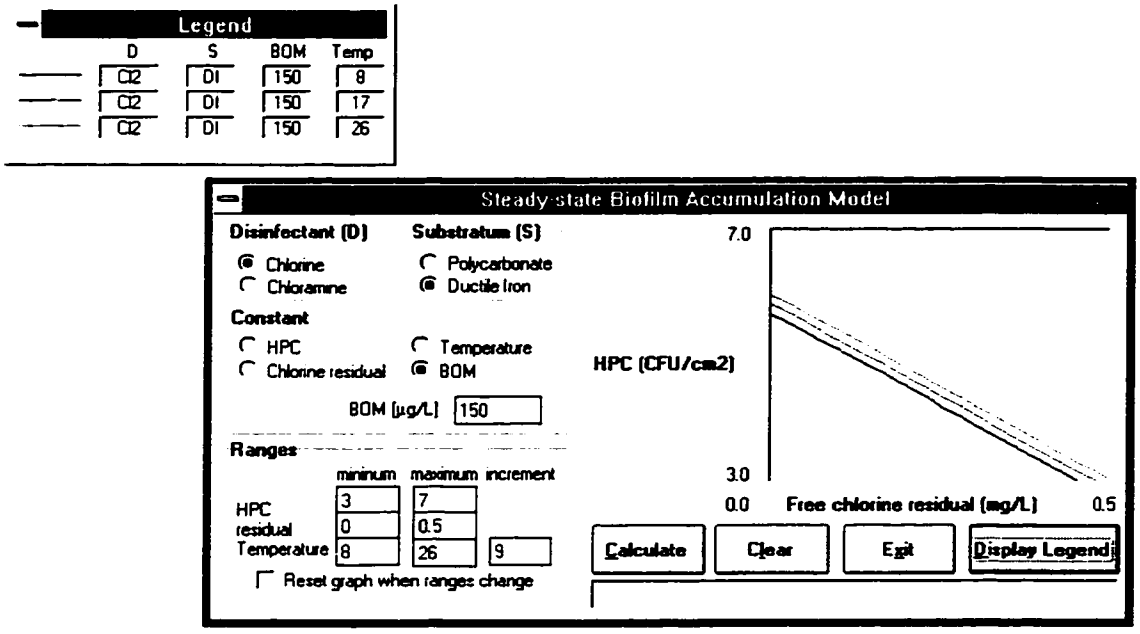


Figure 9.17: Steady-state biofilm accumulation model
Constants: ductile iron, and BOM (150 µg/L)
Variables: free chlorine residual, HPC, and temperature

The sensitivity of HPC numbers with respect to temperature appears to be higher at elevated BOM levels (indicated by the greater distance between the temperature contours in Figure 9.18). For stated system conditions, HPC numbers increase from $10^{4.3}$ CFU/cm^2 to $10^{4.8}$ CFU/cm^2 as a result of the increase of BOM concentration from 150 µg/L to 600 µg/L at 26°C. At 8°C, steady-state biofilm HPCs dropped from about 10^4 CFU/cm^2 to $10^{3.8}$ CFU/cm^2 according to the model. While the first prediction, that is a positive correlation between HPCs and the BOM level may be easily accepted, the second prediction (drop of HPCs by the increase of BOM) is difficult to explain and may be contributed to measurement error.

Figure 9.17 and Figure 9.18 show a linear relationship between free chlorine residual and the system response. This linear relationship might be somewhat surprising to the reader, at first sight at least, since potential quadratic effects of the disinfectant residual, *'c'*, were investigated at three distinct levels (0.1 mg/L, 0.3 mg/L, and 0.8 mg/L) in the real water experiments (Table 8.1). Since 0.8 mg/L residual level was adopted only for experiments with monochloramine (R11 and R12), free chlorine residuals were investigated, in fact, only at two levels (0.1 and 0.3 mg/L). This explains the 'phenomenon' of linearity.

Nomographs

The interface has the ability to display complex information in a compressed form. Figure 9.19 shows a sample nomograph with 24 displayed curves. Each curve is indicated by different color and/or line style. The legend of the actual interface in Figure 9.19 shows that each curve was obtained with a different combination of system variables. Any combination of the variables at any level can be depicted by the nomograph. Note that the 'X' is removed from the 'Reset graph when ranges change' display of the interface to allow the superimposing of curves (Section 9.5). This kind of nomographic model representation may be of great value in cases where the user/reader has adequate time to spend on analyzing the model output.

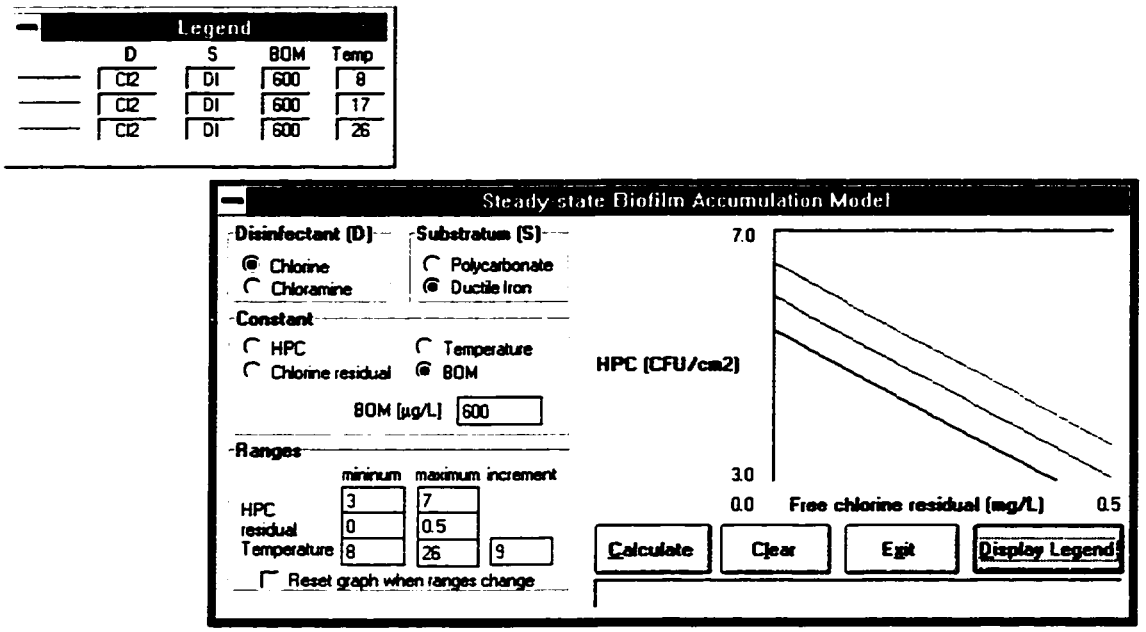


Figure 9.18: Steady-state biofilm accumulation model
Constants: ductile iron, and BOM (600 µg/L)
Variables: free chlorine residual, HPC, and temperature

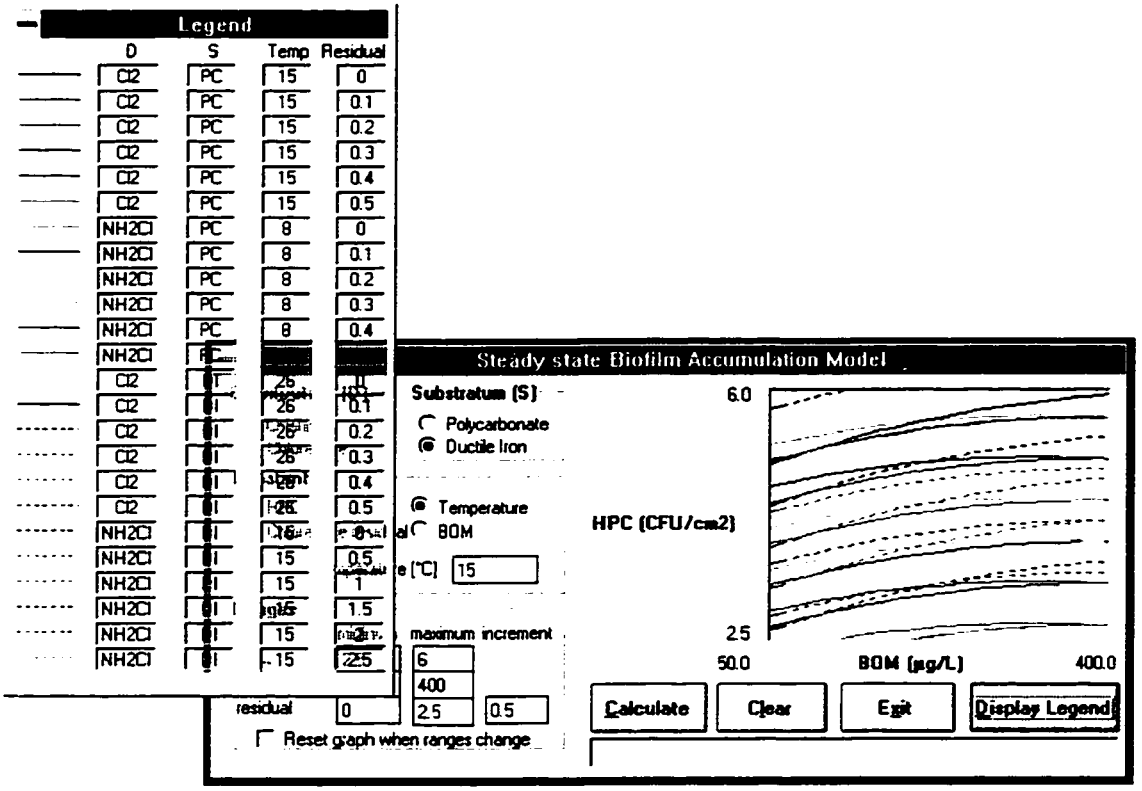


Figure 9.19: Steady-state biofilm accumulation model - HPC vs. BOM nomograph
Variables: BOM, disinfectant type, disinfectant residual, substratum, temperature

9.4.5 SUMMARY

Snapshots of the model were presented and analysed in Section 9.4.4. One of the 'interface variables' was held constant in each of these analyses. The relationship of the actual three 'interface variables' were evaluated for essentially *add hoc* selected scenarios. An HPC number of about 10^4 CFU/cm^2 may be considered as a realistical or achievable biofilm bacterial concentration in disinfected (not sterilized) distribution systems. Although not a specific indicator organism, HPC numbers at this level may indicate a reduced likelihood of pathogens in a drinking water distribution system. Therefore, whenever it was possible, HPC numbers were kept or read at the 10^4 CFU/cm^2. Naturally, any other HPC value could have been tested.

Table 9.14 shows a summary of the 'interface variables' which were considered in the model discussion (Section 9.4.4.1 through Section 9.4.4.5). To facilitate the understanding of these important data, conditions evaluated at the 10^4 CFU/cm^2 level are repeated in a bar graph form in Figure 9.20. Disinfectant types and BOM concentrations are designated to the two abscissas in the 3-D bar graph. The concentration of the actual disinfectant could be read on the ordinate. Temperature values are shown on top of the bars. Bars representing polycarbonate substrata are without shading. A single shaded bar in the figure illustrates ductile iron surface. The height of the bars shows the required amounts of disinfectant residuals (both free chlorine and monochloramine) to suppress steady-state biofilm HPC numbers to 10^4 CFU/cm^2 on either polycarbonate or ductile iron substrata at different temperatures and BOM concentrations. The monochloramine data clearly show the BOM effect. For an HPC number of 10^4 CFU/cm^2 on polycarbonate supporting surface in the presence of 50 μg/L and 300 μg/L BOM concentration, the required amounts of monochloramine residuals are 0.1 and 0.32 mg/L, respectively. Since temperature has a positive correlation with HPCs (Figure 9.17 and Figure 9.18), without the decrease of temperature from 22°C to 15°C in this analysis, the difference in residuals would have been even greater.

Table 9.14: Modeling Results - Summary

Reference	BOM (µg/L)	Disinfectant type	Disinfectant residual (mg/L)	Temperature (°C)	Substratum	Steady-state biofilm log HPC (CFU/cm^2)
Figure 9.10*	400	free chlorine	0.3	10	polycarbonate	4
Figure 9.11	180	free chlorine	0.3	10	polycarbonate	3
Figure 9.12*	50	monochloramine	0.1	22	polycarbonate	4
Figure 9.13	50	monochloramine	0.3	22	polycarbonate	3
Figure 9.14	50	free chlorine	0.3	22	polycarbonate	2.5
Figure 9.15	50	free chlorine	0.3	22	ductile iron	3.1
Figure 9.16*	300	free chlorine	0.28	15	polycarbonate	4
Figure 9.17*	300	free chlorine	0.24	8	polycarbonate	4
Figure 9.17*	300	free chlorine	0.35	26	polycarbonate	4
Figure 9.18*	300	monochloramine	0.32	15	polycarbonate	4
Figure 9.18	300	monochloramine	0.5	15	polycarbonate	3
Figure 9.19*	150	free chlorine	0.3	8	ductile iron	4
Figure 9.19	150	free chlorine	0.3	26	ductile iron	4.3
Figure 9.20	600	free chlorine	0.3	8	ductile iron	3.8
Figure 9.20	600	free chlorine	0.3	26	ductile iron	4.8

* Experiments shown in Figure 9.20

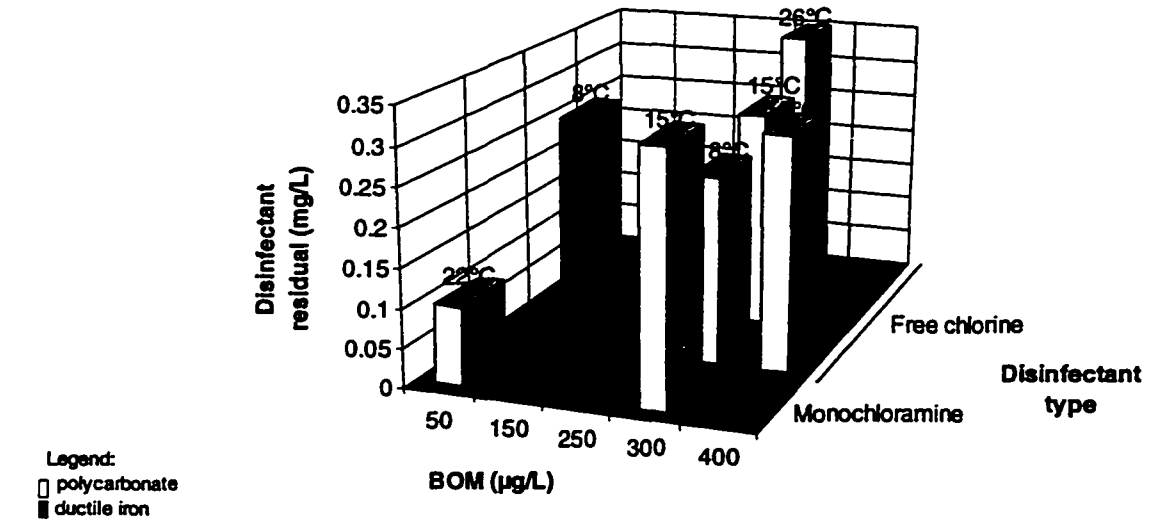


Figure 9.20: Predicted disinfectant residuals for a 10^4 CFU/cm^2 steady-state biofilm HPC number (ref. Table 9.13)

Comparison of the data obtained with free chlorine residual and 300 mg/L BOM shows a temperature effect. For an HPC number of 10^4 CFU/cm^2 on polycarbonate in the presence of 300 µg/L BOM concentration, the required free chlorine residual is about 0.24 mg/L and 0.35 mg/L at 8°C and 26°C, respectively. At 15°C, the corresponding value is 0.28 mg/L. This shows that under the conditions tested, the effect of temperature on net accumulation of HPCs

is essentially linear. Actual distribution systems operated under these conditions may need about 0.1 mg/L higher free chlorine residuals during summer (in contrast to winter) operation for the same year around bacteriological water quality.

Free chlorine residual results with 150 and 400 mg/L BOM supplements (Figure 9.20) show the combined effects of BOM, and substratum. Required disinfectant residuals to maintain 10^4 CFU/cm^2 steady-state HPC numbers on ductile iron with 150 µg/L BOM and on polycarbonate with 400 µg/L BOM were the same (0.3 mg/L). This suggests that at the same temperature, the reported higher HPC supporting characteristics of ductile iron surfaces (Figure 9.12 and Figure 9.13) may be offset by 250 µg/L BOM decrease. The discussion in the previous paragraph suggests that the increase of temperature from 8°C to 10°C in this comparison could be offset by about a 0.01 mg/L increase in free chlorine residual, a negligible value.

Comparison of free chlorine and monochloramine residual data at 15°C (Figure 9.20) shows that in the presence of 300 mg/L BOM, 0.04 mg/L higher monochloramine residual was necessary to suppress steady-state HPC numbers to 10^4 CFU/cm^2 on polycarbonate surfaces.

CHAPTER 10: CONCLUSIONS AND RECOMMENDATIONS

10.1 CONCLUSIONS

1. Among the investigated variables (BOM, disinfectant type, disinfectant concentration, shear, temperature, and substratum) the disinfectant residual appears to have the greatest impact on net accumulation of HPCs. Increasing the free chlorine or a monochloramine residual from zero to 0.5 mg/L reduced HPC numbers on polycarbonate substrata by 3 to 4 orders of magnitude. Under the conditions tested, approximately a 1 to 4 chlorine to monochloramine residual ratio must be maintained to achieve equal reductions in HPC numbers on ductile iron (corrosive) surfaces. To achieve similar steady-state biofilm HPCs, an established biofilm requires a higher disinfectant dosage than a system where biofilm is initially absent.

2. Experimental results showed a positive correlation between BOM and bacterial accumulation. In general, the effect of BOM on bacterial accumulation appears to be greater at higher temperatures and increased shear conditions. In particular, the rate of increase of net HPC accumulation appears to decrease (i.e. flatten) with an increase in BOM supplement. For a surface water tested, biological filtration reduced biofilm HPC numbers by about 0.5 log.

3. Under the conditions tested, steady-state net accumulation of HPCs was at least one order of magnitude higher on mild steel than on either polycarbonate or stainless steel 304 substrata. Mild steel and polycarbonate substrata appeared to bracket the corrosion behavior of ductile iron supporting surfaces.

4. Bench scale experiments with gradually increasing disinfectant residuals on different supporting surfaces suggested that to suppress biofilm HPC numbers to about 10^3 CFU/cm^2, the following disinfectant residuals must be present: 0.5 mg/L free chlorine residual on polycarbonate; 0.5 mg/L monochloramine residual on polycarbonate; 0.5 mg/L free chlorine residual on ductile iron; and 2.0 mg/L monochloramine residual on ductile iron.

5. The effect of shear stress on the system response appears to be influenced by BOM levels. In the presence of a BOM supplement, steady-state biofilm HPCs were little affected by shear conditions. Without a BOM supplement, shear appeared to be an important factor. This suggests that (1) biofilm accumulation is bioreaction limited at higher BOM conditions, and (2) mass transfer is likely the rate limiting process at lower BOM levels.

6. HPC numbers were less affected by temperature than by other factors such as disinfectant residual or BOM level.

7. Modeling results suggest that the interaction between BOM and temperature is significant at the 5% level. The relative importance of this interaction compared to the main effects of the other parameter estimates is low (second lowest after disinfectant type). The interaction between temperature and shear stress appeared also to affect HPC numbers.

8. The close agreement of trials with synthetic and real waters suggests the usefulness of synthetic waters for controlled laboratory experimentation.

9. Linear regression results between suspended and/or steady-state biofilm HPC numbers, and physical system conditions (e.g. temperature, turbidity) showed typically poor correlation. Higher correlation strength (R^2 = 0.81) was demonstrated only between suspeneded HPCs and turbidity in AR effluents.

10. When disinfection was discontinued, HPC bacterial numbers were reestablished to the same or a higher level then they had achieved initially prior to the start of disinfection in the bench scale AR system. This suggests that for systems which rely on a disinfectant residual to control HPC accumulation, even temporary operation without a residual could lead to higher levels of accumulation.

11. The developed steady-state biofilm accumulation model described both synthetic and real water data well within one log accuracy. The fit to real water data was typically less than 0.1 log. The excellent fit of the model to real water data could be the combined effect of increased prior knowledge and the relatively narrow range of the investigated variables.

12. The developed steady-state biofilm accumulation model may predict average biofilm conditions in smaller well defined networks with relatively uniform pipe diameter and overall looping configuration. These predictions are subject to uncertainty due to the extreme complexity of the biofilm phenomena. Trends and relative predictions of the model may be applicable to more complex distribution networks.

10.2 RECOMMENDATIONS

10.2.1 RECOMMENDATIONS FOR DESIGN AND/OR OPERATION

In drinking water distribution systems where the biological water stability is achieved by the provision of a disinfectant residual, operation - even temporarily - without the presence of a

disinfectant residual should be avoided to minimize the regrowth of heterotrophic microorganisms in the system.

The experiments completed in this research investigated net accumulation of HPC numbers in 'young' biofilms with potentially less diversified habitats. Therefore, some caution should be exercised at extrapolating 'young' biofilm data to established 'older' biofilms in distribution systems.

The design of flow velocities in the distribution system must often comply with two fundamental requirements, (1) should be low enough for low friction loss and lessen the operating (pumping) cost, and (2) high enough to avoid disinfectant-residual-free operation due to excessive residence times. To help the design of distribution systems, the following strategic recommendations are made:

1. Treated surface water supplied distribution systems operating with higher disinfectant residuals could be designed for higher normal operating flow velocities to minimize bacterial accumulation potential.

2. Groundwater supplied distribution systems operated in the absence or in the presence of a low disinfectant residual could be designed for lower normal operating flow velocities to minimize bacterial regrowth potential. Since biofilm accumulation was shown to be mass transfer (diffusion) controlled under these conditions, the flow velocity is directly related to the supply of bacterial substrate to the cell wall. Larger distribution systems operated with low flow velocity may require additional on-line disinfection facilities at strategic points of the system (e.g. reservoirs). While pumpage related operating costs in such systems are expected to be reduced, the capital cost will be higher due to the required larger pipe size.

3. Non-corrosive plastic materials, such as polycarbonate, appear to encourage biofilm bacterial accumulation to a lesser degree and may, therefore, be the recommended pipe material for the design of new drinking water distribution systems.

4. Establishment of a disinfectant residual in distribution systems containing both plastic and metallic pipe materials should be based on, from a regrowth viewpoint, the more conservative metallic pipe material.

5. Biological removal of organic matter at the site of the treatment facility and the maintenance of a low disinfectant residual level appears to be the best practice to minimize regrowth potential in the drinking water distribution system.

10.2.2 RECOMMENDATIONS FOR FUTURE RESEARCH

1. If turbidity is to be a reliable surrogate parameter for suspended HPCs, this should be confirmed.

2. The stomacher-based biofilm removal technique appears to be superior to other removal methods and is , therefore, strongly recommended for future research.

3. The complex effect of shear stress and temperature on net accumulation of HPC numbers should be further investigated and the recommended limitations of the overall biofilm accumulation phenomena confirmed.

4. The performance of the developed steady-state biofilm accumulation model should be verified by sets of data collected from actual distribution systems.

5. By the completion of the essentially factorial design used in this research the accuracy of the developed steady-state biofilm accumulation model could be further increased.

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Appendices

Appendix A: Optimal Design of the Third Segment of Experiments with Synthetic Water

A/1 'F' Matrix

A/2 'H' Matrix

A/3 Replicate Steady-State Biofilm HPC Numbers (Preliminary Experiments)

A/4 MATLAB Codes to Calculate 'M' Determinants

A/5 'M' Determinants

A/1

A/2

Appendix A/3: Replicate Steady-State Biofilm HPC Numbers (Preliminary Experiments)

Exp #	Replicate Sample															
	#1		#2		#3		#4		#5		#6		#7		#8	
		log		log		log		log		log		log		log		log
P2	4.1E+6	6.61	8.5E+6	6.93	1.2E+7	7.08	5.0E+6	6.69	8.8E+6	6.94	6.1E+6	6.78	-	-	-	-
P3/1	2.7E+7	7.43	2.8E+7	7.44	3.6E+7	7.55	-	-	-	-	-	-	-	-	-	-
P3/2	3.6E+7	7.55	3.3E+7	7.52	3.6E+7	7.55	-	-	-	-	-	-	-	-	-	-
P4/1	1.2E+8	8.09	2.1E+8	8.33	8.3E+7	7.92	-	-	-	-	-	-	-	-	-	-
P4/2	2.1E+8	8.33	1.7E+8	8.22	8.8E+7	7.94	-	-	-	-	-	-	-	-	-	-
P7/1	3.6E+4	4.55	2.5E+4	4.39	4.1E+4	4.62	-	-	-	-	-	-	-	-	-	-
P7/2	3.0E+4	4.48	9.9E+4	5.00	3.6E+4	4.55	-	-	-	-	-	-	-	-	-	-
P8/1	3.3E+6	6.52	1.7E+6	6.24	1.1E+6	6.03	-	-	-	-	-	-	-	-	-	-
P8/2	2.8E+6	6.44	2.3E+6	6.35	2.8E+6	6.44	-	-	-	-	-	-	-	-	-	-
P9	9.6E+4	4.98	1.0E+5	5.01	7.2E+4	4.85	8.3E+4	4.92	9.1E+4	4.96	1.2E+5	5.06	-	-	-	-
P10	6.3E+4	4.80	4.1E+4	4.62	4.4E+4	4.64	4.1E+4	4.62	6.9E+4	4.84	4.4E+4	4.64	-	-	-	-
P15/1	3.9E+4	4.59	3.3E+4	4.52	3.6E+4	4.55	4.1E+4	4.62	-	-	-	-	-	-	-	-
P15/2	4.1E+4	4.62	4.4E+4	4.64	3.3E+4	4.52	5.0E+4	4.69	-	-	-	-	-	-	-	-
P16/1	1.4E+5	5.14	1.9E+5	5.27	1.8E+5	5.25	3.6E+5	5.55	-	-	-	-	-	-	-	-
P16/2	3.3E+5	5.52	2.2E+5	5.34	8.0E+4	4.90	2.2E+5	5.34	-	-	-	-	-	-	-	-
P17	6.6E+5	5.82	7.7E+5	5.89	5.0E+5	5.70	7.2E+5	5.86	3.6E+5	5.56	3.9E+5	5.59	3.9E+5	5.59	-	-
P18	8.3E+7	7.92	1.1E+8	8.04	1.4E+8	8.15	1.2E+8	8.08	8.3E+7	7.92	8.0E+7	7.90	9.1E+7	7.96	-	-
P19	5.8E+6	6.76	5.5E+6	6.74	8.0E+6	6.90	6.6E+6	6.82	5.5E+6	6.74	5.5E+6	6.74	6.9E+6	6.84	6.9E+6	6.84
P20	1.4E+5	5.14	1.8E+5	5.26	1.4E+5	5.14	1.6E+5	5.19	1.1E+5	5.05	1.5E+5	5.18	1.6E+5	5.21	1.2E+5	5.08
P21	1.7E+8	8.22	1.8E+8	8.24	3.9E+8	8.59	2.8E+8	8.44	1.9E+8	8.28	2.0E+8	8.31	1.2E+8	8.08	2.1E+8	8.32
P22/1	2.4E+7	7.38	2.6E+7	7.41	2.5E+7	7.40	-	-	-	-	-	-	-	-	-	-
P22/2	4.4E+7	7.64	2.8E+7	7.45	2.5E+7	7.40	-	-	-	-	-	-	-	-	-	-

B/5

Appendix B: Preliminary Investigations

B/1 Testing if Two Means Differ from Each Other - Biofilm Removal Method

B/2 Testing if Two Means Differ from Each Other - Sampling Reproducibility

B/3 Regression Analyses of AR Effluent HPCs and Steady-State Biofilm HPCs

B/4 Decoding of Coded Real Water Sources

Appendix B/1: Testing if Two Means Differ from Each Other - Biofilm Removal Method (ref. P13, P14 and Figure 6.2)

Biofilm HPC

	Exp #P13/1 [knife] (CFU/cm^2)	Exp #P13/2 [stomacher] (CFU/cm^2)	Exp #P14/1 [knife] (CFU/cm^2)	Exp #P14/2 [stomacher] (CFU/cm^2)
X_1	1.7327E+03	1.1001E+04	3.3003E+04	4.4004E+04
X_2	8.2508E+03	1.4851E+04	2.6128E+05	1.3751E+05
X_3	8.2508E+03	1.7877E+04	8.2508E+04	1.8702E+05
X_4	4.1254E+03	8.8009E+03	7.7008E+05	1.0726E+06
X_5	4.1254E+03	2.7503E+04	9.0759E+05	9.6260E+05
X_6	4.1254E+03	4.1254E+04	1.3751E+06	9.3509E+05

sum $X_i = X_1 + X_2 + X_3 + X_4 + X_5$
ave X_i = (sum X_i) / 6
sum $(X_i^2 = X_1^2 + X_2^2 + X_3^2 + X_4^2 + X_5^2$

sum X_i	3.0611E+04	1.2129E+05	3.4296E+06	3.3388E+06
ave X_i	5.1018E+03	2.0215E+04	5.7160E+05	5.5647E+05
sum(X_i^2)	1.9021E+08	3.1969E+09	3.3839E+12	3.0073E+12

S^2 (Exp #P14)	2.5729E+11
S (Exp #P14)	5.0724E+05
S^2 (Exp #P13)	7.7921E+07
S (Exp #P13)	8.8273E+03

$t = [ave(X_1) + ave(X_2)] / S[(1/n_1) + (1/n_2)]^{0.5}$

t (Exp #P13)	2.965353751
t (Exp #P14)	0.051652251

For 10 degree of freedom and 95% confidence, the critical value of t is 2.228
t (AR #1) > 2.306
t (AR #2) < 2.306

Consequently, for the polycarbonate material (Exp #P13), the difference cannot be due to chance alone. It is concluded that the stomacher and utility knife removal techniques from the polycarbonate surface are statistically different based on their HPC numbers expressed as CFU/cm^2. This conclusion is based on a 95% confidence level.

On the other hand, for the mild steel material (Exp #P14), the difference between the stomacher and utility knife removal techniques is not significantly large, i.e. it could quite easily be chance that made the result seem different. This conclusion is based on HPC numbers expressed as CFU/cm^2. 95% confidence level was applied.

Appendix B/2: Testing if Two Means Differ from Each Other - Sampling Reproducibility (ref. P11, P12, Figure 6.4 and Figure 6.5)

Biofilm HPC (ref. Figure 6.4)

	Exp #P11/1 [knife] (CFU/cm^2)	Exp #P11/2 [stomacher] (CFU/cm^2)	Exp #P12/1 [knife] (CFU/cm^2)	Exp #P12/2 [stomacher] (CFU/cm^2)
X_1	4.6E+04	1.1E+06	5.4E+04	4.3E+05
X_2	1.8E+06	3.7E+06	9.0E+05	3.1E+06
X_3	1.0E+06	3.7E+06	3.2E+05	1.2E+06
X_4	3.2E+05	1.1E+06	3.0E+05	2.3E+06
X_5	3.0E+05	3.8E+06	3.0E+05	1.1E+06

sum $X_i = X_1 + X_2 + X_3 + X_4 + X_5$
ave X_i = (sum X_i) / 5
sum $(X_i^2) = X_1^2 + X_2^2 + X_3^2 + X_4^2 + X_5^2$

sum X_i	3.4660E+06	1.3400E+07	1.8740E+06	8.1300E+06
ave X_i	6.9320E+05	2.6800E+06	3.7480E+05	1.6260E+06
sum(X_i^2)	4.4345E+12	4.4240E+13	1.0953E+12	1.7735E+13

$S^2 = [\text{sum}(X_1^2) + \text{sum}(X_2^2) - (1/n_1)(\text{sum}(X_1)^2 - (1/n_2)(\text{sum}(X_2)^2] / (n_1 + n_2 - 2)$

S^2 (knife)	3.03103E+11
S (knife)	5.5055E+05
S^2 (stomacher)	1.60544E+12
S (stomacher)	1.2671E+06

$t = [\text{ave}(X_1) + \text{ave}(X_2)] / S[(1/n_1) + (1/n_2)]^{0.5}$

t (knife)	0.914424393
t (stomacher)	1.315265945

For 8 degree of freedom and 95% confidence, the critical value of t is 2.306
t (knife) < 2.306
t (stomacher) < 2.306

Consequently, while the biofilm mean seems typically slightly lower in Exp # P12, the difference is not significantly large, i.e. it could quite easily be chance that made the result seem different. This conclusion is true for both the knife and the stomacher removal techniques and based on a 95% confidence level.

Suspended HPC (ref. Figure 6.5)

	Exp #p11/1 [AR influent] (CFU/mL)	Exp #p11/2 [AR effluent] (CFU/mL)	Exp #p12/1 [AR influent] (CFU/mL)	Exp #p12/2 [AR effluent] (CFU/mL)
X_1	2.4E+04	2.1E+04	1.0E+04	1.1E+04
X_2	1.9E+04	2.8E+04	1.5E+04	1.2E+04
X_3	1.1E+04	2.6E+04	9.0E+03	1.9E+04
X_4	3.2E+04	2.1E+04	1.6E+04	1.7E+04
X_5	1.7E+04	3.3E+04	1.1E+04	9.2E+03
sum Xi	1.0300E+05	1.2900E+05	6.1000E+04	6.8200E+04
ave Xi	2.0600E+04	2.5800E+04	1.2200E+04	1.3640E+04
sum(Xi2)	2.3710E+09	3.4310E+09	7.8300E+08	9.9964E+08

S^2 (influent)	3.60000E+07
S (influent)	6.00000E+03
S^2 (effluent)	2.15240E+07
S (effluent)	4.63940E+03
t (influent)	5.42748E+00
t (effluent)	4.14421E+00

For 8 degree of freedom and 95% confidence, the critical value of t is 2.306
t (influent) > 2.306
t (effluent) > 2.306

Consequently, the difference cannot reasonably be due to chance alone. It is concluded that experiment #P11 and #P12 are statistically different from each other based on their suspended HPC accumulation in both
reactor influent and effluent. This conclusion is based on a 95% confidence level.

Appendix B/3: Regression Analysis of AR Effluent HPCs vs. Steady-State Biofilm HPCs

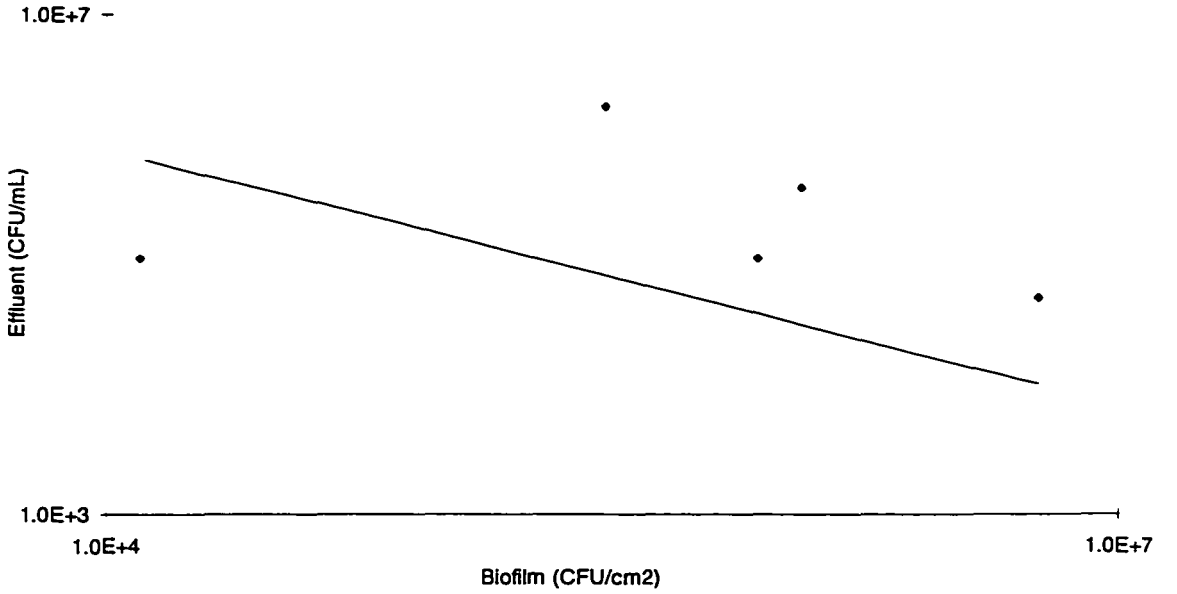


Figure B/3.1: Correlation between suspended HPCs and steady-state biofilm HPCs
$R^2 = 0.13$; n = 5; mild steel substratum (ref. Table 6.2)

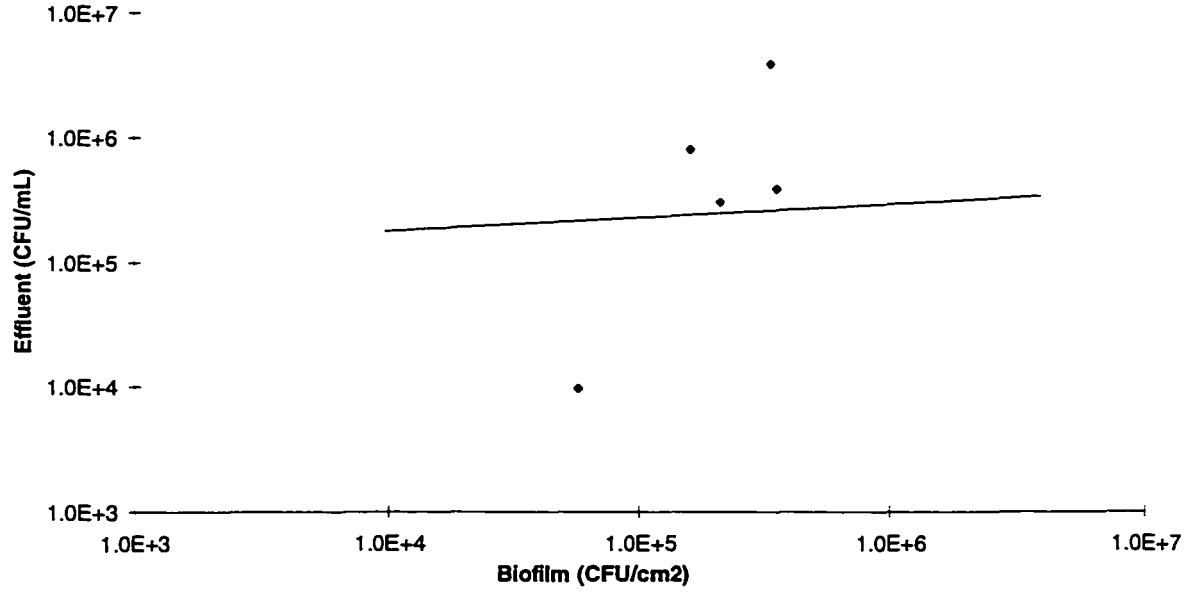


Figure B/3.2: Correlation between suspended HPCs and steady-state biofilm HPCs
$R^2 = 0.28$; n = 5; mild steel substratum (ref. Table 6.2)

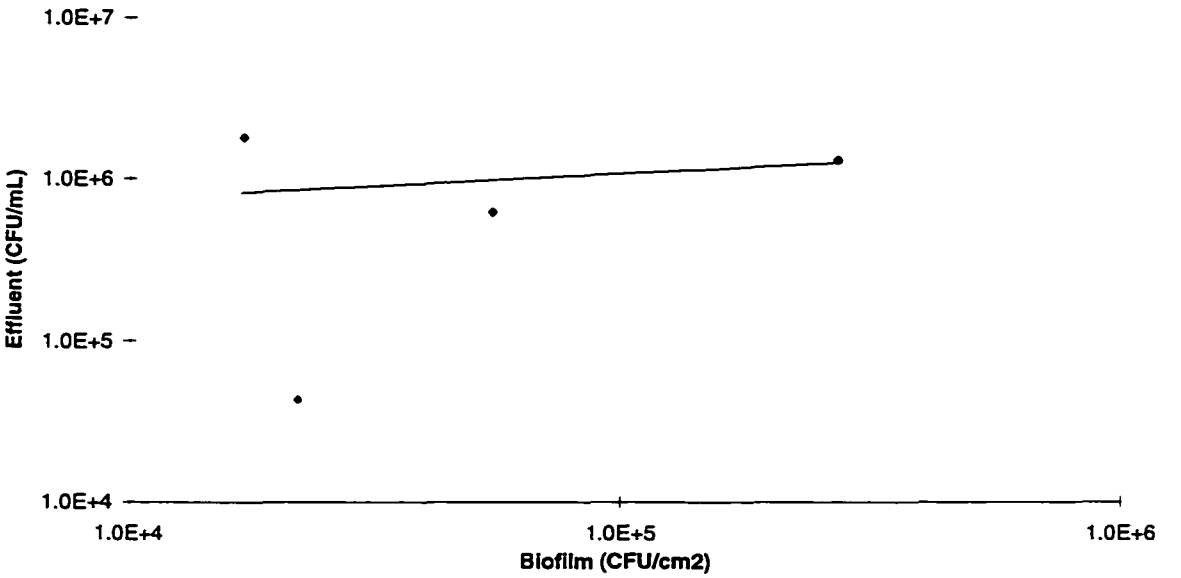


Figure B/3.3: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.07$; n = 4; polycarbonate substratum (ref. Table 6.2)

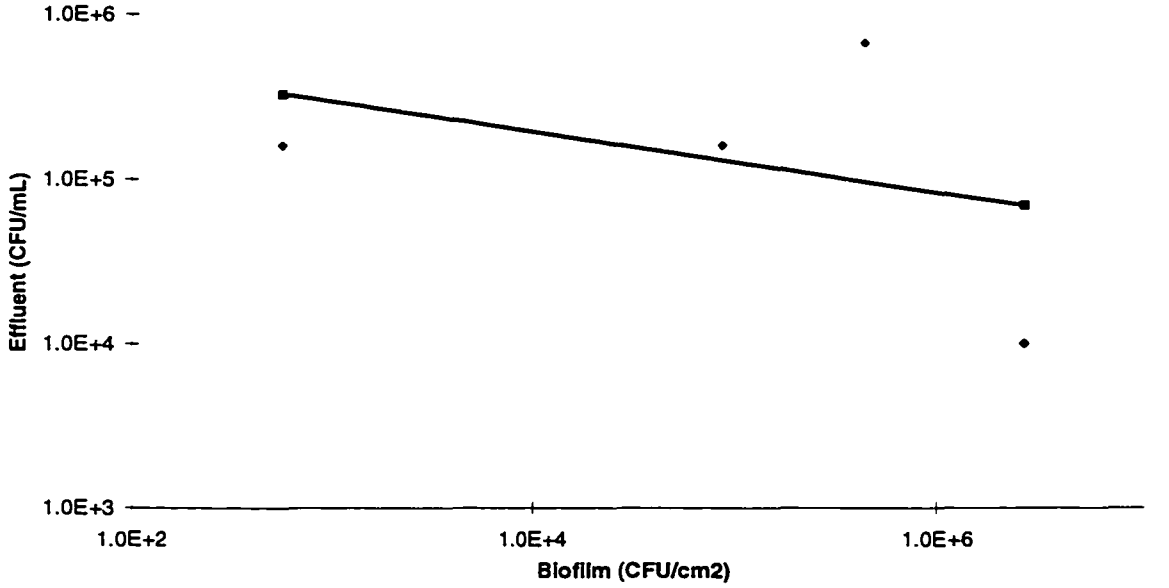


Figure B/3.4: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.17$; n = 4; polycarbonate substratum (ref. Table 6.2)

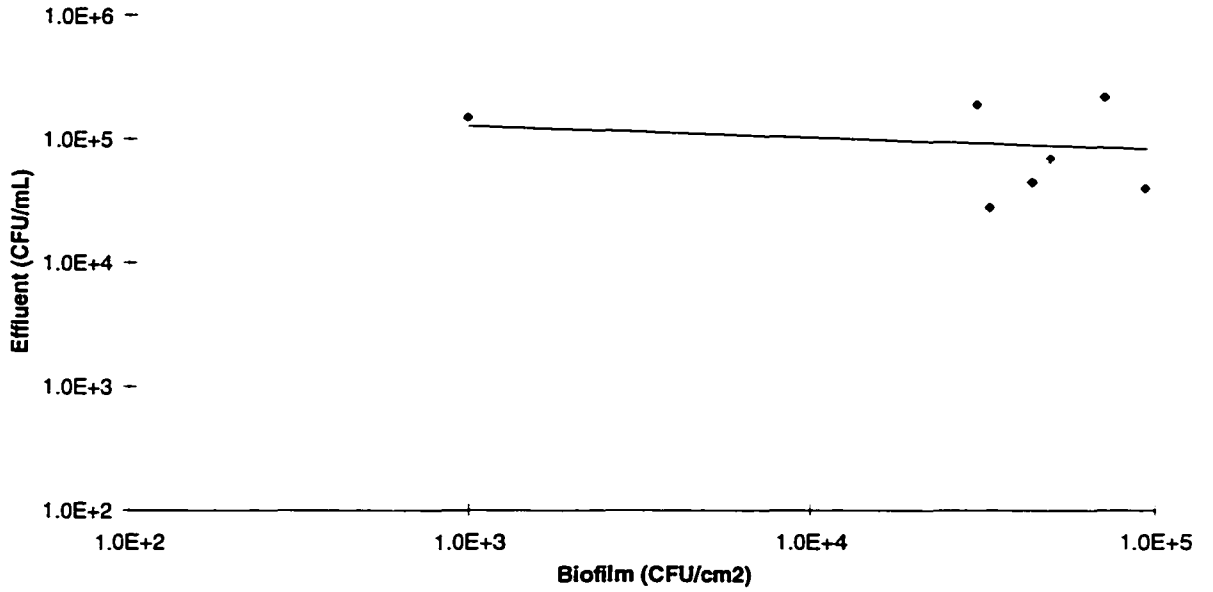


Figure B/3.5: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.03$; n = 7; SS 304 substratum (ref. Table 6.2)

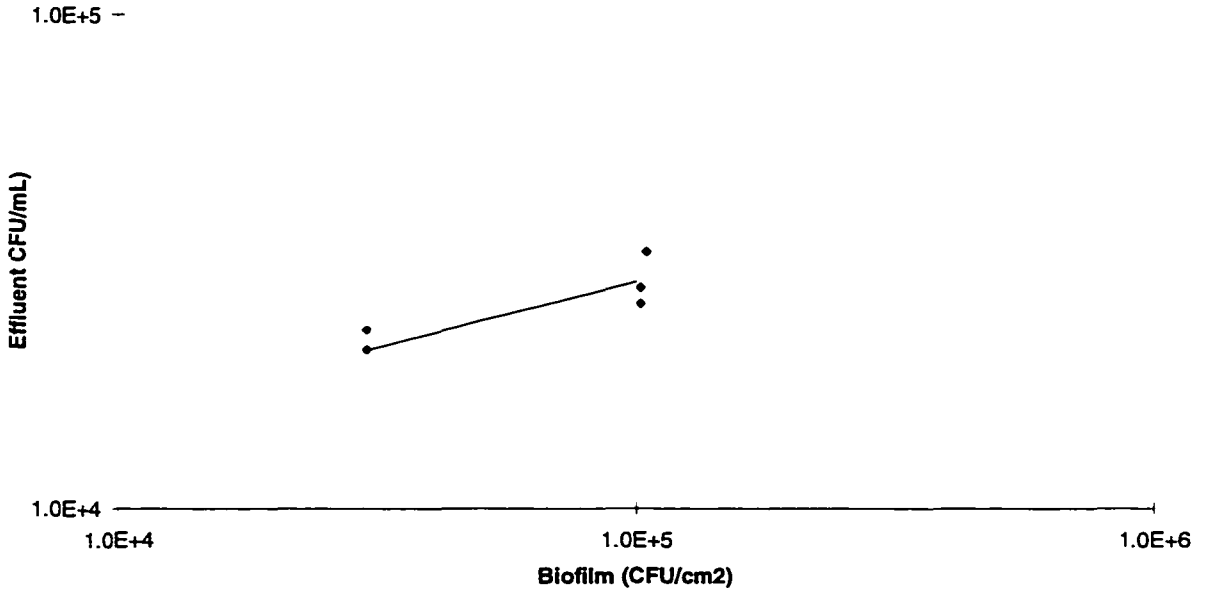


Figure B/3.6: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.77$; n = 5; polycarbonate substratum (ref. Table 6.2)

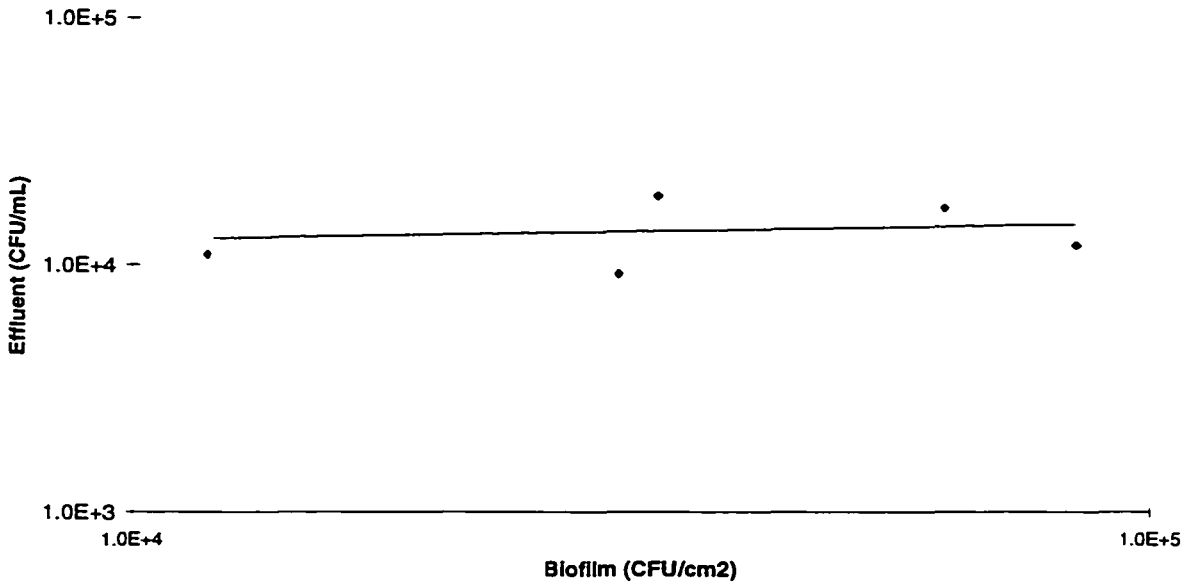


Figure B/3.7: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.03$; n = 5; polycarbonate substratum (ref. Table 6.2)

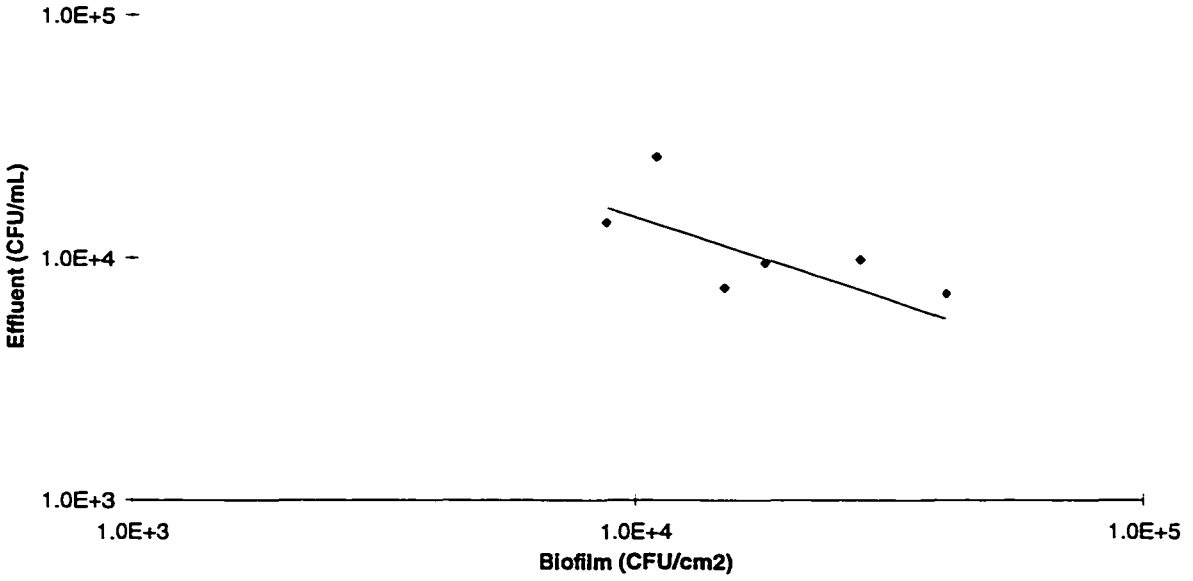


Figure B/3.8: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.31$; n = 6; polycarbonate substratum (ref. Table 6.2)

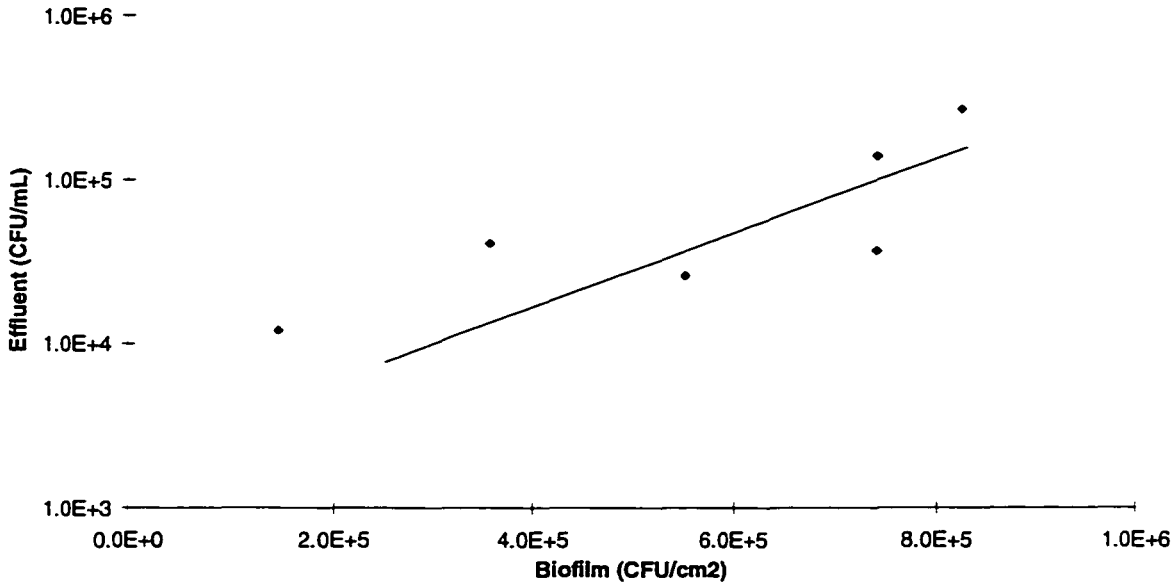


Figure B/3.9: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.46$; n = 6; polycarbonate substratum (ref. Table 6.2)

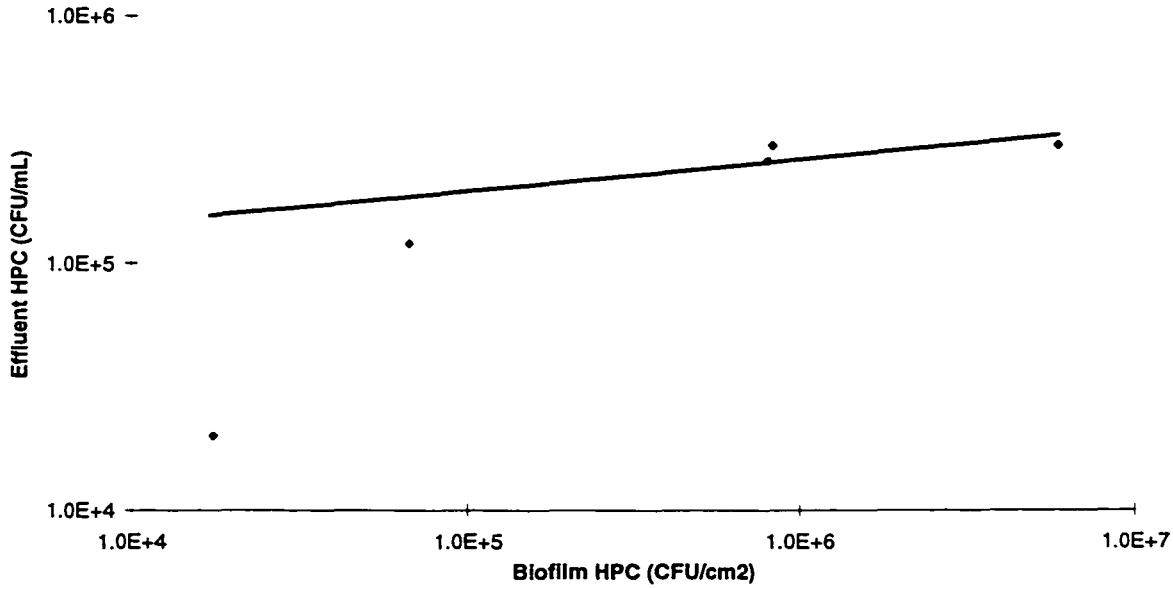


Figure B/3.10: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.33$; n = 5; polycarbonate substratum (ref. Table 6.2)

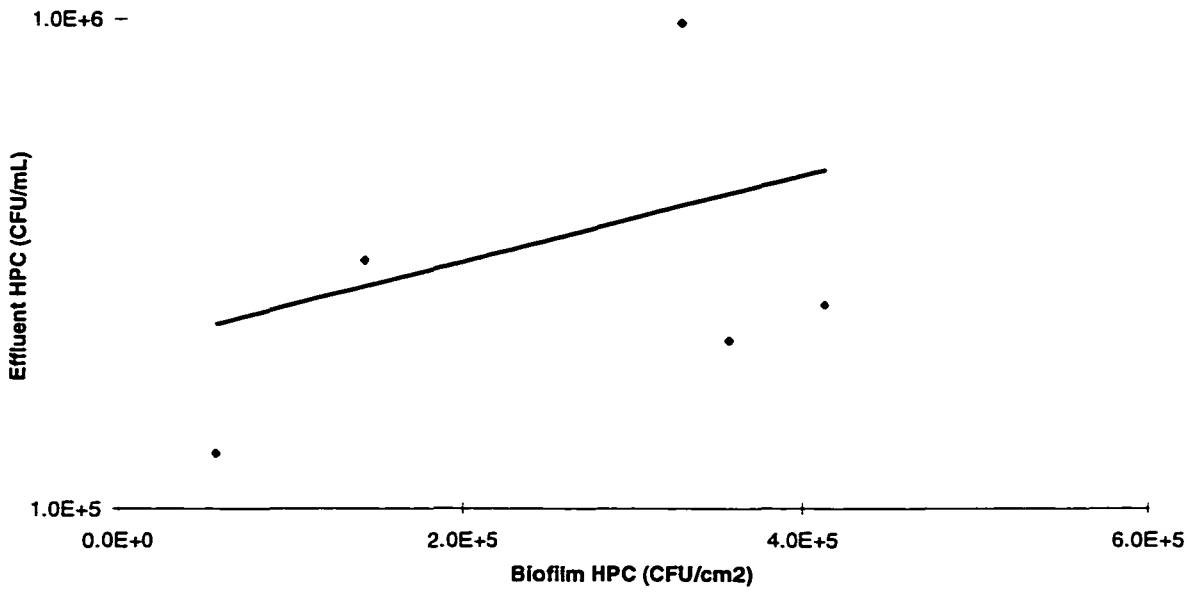


Figure B/3.11: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.10$; n = 5; polycarbonate substratum (ref. Table 6.2)

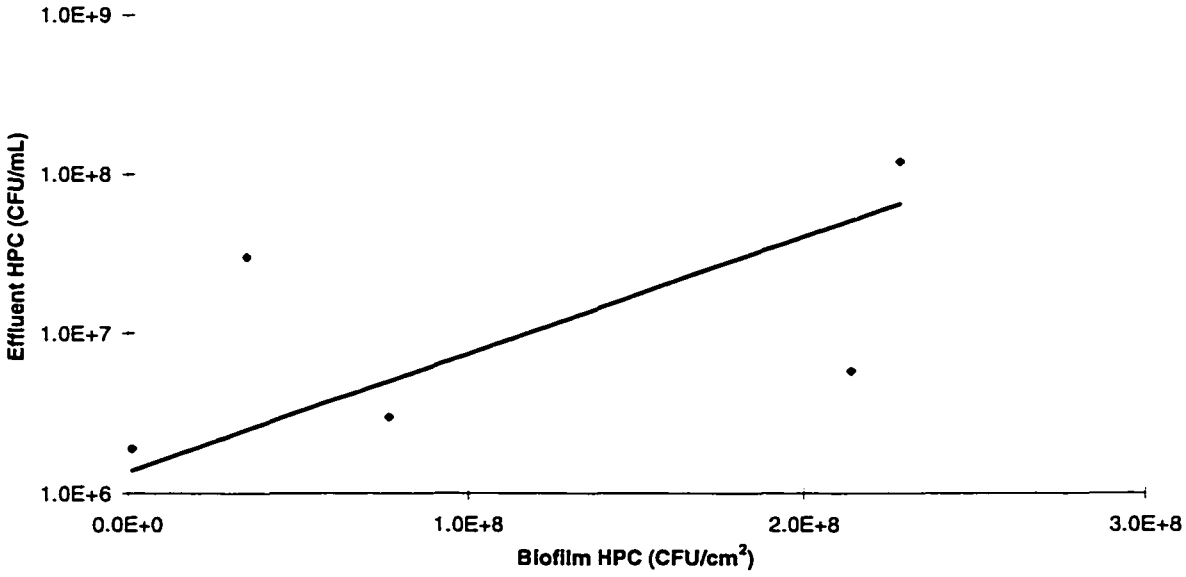


Figure B/3.12: Correlation between suspended HPCs and steady-state biofilm HPCs $R^2 = 0.33$; n = 5; polycarbonate substratum (ref. Table 6.2)

Appendix B/4: Decoding of Coded Real Water Sources

Code	Name	Water Source
A	St. Clements WTP	ground water
B	Linwood WTP	ground water
C	Wellesley WTP	ground water
D	Greenbrook WTP	ground water
E	Mannheim WTP	surface water

Appendix C: Net Accumulation of Biofilm HPCs - Synthetic Water Experiments

Appendix C: Net Accumulation of Biofilm HPCs - Synthetic Water Experiments

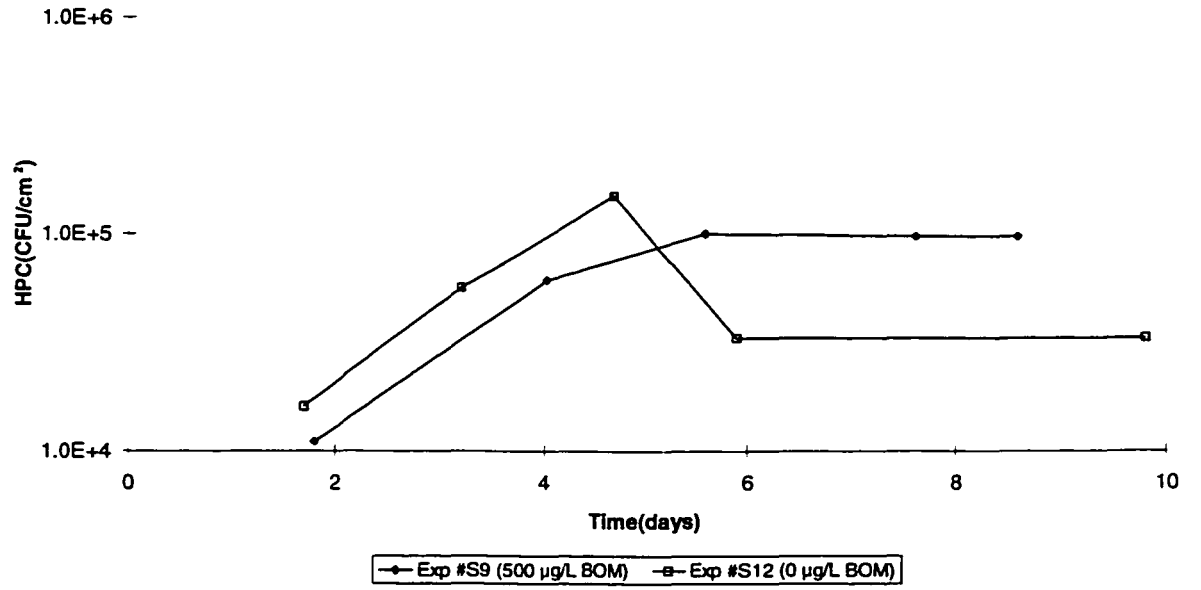


Figure C/1.1: Net accumulation of HPCs on polycarbonate substrata
Constants: 0 mg/L disinfectant residual, 0.4 N/m^2 shear stress, 8 °C (ref. Figure 7.2)

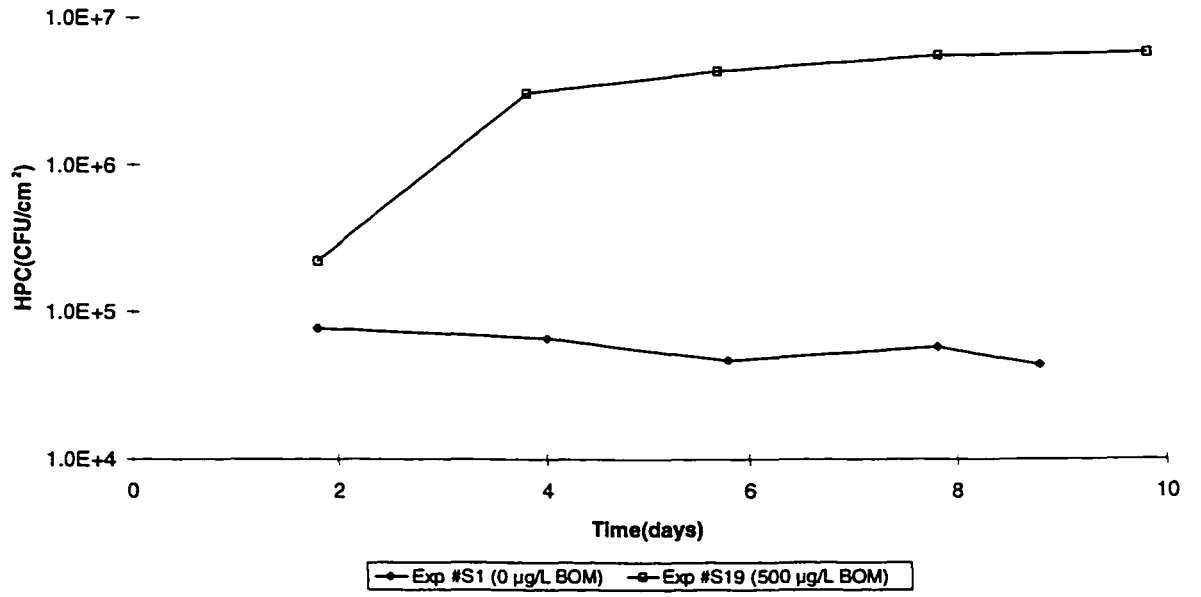


Figure C/1.2: Net accumulation of HPCs on polycarbonate substrata
Constants: 0 mg/L disinfectant residual, 0.4 N/m^2 shear stress, 26 °C (ref. Figure 7.2)

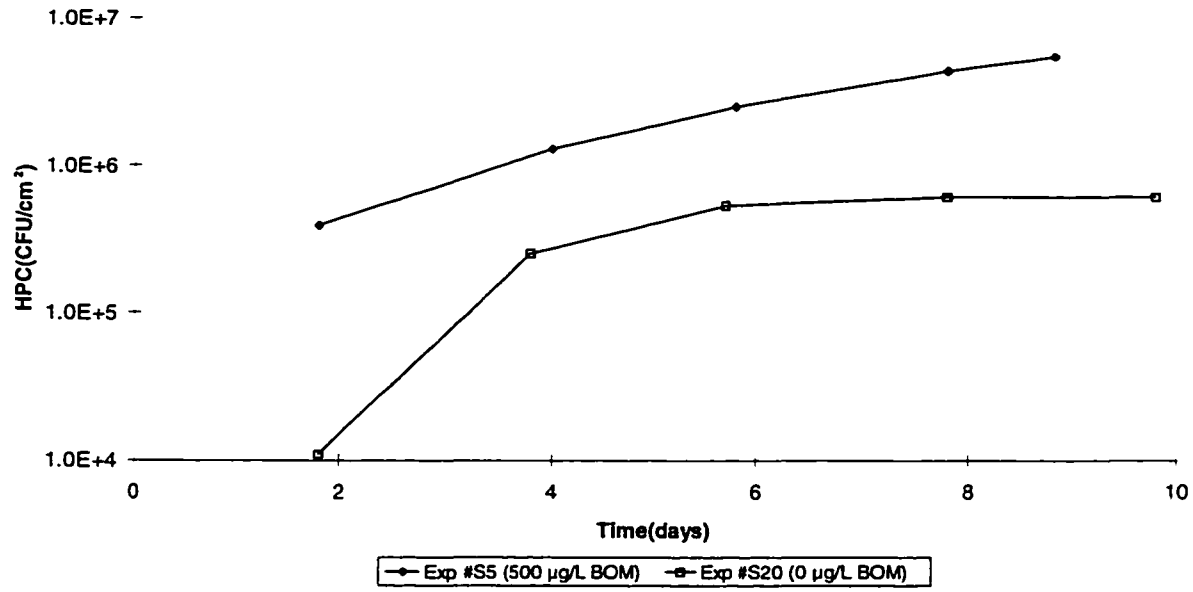


Figure C/1.3: Net accumulation of HPCs on polycarbonate substrata
Constants: 0 mg/L disinfectant residual, 2.0 N/m^2 shear stress, 26 °C (ref. Figure 7.2)

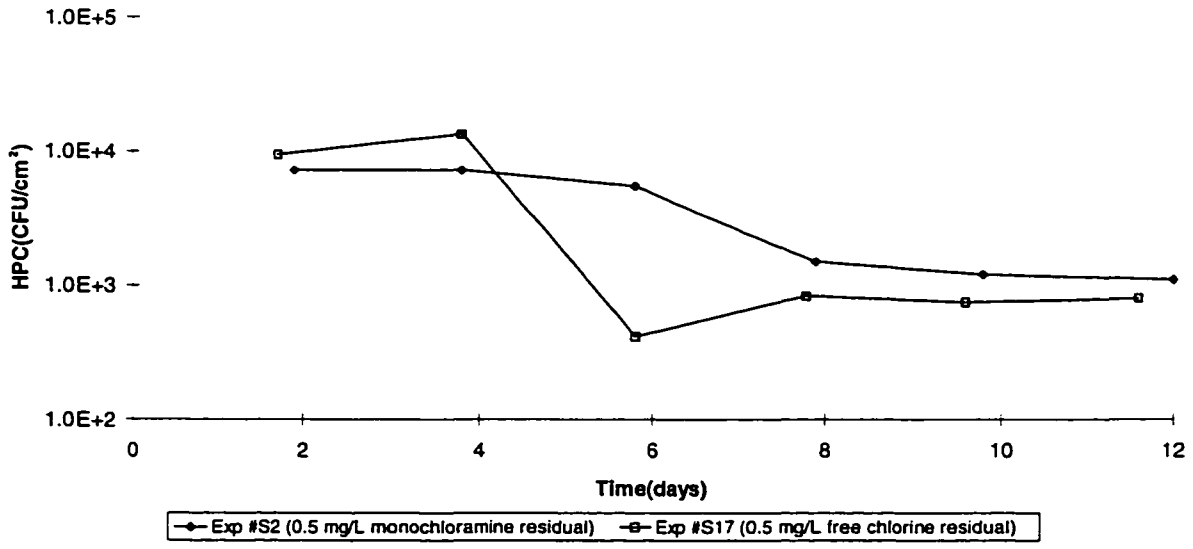


Figure C/1.4: Net accumulation of HPCs on polycarbonate substrata
Constants: 0 µg/L BOM supplement, 0.4 N/m^2 shear stress, 8 °C
Note: both disinfectants applied from day #4 (ref. Figure 7.3)

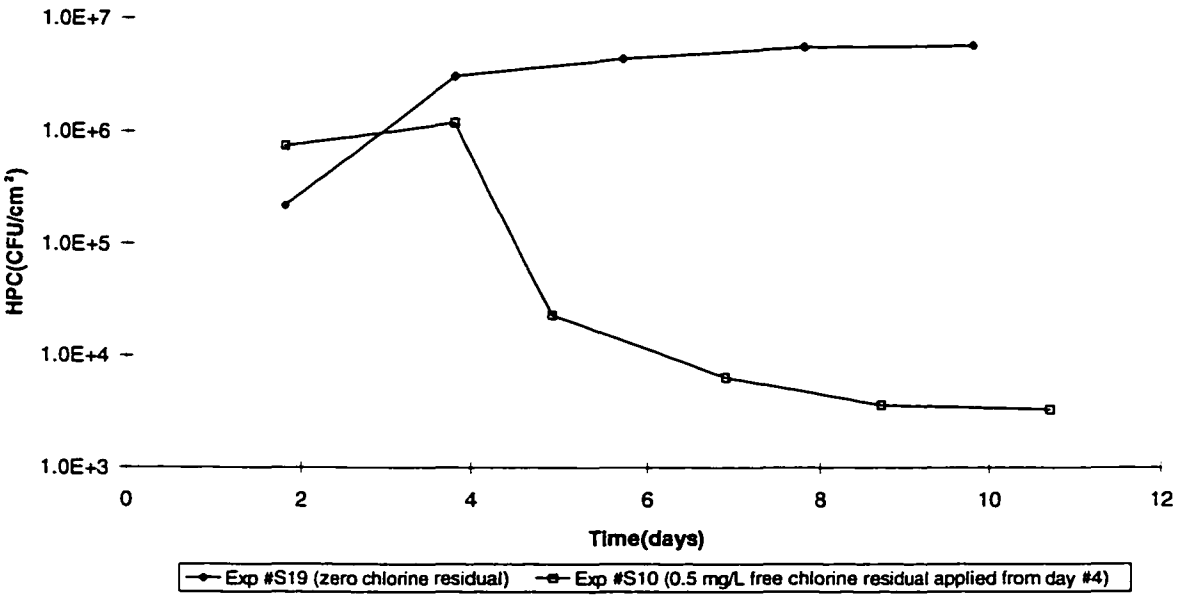


Figure C/1.5: Net accumulation of HPCs on polycarbonate substrata
Constants: 500 µg/L BOM supplement, 0.4 N/m^2 shear stress, 26 °C (ref. Figure 7.3)

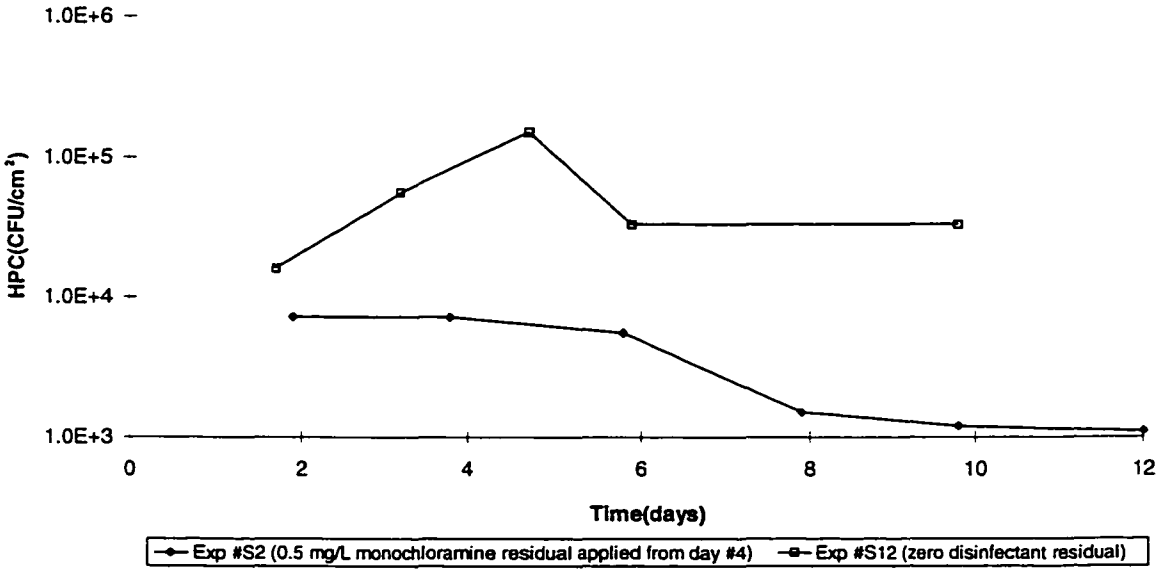


Figure C/1.6: Net accumulation of HPCs on polycarbonate substrata
Constants: 0 µg/L BOM, 0.4 N/m^2 shear stress, 8 °C (ref. Figure 7.3)

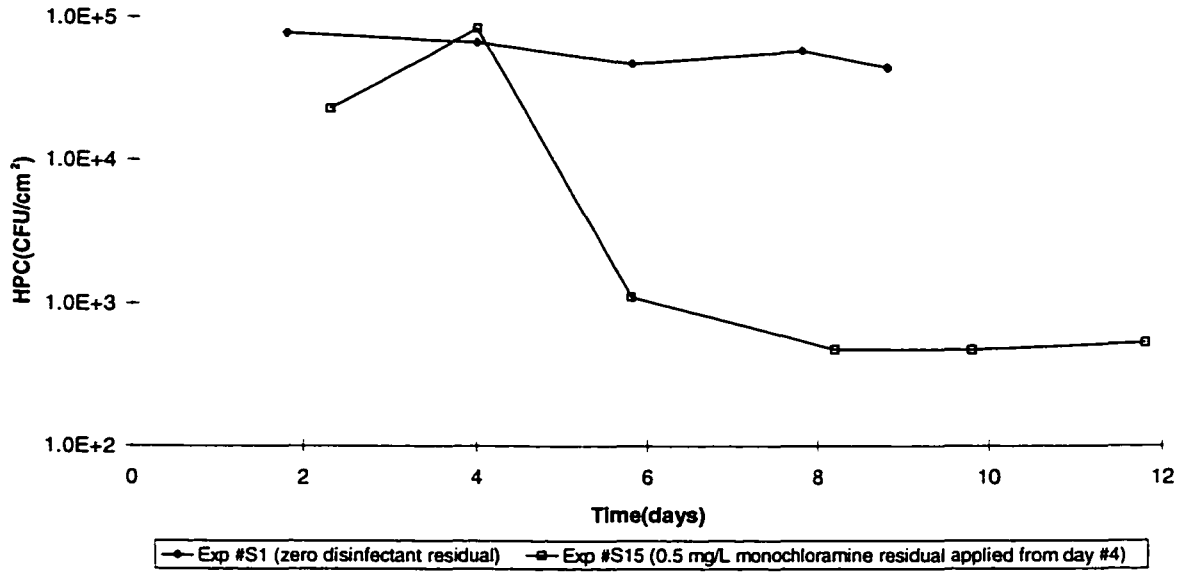


Figure C/1.7: Net accumulation of HPCs on polycarbonate substrata
Constants: 0 µg/L BOM, 0.4 N/m^2 shear stress, 26 °C (ref. Fig 7.3)

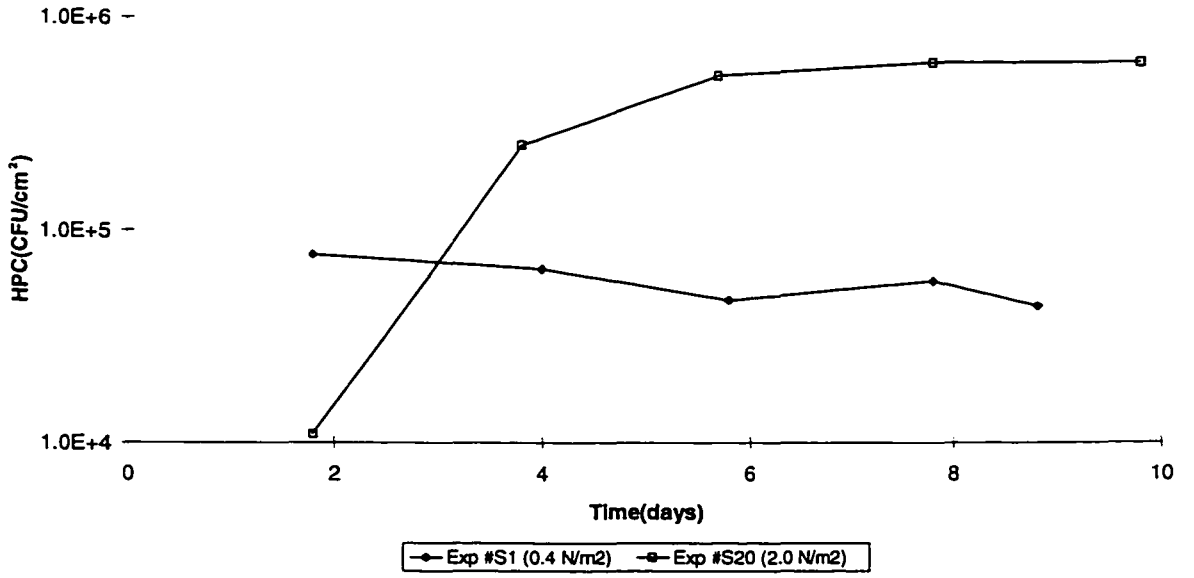


Figure C/1.8: Net accumulation of HPCs on polycarbonate substrata
Constants: 0 µg/L BOM, 0 mg/L disinfectant residual, 26 °C (ref. Figure 7.5)

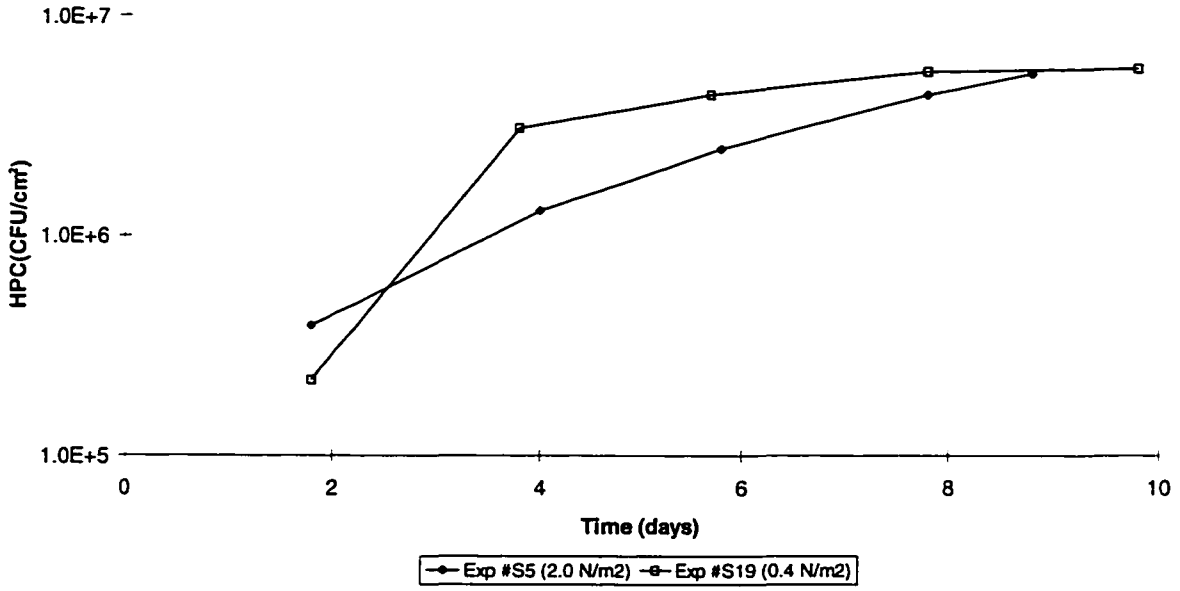


Figure C/1.9: Net accumulation of HPCs on polycarbonate substrata
Constants: 500 µg/L BOM, 0 mg/L disinfectant residual, 26 °C (ref. Figure 7.5)

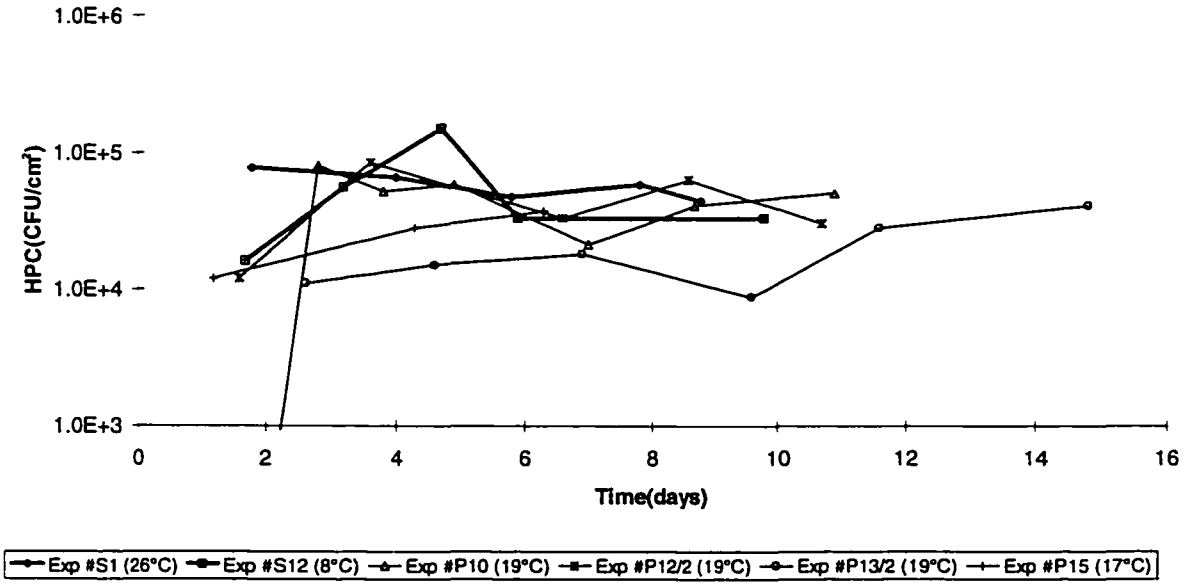


Figure C/1.10: Net accumulation of HPCs on polycarbonate substrata
Constants: 0 µg/L BOM, 0 mg/L disinfectant residual, 0.4 N/m^2 shear stress

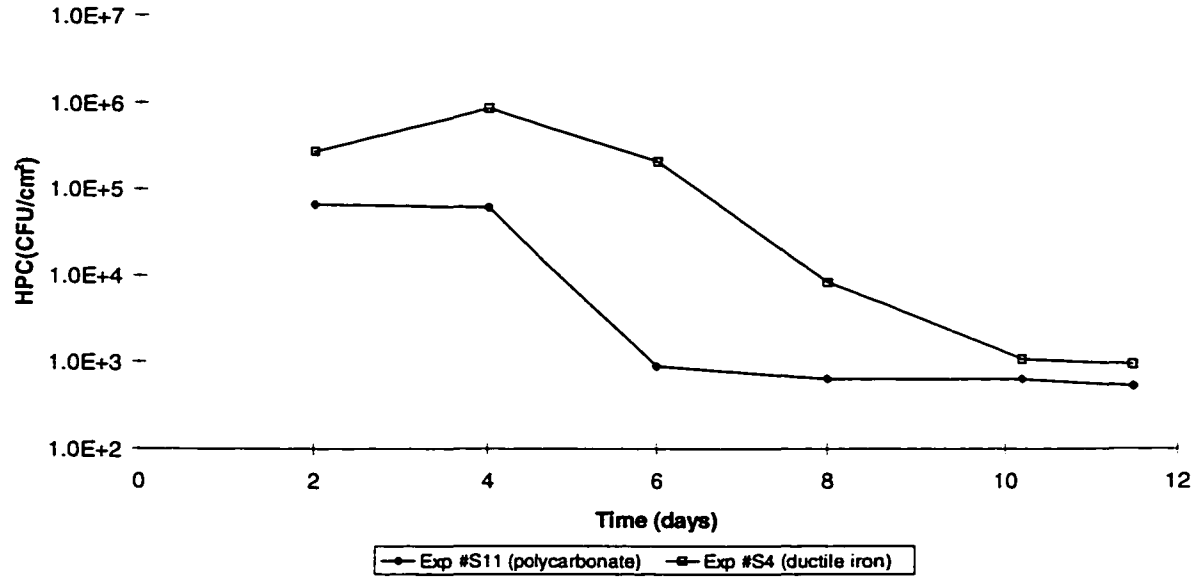


Figure C/1.11: Net accumulation of biofilm HPCs
Constants: 0 µg/L BOM supplement, 0.5 mg/L free chlorine residual, 2.0 N/m^2 shear stress, 26 °C
(ref. Figure 7.9)

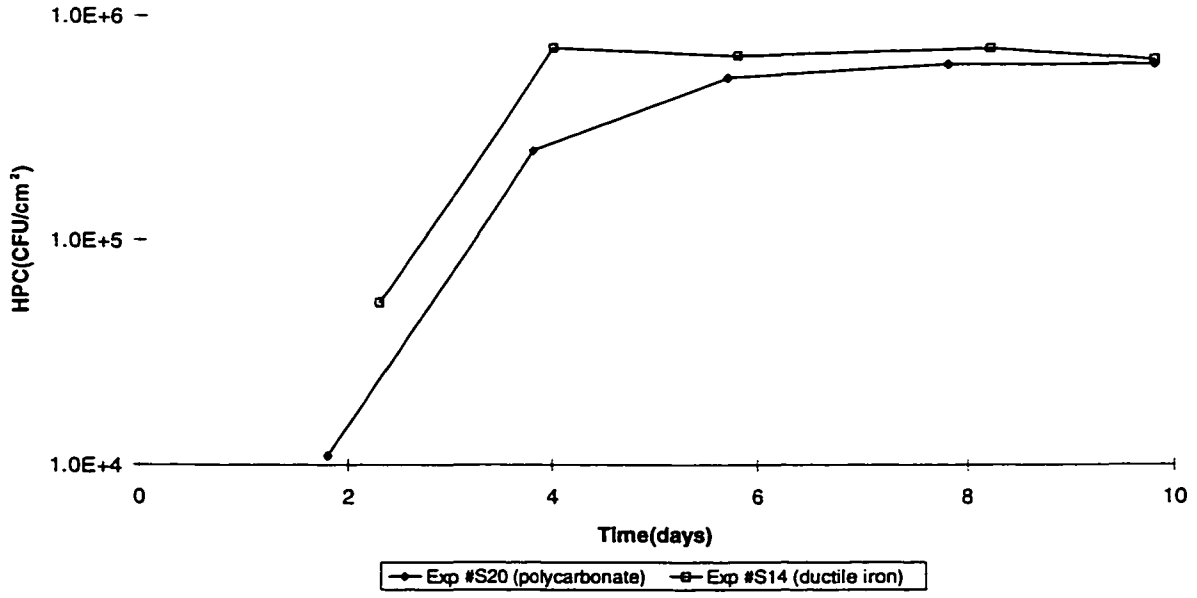


Figure C/1.12: Net accumulation of biofilm HPCs
Constants: 0 µg/L BOM, 0 mg/L disinfectant residual, 2.0 N/m^2 shear stress, 26 °C
(ref. Figure 7.9)

Appendix D: Synthetic Water Models

D/1 Linear Synthetic Water Models - Prior Data, Parameter Estimates, Covariance and Correlation Matrices, Tests

D/2 Quadratic Synthetic Water Models - Prior data, Parameter Estimates, Covariance and Correlation Matrices, Tests

D/3 Residual Plots -Synthetic Water Models

Appendix D/1: Linear Synthetic Water Models - Prior Data, Parameter Estimates, Covariance and Correlation Matrices, Tests

```
format short e
a = [5;0.8;0.7;-1.5;0.4;0.7;1.0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0]
y =
[4.799;3.041;3.839;2.973;6.740;2.724;6.903;3.204;4.987;3.519;2.724;4.519;4.724;5.826;2.699;3.114;2.903;
2.919;6.763;5.785;4.663;4.602;4.602;4.568;5.740;5.519]
u1 = [0.6990 1 1 1 1 1 1 0.0016 0.6021 0.3010 0.4771 0.0016 0.6990 0.0016 0.3010 0.6990 0.3010 0.3010
0.6990 0.3010 0.3010 0.3010 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016
0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016 0.0016]
u = diag(u1)
s=0.0189
x=[1    -1    -1    -1    -1    1    -1    1    1    1    -1    1    1
       1    -1    1    1    -1    1    -1    1    -1    -1    -1    1
       -1    -1    1    -1    1    -1    1    -1    1    -1    1    -1
       1    1    -1    1    1;
1      -1    1    1    -1    -1    -1    -1    -1    1    1    1    1
       -1    -1    -1    -1    -1    -1    1    1    1    -1    1    1
       1    1    1    1    -1    -1    -1    -1    -1    -1    1    1
       1    1    1    1    -1;
1      1    1    1    -1    1    1    1    1    -1    1    1    1
       -1    1    1    -1    1    1    -1    -1    1    1    -1    1
       1    -1    1    1    -1    -1    1    -1    1    1    -1    -1
       1    -1    -1    1    -1;
1      -1    -1    1    1    1    1    1    -1    -1    -1    -1    -1
       -1    -1    -1    1    1    1    1    1    1    1    1    1
       1    -1    -1    -1    -1    -1    -1    -1    -1    -1    -1    -1
       -1    1    1    1    1;
1      1    1    -1    1    1    -1    1    -1    1    1    -1    -1
       1    1    -1    -1    -1    1    1    -1    -1    -1    1    1
       -1    -1    -1    1    1    -1    -1    -1    -1    1    1    -1
       -1    -1    1    1    -1;
1      1    -1    1    1    -1    -1    -1    1    1    -1    -1    -1
       -1    1    1    1    -1    -1    -1    -1    1    -1    -1    1
       1    1    -1    -1    -1    -1    1    -1    1    1    1    1
       -1    -1    -1    1    1;
1      1    -1    -1    -1    -1    1    -1    -1    -1    -1    1    1
       1    1    -1    1    1    -1    1    -1    -1    1    1    1
       -1    1    1    -1    1    -1    -1    -1    -1    1    -1    1
       1    -1    1    1    1;
1      1    1    1    1    1    -1    1    1    1    1    -1    1
       1    1    -1    1    1    -1    1    -1    -1    1    1    1
       -1    1    1    -1    1    -1    -1    1    1    -1    1    -1
       -1    1    -1    -1    -1;
1      1    -1    -1    -1    -1    -1    -1    -1    -1    -1    -1    1
       1    1    1    1    1    1    1    1    1    1    1    1
       1    1    1    1    1    1    1    -1    -1    -1    -1    -1
       -1    -1    -1    -1    -1;
1      1    -1    1    -1    1    -1    -1    1    -1    1    -1    -1
       1    -1    1    -1    1    -1    -1    1    -1    -1    1    -1
       1    -1    1    -1    -1    1    -1    1    -1    1    1    -1
       1    -1    1    -1    1;
```

```
 1    -1   -1    1    1    1   -1    1   -1   -1   -1    1   -1
      -1   -1    1    1    1   -1    1   -1   -1    1    1    1
      -1   -1   -1    1   -1    1    1   -1   -1    1   -1    1
       1    1   -1   -1   -1;
 1    -1    1   -1   -1   -1   -1   -1    1    1    1    1   -1
      -1   -1   -1    1    1    1    1    1    1    1    1    1
       1   -1   -1   -1   -1   -1   -1    1    1    1    1    1
       1   -1   -1   -1   -1;
 1    -1   -1   -1    1   -1   -1    1    1   -1    1    1    1
      -1    1    1   -1    1    1   -1   -1    1   -1    1   -1
      -1    1   -1   -1    1    1   -1    1   -1   -1    1    1
      -1    1    1   -1    1;
 1    -1    1   -1    1    1    1   -1    1   -1   -1   -1   -1
       1    1    1   -1   -1   -1    1    1    1    1   -1   -1
      -1    1    1    1   -1   -1   -1   -1   -1   -1    1    1
       1   -1   -1   -1    1;
 1    -1    1    1   -1    1   -1   -1   -1    1   -1    1    1
      -1    1   -1   -1    1   -1   -1    1   -1   -1    1   -1
       1    1   -1    1    1   -1    1   -1    1   -1   -1    1
      -1   -1    1   -1    1;
 1    1    -1    1   -1   -1    1   -1    1   -1   -1    1   -1
      1     1    -1   -1   -1    1    1   -1   -1   -1    1    1
     -1    -1   -1    1    1   -1   -1    1    1   -1   -1    1
      1     1   -1   -1    1;
 1   -1    -1    1   -1   -1   -1    1   -1    1    1    1   -1
      1     1    1   -1   -1   -1    1    1    1    1   -1   -1
     -1     1    1    1   -1   -1   -1    1    1    1   -1   -1
     -1     1    1    1   -1;
 1    1     1    1    1   -1   -1    1    1    1   -1   -1    1
      1    -1   -1    1   -1   -1   -1   -1    1    1    1   -1
     -1     1   -1   -1   -1   -1    1    1   -1   -1   -1   -1
      1    -1   -1    1    1;
 1    1    -1   -1   -1    1   -1   -1   -1   -1    1   -1    1
      1    -1    1    1   -1    1   -1    1   -1    1    1   -1
      1     1   -1    1   -1    1   -1   -1    1   -1    1   -1
      1     1   -1    1    1;
 1   -1    -1   -1    1    1   -1    1    1   -1   -1    1    1
     -1    -1    1   -1   -1    1    1   -1   -1   -1    1    1
     -1     1    1   -1   -1    1    1    1    1   -1   -1    1
      1    -1    1    1   -1;
 1   -1    -1   -1   -1    0   -1    1    1    1    0    1    1
      1     0    1    1    0    1    0    1    0   -1   -1    0
     -1    -1    0   -1    0   -1    0   -1    0   -1    0   -1
      0     0   -1    0    0;
 1   -1    -1   -1   -1    0   -1    1    1    1    0    1    1
      1     0    1    1    0    1    0    1    0   -1   -1    0
     -1    -1    0   -1    0   -1    0   -1    0   -1    0   -1
      0     0   -1    0    0;
 1   -1    -1   -1   -1    0   -1    1    1    1    0    1    1
      1     0    1    1    0    1    0    1    0   -1   -1    0
     -1    -1    0   -1    0   -1    0   -1    0   -1    0   -1
      0     0   -1    0    0;
 1   -1    -1   -1   -1    0   -1    1    1    1    0    1    1
      1     0    1    1    0    1    0    1    0   -1   -1    0
```

```
        -1    -1    0     -1    0     -1    0     -1    0     -1    0     -1
        0     0     -1    0     0;
1       -1    -1    -1    0     0     -1    1     1     0     0     1     1
        0     0     1     0     0     1     0     0     0     -1    0     0
        -1    0     0     -1    0     0     0     0     0     -1    0     0
        0     0     0     0     0;
1       -1    -1    -1    1     0     -1    1     1     -1    0     1     1
        -1    0     1     -1    0     1     0     -1    0     -1    1     0
        -1    1     0     -1    0     1     0     1     0     -1    0     1
        0     0     1     0     0]
```

```
covmat = inv(inv(u)+(1/s)*x'*x)
parest = inv(inv(u)+(1/s)*x'*x)*(inv(u)*a+(1/s)*x'*y)

wk1write('a',a)
wk1write('y',y)
wk1write('u1',u1)
wk1write('u',u)
wk1write('x',x)
wk1write('covmat',covmat)
wk1write('parest',parest)
```

```
Parameter Estimate:

        1        4.567          ef         0.109
        a        0.371          abc        0.006
        b       -0.235          abd        0.001
        c       -1.578          abe       -0.015
        d       -0.057          abf        0.015
        e        0.396          acd        0.017
        f        0.354          ace       -0.015
        ab      -0.009          acf       -0.008
        ac      -0.224          ade       -0.030
        ad       0.036          adf        0.002
        ae       0.242          aef        0.003
        af       0.003          bcd        0.010
        bc       0.090          bce        0.026
        bd      -0.016          bcf        0.009
        be      -0.144          bde       -0.023
        bf      -0.145          bdf        0.010
        cd      -0.181          bef       -0.003
        ce      -0.160          cde       -0.029
        cf      -0.290          cdf        0.010
        de       0.025          cef        0.011
        df      -0.229          def       -0.004
```

Posterior Covariance Matrix - Linear Synthetic Water Model

0.0396	0.0106	0.0229	0.0088	0.0397	-0.0488	0.0456	-0.0005	-0.0135	0.0285	0.0021	-0.0002	0.0056	-0.0008
0.0106	*0.0126*	0.0023	-0.0008	0.0131	-0.0107	0.0142	0.0002	-0.0042	0.0152	-0.0021	0.0015	0.0030	-0.0010
0.0229	0.0023	*0.0295*	0.0127	0.0260	-0.0405	0.0245	0.0003	-0.0087	0.0083	0.0003	-0.0002	0.0020	-0.0002
0.0088	-0.0008	0.0127	*0.0108*	0.0137	-0.0193	0.0103	-0.0003	-0.0035	0.0023	0.0013	-0.0001	0.0025	0.0002
0.0397	0.0131	0.0260	0.0137	*0.0543*	-0.0604	0.0473	-0.0007	-0.0137	0.0285	0.0033	0.0013	0.0091	-0.0006
-0.0488	-0.0107	-0.0405	-0.0193	-0.0604	*0.0795*	-0.0566	-0.0002	0.0167	-0.0294	-0.0030	0.0004	-0.0082	0.0009
0.0456	0.0142	0.0245	0.0103	0.0473	-0.0566	*0.0579*	-0.0008	-0.0161	0.0393	0.0022	-0.0003	0.0080	-0.0009
-0.0005	0.0002	0.0003	-0.0003	-0.0007	-0.0002	-0.0008	*0.0014*	0.0004	-0.0014	-0.0002	0.0000	-0.0004	-0.0002
-0.0135	-0.0042	-0.0087	-0.0035	-0.0137	0.0167	-0.0161	0.0004	*0.0088*	-0.0116	0.0002	0.0001	-0.0020	-0.0001
0.0285	0.0152	0.0083	0.0023	0.0285	-0.0294	0.0393	-0.0014	-0.0116	*0.0382*	-0.0021	-0.0001	0.0058	-0.0006
0.0021	-0.0021	0.0003	0.0013	0.0033	-0.0030	0.0022	-0.0002	0.0002	-0.0021	*0.0055*	0.0000	0.0001	-0.0001
-0.0002	0.0015	-0.0002	-0.0001	0.0013	0.0004	-0.0003	0.0000	0.0001	-0.0001	0.0000	*0.0016*	-0.0001	0.0000
0.0056	0.0030	0.0020	0.0025	0.0091	-0.0082	0.0080	-0.0004	-0.0020	0.0058	0.0001	-0.0001	*0.0052*	-0.0002
-0.0008	-0.0010	-0.0002	0.0002	-0.0006	0.0009	-0.0009	-0.0002	-0.0001	-0.0006	-0.0001	0.0000	-0.0002	*0.0014*
-0.0188	-0.0097	-0.0076	-0.0012	-0.0175	0.0206	-0.0240	0.0006	0.0073	-0.0205	0.0012	0.0001	-0.0031	0.0001
0.0422	0.0135	0.0346	0.0153	0.0474	-0.0621	0.0531	-0.0008	-0.0172	0.0351	-0.0003	-0.0003	0.0072	-0.0007
-0.0097	-0.0088	0.0006	0.0019	-0.0115	0.0102	-0.0138	0.0004	0.0044	-0.0164	0.0025	0.0001	-0.0032	0.0001
0.0170	0.0092	0.0086	0.0024	0.0199	-0.0228	0.0220	-0.0005	-0.0070	0.0197	-0.0020	-0.0001	0.0028	0.0001
0.0160	0.0034	0.0157	0.0101	0.0208	-0.0271	0.0191	-0.0006	-0.0069	0.0116	-0.0001	-0.0001	0.0028	0.0001
-0.0023	0.0023	-0.0013	-0.0002	-0.0026	0.0035	-0.0010	-0.0002	0.0007	0.0022	-0.0011	0.0000	0.0013	-0.0002
0.0267	0.0096	0.0206	0.0114	0.0394	-0.0446	0.0311	0.0007	-0.0077	0.0150	0.0029	0.0013	0.0067	-0.0013
-0.0454	-0.0087	-0.0387	-0.0193	-0.0565	0.0743	-0.0529	-0.0001	0.0149	-0.0267	-0.0035	0.0003	-0.0075	0.0012
0.0003	0.0004	-0.0007	0.0001	0.0010	-0.0009	0.0006	-0.0001	0.0002	0.0016	-0.0002	0.0000	0.0004	-0.0001
-0.0003	-0.0001	-0.0002	-0.0001	-0.0003	0.0004	0.0012	0.0000	0.0001	0.0013	0.0000	0.0000	-0.0001	0.0000
-0.0001	-0.0004	0.0003	-0.0001	0.0006	-0.0007	-0.0001	0.0001	0.0001	-0.0007	0.0005	0.0000	0.0001	0.0001
0.0009	-0.0005	0.0004	-0.0002	0.0009	-0.0012	0.0011	0.0002	0.0000	0.0007	0.0001	0.0000	0.0002	0.0002
0.0010	0.0004	0.0006	0.0004	0.0004	-0.0010	0.0018	0.0000	-0.0002	0.0017	-0.0001	0.0000	-0.0007	0.0001
0.0006	-0.0008	0.0009	0.0007	0.0009	-0.0014	0.0000	0.0000	-0.0005	-0.0005	0.0003	0.0000	-0.0007	0.0000
-0.0002	0.0003	-0.0021	-0.0011	0.0001	0.0011	0.0000	0.0000	0.0011	0.0009	0.0001	0.0000	0.0002	0.0000
0.0002	-0.0001	-0.0002	0.0001	-0.0005	0.0009	0.0000	-0.0001	0.0002	-0.0002	0.0004	0.0000	-0.0002	-0.0001
0.0013	-0.0001	-0.0001	0.0000	-0.0003	0.0004	0.0012	0.0000	0.0001	0.0013	0.0000	0.0000	-0.0001	0.0000
0.0009	-0.0002	-0.0005	-0.0003	0.0007	-0.0005	0.0008	0.0000	0.0002	-0.0004	0.0015	0.0000	-0.0001	0.0000
0.0016	0.0004	0.0007	0.0000	0.0007	-0.0005	0.0019	-0.0001	-0.0013	0.0014	-0.0002	0.0000	0.0004	-0.0001
0.0020	0.0012	0.0007	-0.0002	0.0025	-0.0025	0.0023	0.0001	-0.0010	0.0016	-0.0002	0.0000	0.0005	0.0001
0.0002	-0.0004	0.0005	0.0010	0.0014	-0.0010	-0.0001	0.0000	0.0005	-0.0009	-0.0001	0.0000	0.0013	0.0000
-0.0004	-0.0001	-0.0003	0.0005	-0.0008	0.0003	-0.0001	0.0001	-0.0004	0.0001	-0.0010	0.0000	0.0003	0.0001
0.0003	0.0013	-0.0004	0.0002	0.0005	0.0005	0.0006	0.0002	-0.0003	0.0013	0.0001	0.0000	0.0004	0.0002
-0.0009	0.0002	-0.0011	0.0002	0.0008	0.0006	-0.0007	0.0000	-0.0003	0.0005	0.0000	0.0000	0.0001	0.0000
-0.0001	0.0013	-0.0003	-0.0015	-0.0008	0.0012	-0.0004	0.0000	0.0003	0.0009	-0.0011	0.0000	-0.0003	-0.0001
0.0003	-0.0003	0.0021	0.0011	0.0000	-0.0012	0.0001	0.0000	0.0004	-0.0008	-0.0001	0.0000	-0.0002	0.0000
-0.0003	-0.0005	0.0016	0.0008	0.0008	-0.0017	-0.0007	0.0000	0.0007	-0.0013	-0.0001	0.0000	-0.0004	0.0000
-0.0009	0.0002	0.0005	0.0003	-0.0007	0.0005	-0.0008	0.0000	-0.0002	0.0004	0.0001	0.0000	0.0001	0.0000

Square root of the diagonal elements:

0.19897	0.11234	0.17171	0.10406	0.23294	0.28187	0.2406	0.03765	0.09361	0.19537	0.07402	0.03985	0.07213	0.03721

-0.0188	0.0422	-0.0097	0.0170	0.0160	-0.0023	0.0267	-0.0454	0.0003	-0.0003	-0.0001	0.0009	0.0010	0.0006
-0.0097	0.0135	-0.0088	0.0092	0.0034	0.0023	0.0096	-0.0087	0.0004	-0.0001	-0.0004	-0.0005	0.0004	-0.0008
-0.0076	0.0346	0.0006	0.0086	0.0157	-0.0013	0.0206	-0.0387	-0.0007	-0.0002	0.0003	0.0004	0.0006	0.0009
-0.0012	0.0153	0.0019	0.0024	0.0101	-0.0002	0.0114	-0.0193	0.0001	-0.0001	-0.0001	-0.0002	0.0004	0.0007
-0.0175	0.0474	-0.0115	0.0199	0.0208	-0.0026	0.0394	-0.0565	0.0010	-0.0003	0.0006	0.0009	0.0004	0.0009
0.0206	-0.0621	0.0102	-0.0228	-0.0271	0.0035	-0.0446	0.0743	-0.0009	0.0004	-0.0007	-0.0012	-0.0010	-0.0014
-0.0240	0.0531	-0.0138	0.0220	0.0191	-0.0010	0.0311	-0.0529	0.0006	0.0012	-0.0001	0.0011	0.0018	0.0000
0.0006	-0.0008	0.0004	-0.0005	-0.0006	-0.0002	0.0007	-0.0001	-0.0001	0.0000	0.0001	0.0002	0.0000	0.0000
0.0073	-0.0172	0.0044	-0.0070	-0.0069	0.0007	-0.0077	0.0149	0.0002	0.0001	0.0001	0.0000	-0.0002	-0.0005
-0.0205	0.0351	-0.0164	0.0197	0.0116	0.0022	0.0150	-0.0267	0.0016	0.0013	-0.0007	0.0007	0.0017	-0.0005
0.0012	-0.0003	0.0025	-0.0020	-0.0001	-0.0011	0.0029	-0.0035	-0.0002	0.0000	0.0005	0.0001	-0.0001	0.0003
0.0001	-0.0003	0.0001	-0.0001	-0.0001	0.0000	0.0013	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0031	0.0072	-0.0032	0.0028	0.0028	0.0013	0.0067	-0.0075	0.0004	-0.0001	0.0001	0.0002	-0.0007	-0.0007
0.0001	-0.0007	0.0001	0.0001	0.0001	-0.0002	-0.0013	0.0012	-0.0001	0.0000	0.0001	0.0002	0.0001	0.0000
0.0166	-0.0228	0.0104	-0.0126	-0.0065	-0.0016	-0.0090	0.0175	-0.0005	0.0002	0.0005	-0.0001	-0.0012	0.0005
-0.0228	*0.0618*	-0.0113	0.0225	0.0235	0.0013	0.0321	-0.0583	0.0005	0.0012	-0.0001	0.0009	0.0016	-0.0001
0.0104	-0.0113	*0.0127*	-0.0103	-0.0025	-0.0016	-0.0060	0.0079	-0.0015	0.0001	0.0000	-0.0001	-0.0004	0.0009
-0.0126	0.0225	-0.0103	*0.0150*	0.0082	0.0011	0.0119	-0.0201	0.0005	-0.0002	-0.0003	0.0000	0.0012	-0.0002
-0.0065	0.0235	-0.0025	0.0082	*0.0144*	-0.0006	0.0148	-0.0264	0.0005	-0.0001	-0.0004	0.0000	0.0013	0.0012
-0.0016	0.0013	-0.0016	0.0011	-0.0006	*0.0055*	-0.0024	0.0034	-0.0001	0.0000	-0.0010	0.0002	0.0001	-0.0011
-0.0090	0.0321	-0.0060	0.0119	0.0148	-0.0024	*0.0339*	-0.0424	0.0006	-0.0002	0.0011	-0.0001	0.0001	0.0003
0.0175	-0.0583	0.0079	-0.0201	-0.0264	0.0034	-0.0424	*0.0721*	-0.0011	0.0004	-0.0007	-0.0014	-0.0013	-0.0014
-0.0005	0.0005	-0.0015	0.0005	0.0005	-0.0001	0.0006	-0.0011	*0.0014*	0.0000	0.0001	0.0001	-0.0001	-0.0001
0.0002	0.0012	0.0001	-0.0002	-0.0001	0.0000	-0.0002	0.0004	0.0000	*0.0016*	0.0000	0.0000	0.0000	0.0000
0.0005	-0.0001	0.0000	-0.0003	-0.0004	-0.0010	0.0011	-0.0007	0.0001	0.0000	*0.0013*	-0.0001	0.0000	-0.0001
-0.0001	0.0009	-0.0001	0.0000	0.0000	0.0002	-0.0001	-0.0014	0.0001	0.0000	-0.0001	*0.0014*	-0.0001	0.0000
-0.0012	0.0016	-0.0004	0.0012	0.0013	0.0001	0.0001	-0.0013	-0.0001	0.0000	0.0000	-0.0001	*0.0015*	0.0000
0.0005	-0.0001	0.0009	-0.0002	0.0012	-0.0011	0.0003	-0.0014	-0.0001	0.0000	-0.0001	0.0000	0.0000	*0.0014*
-0.0006	-0.0014	-0.0003	0.0006	-0.0008	-0.0001	-0.0004	0.0012	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0007	0.0001	0.0003	-0.0005	-0.0006	0.0003	-0.0005	0.0011	0.0000	0.0000	-0.0001	0.0001	0.0000	-0.0001
0.0002	-0.0004	0.0001	-0.0002	-0.0001	0.0000	-0.0002	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0003	-0.0008	0.0001	-0.0003	-0.0004	0.0001	0.0009	-0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0003	0.0018	0.0002	0.0004	0.0004	-0.0001	0.0004	-0.0007	-0.0002	0.0000	0.0001	0.0001	-0.0001	-0.0001
-0.0017	0.0020	-0.0013	0.0016	0.0001	0.0001	0.0014	-0.0015	-0.0001	0.0000	0.0000	-0.0001	-0.0002	-0.0001
0.0006	-0.0002	0.0003	-0.0006	0.0008	0.0001	0.0018	-0.0011	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0001	0.0000	-0.0003	0.0003	0.0003	0.0005	-0.0008	0.0005	0.0001	0.0000	-0.0002	-0.0001	0.0000	-0.0001
-0.0005	0.0006	-0.0003	0.0004	0.0005	0.0002	0.0007	0.0003	0.0001	0.0000	-0.0001	-0.0002	0.0000	0.0000
0.0012	-0.0007	-0.0002	0.0003	0.0004	0.0000	0.0006	0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0005	-0.0002	-0.0011	0.0008	-0.0007	0.0003	-0.0006	0.0014	0.0000	0.0000	-0.0001	0.0001	0.0000	-0.0001
0.0005	0.0015	0.0018	-0.0006	0.0009	0.0001	0.0004	-0.0013	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0008	0.0007	0.0004	0.0007	0.0005	0.0001	0.0014	-0.0019	0.0000	0.0000	0.0000	0.0000	-0.0001	0.0000
-0.0003	0.0008	-0.0001	0.0003	0.0004	0.0015	-0.0009	0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

0.12888	0.24852	0.11263	0.12248	0.11991	0.07405	0.18425	0.26857	0.03693	0.03986	0.03662	0.0373	0.03829	0.03731

-0.0002	0.0002	0.0013	0.0009	0.0016	0.0020	0.0002	-0.0004	0.0003	-0.0009	-0.0001	0.0003	-0.0003	-0.0009
0.0003	-0.0001	-0.0001	-0.0002	0.0004	0.0012	-0.0004	-0.0001	0.0013	0.0002	0.0013	-0.0003	-0.0005	0.0002
-0.0021	-0.0002	-0.0001	-0.0005	0.0007	0.0007	0.0005	-0.0003	-0.0004	-0.0011	-0.0003	0.0021	0.0016	0.0005
-0.0011	0.0001	0.0000	-0.0003	0.0000	-0.0002	0.0010	0.0005	0.0002	0.0002	-0.0015	0.0011	0.0008	0.0003
0.0001	-0.0005	-0.0003	0.0007	0.0007	0.0025	0.0014	-0.0008	0.0005	0.0008	-0.0008	0.0000	0.0008	-0.0007
0.0011	0.0009	0.0004	-0.0005	-0.0005	-0.0025	-0.0010	0.0003	0.0005	0.0006	0.0012	-0.0012	-0.0017	0.0005
0.0000	0.0000	0.0012	0.0008	0.0019	0.0023	-0.0001	-0.0001	0.0006	-0.0007	-0.0004	0.0001	-0.0007	-0.0008
0.0000	-0.0001	0.0000	0.0000	-0.0001	0.0001	0.0000	0.0001	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000
0.0011	0.0002	0.0001	0.0002	-0.0013	-0.0010	0.0005	-0.0004	-0.0003	-0.0003	0.0003	0.0004	0.0007	-0.0002
0.0009	-0.0002	0.0013	-0.0004	0.0014	0.0016	-0.0009	0.0001	0.0013	0.0005	0.0009	-0.0008	-0.0013	0.0004
0.0001	0.0004	0.0000	0.0015	-0.0002	-0.0002	-0.0001	-0.0010	0.0001	0.0000	-0.0011	-0.0001	-0.0001	0.0001
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0002	-0.0002	-0.0001	-0.0001	0.0004	0.0005	0.0013	0.0003	0.0004	0.0001	-0.0003	-0.0002	-0.0004	0.0001
0.0000	-0.0001	0.0000	0.0000	-0.0001	0.0001	0.0000	0.0001	0.0002	0.0000	-0.0001	0.0000	0.0000	0.0000
-0.0006	-0.0007	0.0002	0.0003	-0.0003	-0.0017	0.0006	-0.0001	-0.0005	0.0012	-0.0005	0.0005	0.0008	-0.0003
-0.0014	0.0001	-0.0004	-0.0008	0.0018	0.0020	-0.0002	0.0000	0.0006	-0.0007	-0.0002	0.0015	0.0007	0.0008
-0.0003	0.0003	0.0001	0.0001	0.0002	-0.0013	0.0003	-0.0003	-0.0003	-0.0002	-0.0011	0.0018	0.0004	-0.0001
0.0006	-0.0005	-0.0002	-0.0003	0.0004	0.0016	-0.0006	0.0003	0.0004	0.0003	0.0008	-0.0006	0.0007	0.0003
-0.0008	-0.0006	-0.0001	-0.0004	0.0004	0.0001	0.0008	0.0003	0.0005	0.0004	-0.0007	0.0009	0.0005	0.0004
-0.0001	0.0003	0.0000	0.0001	-0.0001	0.0001	0.0001	0.0005	0.0002	0.0000	0.0003	0.0001	0.0001	0.0015
-0.0004	-0.0005	-0.0002	0.0009	0.0004	0.0014	0.0018	-0.0008	0.0007	0.0006	-0.0006	0.0004	0.0014	-0.0009
0.0012	0.0011	0.0003	-0.0006	-0.0007	-0.0015	-0.0011	0.0005	0.0003	0.0006	0.0014	-0.0013	-0.0019	0.0006
0.0000	0.0000	0.0000	0.0000	-0.0002	-0.0001	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	-0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	-0.0002	-0.0001	0.0000	-0.0001	0.0000	0.0000	0.0000
0.0000	0.0001	0.0000	0.0000	0.0001	-0.0001	0.0000	-0.0001	-0.0002	0.0000	0.0001	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	-0.0001	-0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	-0.0001	0.0000
0.0000	-0.0001	0.0000	0.0000	-0.0001	-0.0001	0.0000	-0.0001	0.0000	0.0000	-0.0001	0.0000	0.0000	0.0000
0.0016	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	*0.0014*	0.0000	0.0000	0.0000	0.0001	0.0000	-0.0001	0.0001	0.0000	-0.0002	0.0000	0.0000	0.0000
0.0000	0.0000	*0.0016*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	*0.0016*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	*0.0014*	-0.0002	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
0.0001	0.0001	0.0000	0.0000	-0.0002	*0.0013*	-0.0001	0.0000	-0.0001	0.0000	0.0000	0.0000	-0.0001	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	-0.0001	*0.0016*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	-0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	*0.0013*	-0.0001	0.0000	-0.0001	0.0000	0.0000	0.0000
0.0000	0.0001	0.0000	0.0000	0.0001	-0.0001	0.0000	-0.0001	*0.0014*	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0016*	0.0000	0.0000	0.0000	0.0000
0.0000	-0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	-0.0001	0.0000	0.0000	*0.0014*	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0016*	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	-0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0015*	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0016*

0.03952	0.03752	0.03985	0.03977	0.03681	0.03581	0.03942	0.03669	0.03763	0.03978	0.0373	0.03941	0.03914	0.03973

Posterior Correlation Matrix - Linear Synthetic Water Model

0.9997	0.4723	0.6696	0.4256	0.8573	-0.8700	0.9527	-0.0654	-0.7248	0.7323	0.1443	-0.0280	0.3920	-0.1068
0.4723	*1.0007*	0.1210	-0.0725	0.5003	-0.3383	0.5238	0.0496	-0.4020	0.6942	-0.2481	0.3365	0.3656	-0.2485
0.6696	0.1210	*1.0001*	0.7121	0.6508	-0.8363	0.5931	0.0510	-0.5413	0.2475	0.0224	-0.0226	0.1606	-0.0386
0.4256	-0.0725	0.7121	*0.9993*	0.5642	-0.6570	0.4099	-0.0724	-0.3641	0.1147	0.1731	-0.0195	0.3370	0.0560
0.8573	0.5003	0.6508	0.5642	*1.0004*	-0.9195	0.8441	-0.0781	-0.6270	0.6263	0.1924	0.1381	0.5440	-0.0749
-0.8700	-0.3383	-0.8363	-0.6570	-0.9195	*0.9998*	-0.8344	-0.0201	0.6340	-0.5346	-0.1423	0.0312	-0.4029	0.0902
0.9527	0.5238	0.5931	0.4099	0.8441	-0.8344	*1.0000*	-0.0891	-0.7171	0.8351	0.1260	-0.0274	0.4629	-0.0981
-0.0654	0.0496	0.0510	-0.0724	-0.0781	-0.0201	-0.0891	*0.9975*	0.1022	-0.1934	-0.0546	-0.0019	-0.1448	-0.1270
-0.7248	-0.4020	-0.5413	-0.3641	-0.6270	0.6340	-0.7171	0.1022	*1.0003*	-0.6348	0.0358	0.0186	-0.3017	-0.0207
0.7323	0.6942	0.2475	0.1147	0.6263	-0.5346	0.8351	-0.1934	-0.6348	*0.9997*	-0.1426	-0.0192	0.4084	-0.0819
0.1443	-0.2481	0.0224	0.1731	0.1924	-0.1423	0.1260	-0.0546	0.0358	-0.1426	*1.0006*	-0.0059	0.0162	-0.0235
-0.0280	0.3365	-0.0226	-0.0195	0.1381	0.0312	-0.0274	-0.0019	0.0186	-0.0192	-0.0059	*0.9977*	-0.0192	0.0064
0.3920	0.3656	0.1606	0.3370	0.5440	-0.4029	0.4629	-0.1448	-0.3017	0.4084	0.0162	-0.0192	*1.0008*	-0.0562
-0.1068	-0.2485	-0.0386	0.0560	-0.0749	0.0902	-0.0981	-0.1270	-0.0207	-0.0819	-0.0235	0.0064	-0.0562	*1.0006*
-0.7322	-0.6673	-0.3451	-0.0915	-0.5822	0.5663	-0.7743	0.1228	0.6055	-0.8126	0.1226	0.0177	-0.3377	0.0125
0.8543	0.4847	0.8098	0.5898	0.8185	-0.8862	0.8878	-0.0830	-0.7392	0.7224	-0.0188	-0.0271	0.4027	-0.0802
-0.4326	-0.6938	0.0326	0.1650	-0.4379	0.3218	-0.5085	0.0877	0.4141	-0.7454	0.2979	0.0144	-0.3990	0.0213
0.6979	0.6714	0.4110	0.1917	0.6965	-0.6596	0.7452	-0.1128	-0.6074	0.8232	-0.2199	-0.0225	0.3209	0.0173
0.6726	0.2519	0.7616	0.8115	0.7445	-0.8018	0.6613	-0.1388	-0.6148	0.4950	-0.0083	-0.0245	0.3186	0.0184
-0.1577	0.2786	-0.0987	-0.0317	-0.1519	0.1654	-0.0575	-0.0549	0.0940	0.1539	-0.1939	0.0045	0.2461	-0.0845
0.7286	0.4656	0.6516	0.5933	0.9174	-0.8581	0.7008	0.0979	-0.4450	0.4163	0.2112	0.1824	0.5069	-0.1852
-0.8494	-0.2892	-0.8401	-0.6920	-0.9026	0.9815	-0.8184	-0.0066	0.5946	-0.5094	-0.1767	0.0308	-0.3898	0.1190
0.0359	0.0986	-0.1077	0.0366	0.1152	-0.0868	0.0682	-0.0876	0.0692	0.2160	-0.0799	-0.0038	0.1674	-0.0702
-0.0404	-0.0300	-0.0237	-0.0154	-0.0361	0.0346	0.1226	0.0071	0.0340	0.1608	0.0028	0.0012	-0.0208	0.0042
-0.0174	-0.1021	0.0415	-0.0148	0.0761	-0.0682	-0.0070	0.0798	0.0172	-0.0962	0.1790	-0.0043	0.0191	0.0965
0.1279	-0.1171	0.0561	-0.0406	0.1012	-0.1141	0.1188	0.1295	0.0071	0.0960	0.0278	0.0001	0.0711	0.1491
0.1308	0.0815	0.0954	0.1089	0.0397	-0.0881	0.1899	0.0335	-0.0611	0.2295	-0.0300	-0.0011	-0.2499	0.0541
0.0867	-0.1971	0.1386	0.1719	0.1093	-0.1315	-0.0054	-0.0271	-0.1313	-0.0712	0.1075	-0.0012	-0.2534	-0.0097
-0.0304	0.0751	-0.3073	-0.2561	0.0068	0.0960	0.0006	-0.0026	0.3021	0.1140	0.0262	0.0008	0.0835	-0.0205
0.0224	-0.0238	-0.0370	0.0186	-0.0603	0.0805	-0.0013	-0.0371	0.0532	-0.0326	0.1580	0.0024	-0.0745	-0.0520
0.1618	-0.0285	-0.0198	-0.0118	-0.0342	0.0319	0.1244	0.0066	0.0317	0.1628	0.0008	0.0011	-0.0196	0.0043
0.1142	-0.0393	-0.0753	-0.0621	0.0787	-0.0474	0.0818	-0.0002	0.0603	-0.0560	0.5192	-0.0037	-0.0452	0.0129
0.2161	0.0877	0.1065	0.0076	0.0815	-0.0486	0.2121	-0.0859	-0.3632	0.1936	-0.0877	-0.0027	0.1551	-0.0670
0.2792	0.3023	0.1187	-0.0670	0.3004	-0.2518	0.2638	0.0501	-0.3077	0.2278	-0.0582	-0.0094	0.1881	0.0950
0.0241	-0.0827	0.0706	0.2510	0.1534	-0.0871	-0.0065	0.0000	0.1285	-0.1161	-0.0337	-0.0073	0.4664	0.0248
-0.0493	-0.0209	-0.0428	0.1239	-0.0964	0.0336	-0.0074	0.0818	-0.1140	0.0201	-0.3664	0.0038	0.1121	0.0702
0.0443	0.3123	-0.0687	0.0574	0.0520	0.0441	0.0686	0.1258	-0.0889	0.1797	0.0504	-0.0045	0.1302	0.1333
-0.1116	0.0439	-0.1567	0.0560	0.0870	0.0499	-0.0776	-0.0041	-0.0685	0.0668	0.0086	-0.0029	0.0402	-0.0091
-0.0164	0.3091	-0.0480	-0.3913	-0.0959	0.1103	-0.0456	-0.0295	0.0889	0.1288	-0.3996	0.0018	-0.0984	-0.0487
0.0416	-0.0622	0.3110	0.2565	0.0038	-0.1051	0.0116	0.0006	0.1184	-0.0990	-0.0310	-0.0012	-0.0757	0.0204
-0.0438	-0.1154	0.2441	0.2048	0.0852	-0.1575	-0.0736	0.0017	0.1819	-0.1660	-0.0382	-0.0050	-0.1245	0.0313
-0.1138	0.0382	0.0763	0.0615	-0.0786	0.0468	-0.0822	0.0011	-0.0617	0.0555	0.0198	0.0037	0.0428	-0.0119

-0.7322	0.8543	-0.4326	0.6979	0.6726	-0.1577	0.7286	-0.8494	0.0359	-0.0404	-0.0174	0.1279	0.1308	0.0867
-0.6673	0.4847	-0.6938	0.6714	0.2519	0.2786	0.4656	-0.2892	0.0986	-0.0300	-0.1021	-0.1171	0.0815	-0.1971
-0.3451	0.8098	0.0326	0.4110	0.7616	-0.0987	0.6516	-0.8401	-0.1077	-0.0237	0.0415	0.0561	0.0954	0.1386
-0.0915	0.5898	0.1650	0.1917	0.8115	-0.0317	0.5933	-0.6920	0.0366	-0.0154	-0.0148	-0.0406	0.1089	0.1719
-0.5822	0.8185	-0.4379	0.6965	0.7445	-0.1519	0.9174	-0.9026	0.1152	-0.0361	0.0761	0.1012	0.0397	0.1093
0.5663	-0.8862	0.3218	-0.6596	-0.8018	0.1654	-0.8581	0.9815	-0.0868	0.0346	-0.0682	-0.1141	-0.0881	-0.1315
-0.7743	0.8878	-0.5085	0.7452	0.6613	-0.0575	0.7008	-0.8184	0.0682	0.1226	-0.0070	0.1188	0.1899	-0.0054
0.1228	-0.0830	0.0877	-0.1128	-0.1388	-0.0549	0.0979	-0.0066	-0.0876	0.0071	0.0798	0.1295	0.0335	-0.0271
0.6055	-0.7392	0.4141	-0.6074	-0.6148	0.0940	-0.4450	0.5946	0.0692	0.0340	0.0172	0.0071	-0.0611	-0.1313
-0.8126	0.7224	-0.7454	0.8232	0.4950	0.1539	0.4163	-0.5094	0.2160	0.1608	-0.0962	0.0960	0.2295	-0.0712
0.1226	-0.0188	0.2979	-0.2199	-0.0083	-0.1939	0.2112	-0.1767	-0.0799	0.0028	0.1790	0.0278	-0.0300	0.1075
0.0177	-0.0271	0.0144	-0.0225	-0.0245	0.0045	0.1824	0.0308	-0.0038	0.0012	-0.0043	0.0001	-0.0011	-0.0012
-0.3377	0.4027	-0.3990	0.3209	0.3186	0.2461	0.5069	-0.3898	0.1674	-0.0208	0.0191	0.0711	-0.2499	-0.2534
0.0125	-0.0802	0.0213	0.0173	0.0184	-0.0845	-0.1852	0.1190	-0.0702	0.0042	0.0965	0.1491	0.0541	-0.0097
0.9997	-0.7106	0.7154	-0.7973	-0.4193	-0.1678	-0.3790	0.5055	-0.0965	0.0388	0.0978	-0.0256	-0.2517	0.0975
-0.7106	*1.0001*	-0.4028	0.7399	0.7873	0.0682	0.7017	-0.8728	0.0576	0.1197	-0.0072	0.1008	0.1670	-0.0113
0.7154	-0.4028	*1.0006*	-0.7491	-0.1870	-0.1907	-0.2912	0.2626	-0.3498	0.0301	-0.0102	-0.0323	-0.0944	0.2209
-0.7973	0.7399	-0.7491	*0.9997*	0.5573	0.1200	0.5288	-0.6114	0.1041	-0.0392	-0.0629	-0.0001	0.2506	-0.0513
-0.4193	0.7873	-0.1870	0.5573	*1.0001*	-0.0635	0.6702	-0.8196	0.1206	-0.0305	-0.0942	0.0005	0.2724	0.2586
-0.1678	0.0682	-0.1907	0.1200	-0.0635	*0.9987*	-0.1777	0.1709	-0.0359	-0.0044	-0.3630	0.0810	0.0269	-0.3999
-0.3790	0.7017	-0.2912	0.5288	0.6702	-0.1777	*0.9995*	-0.8574	0.0922	-0.0276	0.1638	-0.0196	0.0147	0.0416
0.5055	-0.8728	0.2626	-0.6114	-0.8196	0.1709	-0.8574	*0.9998*	-0.1133	0.0336	-0.0666	-0.1426	-0.1251	-0.1352
-0.0965	0.0576	-0.3498	0.1041	0.1206	-0.0359	0.0922	-0.1133	*1.0017*	-0.0071	0.0756	0.0729	-0.0669	-0.0478
0.0388	0.1197	0.0301	-0.0392	-0.0305	-0.0044	-0.0276	0.0336	-0.0071	*0.9979*	0.0027	-0.0051	-0.0102	0.0021
0.0978	-0.0072	-0.0102	-0.0629	-0.0942	-0.3630	0.1638	-0.0666	0.0756	0.0027	*1.0011*	-0.0924	-0.0077	-0.1069
-0.0256	0.1008	-0.0323	-0.0001	0.0005	0.0810	-0.0196	-0.1426	0.0729	-0.0051	-0.0924	*1.0002*	-0.0532	0.0098
-0.2517	0.1670	-0.0944	0.2506	0.2724	0.0269	0.0147	-0.1251	-0.0669	-0.0102	-0.0077	-0.0532	*0.9996*	-0.0258
0.0975	-0.0113	0.2209	-0.0513	0.2586	-0.3999	0.0416	-0.1352	-0.0478	0.0021	-0.1069	0.0098	-0.0258	*1.0005*
-0.1127	-0.1464	-0.0620	0.1298	-0.1749	-0.0249	-0.0503	0.1102	0.0153	-0.0009	0.0104	0.0195	0.0240	0.0207
-0.1498	0.0144	0.0668	-0.1134	-0.1225	0.1200	-0.0693	0.1080	-0.0057	0.0007	-0.0838	0.0500	0.0285	-0.1059
0.0370	-0.0371	0.0292	-0.0366	-0.0269	-0.0017	-0.0259	0.0309	-0.0067	-0.0049	0.0028	-0.0051	-0.0093	0.0009
0.0544	-0.0845	0.0323	-0.0628	-0.0760	0.0174	0.1280	-0.0544	-0.0090	0.0019	-0.0088	-0.0097	-0.0094	-0.0100
-0.0736	0.1930	0.0385	0.0816	0.0867	-0.0300	0.0621	-0.0751	-0.1689	-0.0098	0.0761	0.0689	-0.0729	-0.0543
-0.3765	0.2283	-0.3268	0.3692	0.0309	0.0520	0.2141	-0.1539	-0.1126	-0.0117	-0.0208	-0.0876	-0.1436	-0.0420
0.1129	-0.0188	0.0670	-0.1338	0.1709	0.0295	0.2526	-0.1012	-0.0198	0.0034	-0.0153	-0.0185	-0.0203	-0.0202
-0.0160	0.0018	-0.0756	0.0717	0.0743	0.1821	-0.1145	0.0502	0.0947	-0.0005	-0.1706	-0.0731	0.0122	-0.0815
-0.1100	0.0626	-0.0770	0.0961	0.1205	0.0583	0.1045	0.0303	0.0849	-0.0062	-0.0838	-0.1287	-0.0344	0.0270
0.2417	-0.0699	-0.0408	0.0717	0.0763	-0.0072	0.0766	0.0581	0.0079	0.0000	0.0014	0.0113	0.0162	0.0064
-0.0990	-0.0248	-0.2578	0.1799	-0.1473	0.1084	-0.0934	0.1348	-0.0201	-0.0026	-0.0790	0.0476	0.0162	-0.0976
0.0983	0.1575	0.4050	-0.1149	0.1821	0.0273	0.0577	-0.1182	-0.0105	0.0002	-0.0104	-0.0191	-0.0223	-0.0233
0.1634	0.0728	0.0972	0.1403	0.1083	0.0366	0.1919	-0.1794	-0.0262	0.0040	-0.0176	-0.0267	-0.0344	-0.0278
-0.0537	0.0842	-0.0324	0.0631	0.0769	0.5178	-0.1279	0.0539	0.0098	-0.0019	0.0113	0.0087	0.0093	0.0133

-0.0304	0.0224	0.1618	0.1142	0.2161	0.2792	0.0241	-0.0493	0.0443	-0.1116	-0.0164	0.0416	-0.0438	-0.1138
0.0751	-0.0238	-0.0285	-0.0393	0.0877	0.3023	-0.0827	-0.0209	0.3123	0.0439	0.3091	-0.0622	-0.1154	0.0382
-0.3073	-0.0370	-0.0198	-0.0753	0.1065	0.1187	0.0706	-0.0428	-0.0687	-0.1567	-0.0480	0.3110	0.2441	0.0763
-0.2561	0.0186	-0.0118	-0.0621	0.0076	-0.0670	0.2510	0.1239	0.0574	0.0560	-0.3913	0.2565	0.2048	0.0615
0.0068	-0.0603	-0.0342	0.0787	0.0815	0.3004	0.1534	-0.0964	0.0520	0.0870	-0.0959	0.0038	0.0852	-0.0786
0.0960	0.0805	0.0319	-0.0474	-0.0486	-0.2518	-0.0871	0.0336	0.0441	0.0499	0.1103	-0.1051	-0.1575	0.0468
0.0006	-0.0013	0.1244	0.0818	0.2121	0.2638	-0.0065	-0.0074	0.0686	-0.0776	-0.0456	0.0116	-0.0736	-0.0822
-0.0026	-0.0371	0.0066	-0.0002	-0.0859	0.0501	0.0000	0.0818	0.1258	-0.0041	-0.0295	0.0006	0.0017	0.0011
0.3021	0.0532	0.0317	0.0603	-0.3632	-0.3077	0.1285	-0.1140	-0.0889	-0.0685	0.0889	0.1184	0.1819	-0.0617
0.1140	-0.0326	0.1628	-0.0560	0.1936	0.2278	-0.1161	0.0201	0.1797	0.0668	0.1288	-0.0990	-0.1660	0.0555
0.0262	0.1580	0.0008	0.5192	-0.0877	-0.0582	-0.0337	-0.3664	0.0504	0.0086	-0.3996	-0.0310	-0.0382	0.0198
0.0008	0.0024	0.0011	-0.0037	-0.0027	-0.0094	-0.0073	0.0038	-0.0045	-0.0029	0.0018	-0.0012	-0.0050	0.0037
0.0835	-0.0745	-0.0196	-0.0452	0.1551	0.1881	0.4664	0.1121	0.1302	0.0402	-0.0984	-0.0757	-0.1245	0.0428
-0.0205	-0.0520	0.0043	0.0129	-0.0670	0.0950	0.0248	0.0702	0.1333	-0.0091	-0.0487	0.0204	0.0313	-0.0119
-0.1127	-0.1498	0.0370	0.0544	-0.0736	-0.3765	0.1129	-0.0160	-0.1100	0.2417	-0.0990	0.0983	0.1634	-0.0537
-0.1464	0.0144	-0.0371	-0.0845	0.1930	0.2283	-0.0188	0.0018	0.0626	-0.0699	-0.0248	0.1575	0.0728	0.0842
-0.0620	0.0668	0.0292	0.0323	0.0385	-0.3268	0.0670	-0.0756	-0.0770	-0.0408	-0.2578	0.4050	0.0972	-0.0324
0.1298	-0.1134	-0.0366	-0.0628	0.0816	0.3692	-0.1338	0.0717	0.0961	0.0717	0.1799	-0.1149	0.1403	0.0631
-0.1749	-0.1225	-0.0269	-0.0760	0.0867	0.0309	0.1709	0.0743	0.1205	0.0763	-0.1473	0.1821	0.1083	0.0769
-0.0249	0.1200	-0.0017	0.0174	-0.0300	0.0520	0.0295	0.1821	0.0583	-0.0072	0.1084	0.0273	0.0366	0.5178
-0.0503	-0.0693	-0.0259	0.1280	0.0621	0.2141	0.2526	-0.1145	0.1045	0.0766	-0.0934	0.0577	0.1919	-0.1279
0.1102	0.1080	0.0309	-0.0544	-0.0751	-0.1539	-0.1012	0.0502	0.0303	0.0581	0.1348	-0.1182	-0.1794	0.0539
0.0153	-0.0057	-0.0067	-0.0090	-0.1689	-0.1126	-0.0198	0.0947	0.0849	0.0079	-0.0201	-0.0105	-0.0262	0.0098
-0.0009	0.0007	-0.0049	0.0019	-0.0098	-0.0117	0.0034	-0.0005	-0.0062	0.0000	-0.0026	0.0002	0.0040	-0.0019
0.0104	-0.0838	0.0028	-0.0088	0.0761	-0.0208	-0.0153	-0.1706	-0.0838	0.0014	-0.0790	-0.0104	-0.0176	0.0113
0.0195	0.0500	-0.0051	-0.0097	0.0689	-0.0876	-0.0185	-0.0731	-0.1287	0.0113	0.0476	-0.0191	-0.0267	0.0087
0.0240	0.0285	-0.0093	-0.0094	-0.0729	-0.1436	-0.0203	0.0122	-0.0344	0.0162	0.0162	-0.0223	-0.0344	0.0093
0.0207	-0.1059	0.0009	-0.0100	-0.0543	-0.0420	-0.0202	-0.0815	0.0270	0.0064	-0.0976	-0.0233	-0.0278	0.0133
1.0011	-0.0129	-0.0026	0.0064	0.0234	0.0409	0.0191	-0.0076	0.0036	-0.0077	-0.0207	0.0235	0.0269	-0.0063
-0.0129	*1.0009*	0.0006	0.0058	-0.0067	0.0492	0.0151	-0.0989	0.0392	-0.0020	-0.1324	0.0116	0.0218	-0.0080
-0.0026	0.0006	*0.9977*	-0.0006	-0.0096	-0.0117	0.0028	0.0001	-0.0058	0.0003	-0.0027	0.0020	0.0055	0.0006
0.0064	0.0058	-0.0006	*0.9984*	-0.0095	-0.0218	-0.0123	0.0088	-0.0030	0.0019	0.0124	-0.0073	-0.0123	0.0083
0.0234	-0.0067	-0.0096	-0.0095	*1.0003*	-0.1203	-0.0206	0.0964	0.0841	0.0125	-0.0139	-0.0217	-0.0283	0.0104
0.0409	0.0492	-0.0117	-0.0218	-0.1203	*1.0004*	-0.0439	0.0247	-0.0573	0.0247	0.0292	-0.0341	-0.0623	0.0217
0.0191	0.0151	0.0028	-0.0123	-0.0206	-0.0439	*1.0013*	0.0113	-0.0062	0.0031	0.0232	-0.0210	-0.0304	0.0122
-0.0076	-0.0989	0.0001	0.0088	0.0964	0.0247	0.0113	*0.9996*	-0.0790	-0.0028	-0.1042	0.0095	0.0132	-0.0084
0.0036	0.0392	-0.0058	-0.0030	0.0841	-0.0573	-0.0062	-0.0790	*1.0014*	0.0019	0.0305	-0.0018	-0.0061	0.0021
-0.0077	-0.0020	0.0003	0.0019	0.0125	0.0247	0.0031	-0.0028	0.0019	*0.9992*	-0.0057	0.0087	0.0069	-0.0019
-0.0207	-0.1324	-0.0027	0.0124	-0.0139	0.0292	0.0232	-0.1042	0.0305	-0.0057	*1.0002*	0.0241	0.0268	-0.0110
0.0235	0.0116	0.0020	-0.0073	-0.0217	-0.0341	-0.0210	0.0095	-0.0018	0.0087	0.0241	*1.0003*	-0.0296	0.0072
0.0269	0.0218	0.0055	-0.0123	-0.0283	-0.0623	-0.0304	0.0132	-0.0061	0.0069	0.0268	-0.0296	*1.0019*	0.0122
-0.0063	-0.0080	0.0006	0.0083	0.0104	0.0217	0.0122	-0.0084	0.0021	-0.0019	-0.0110	0.0072	0.0122	*1.0016*

Tests - Linear Synthetic Water Model
test #1: (prior mean)/(prior standard deviation)
test #2: (posterior mean)/(posterior standard deviation)
test #3: (posterior mean - prior mean)/(posterior standard deviation)

	Test #1		Test #2		Test #3	
	formula	ratio	formula	ratio	formula	ratio
1	5/0.699^0.5=	**5.98**	4.567/0.0369^0.5=	**23.77**	(4.567-5)/0.0369^0.5=	**-2.25**
a	0.8/1=	0.80	0.371/0.0126^0.5=	**3.31**	(0.371-0.8)/0.0126^0.5=	**-3.82**
b	0.7/1=	0.70	-0.235/0.0295^0.5=	-1.37	(-0.235-0.7)/0.0295^0.5=	**-5.44**
c	-1.5/1=	-1.50	-1.577/0.0108^0.5=	**-15.18**	(-1.577+1.5)/0.0108^0.5=	-0.74
d	0.4/1=	0.40	-0.0571/0.0543^0.5=	-0.25	(-0.0571-0.4)/0.0543^0.5=	**-1.96**
e	0.7/1=	0.70	0.3956/0.0795=	**4.98**	(0.3956-0.7)/0.0795=	**-3.83**
f	1/1=	1.00	0.3537/0.0579^0.5=	1.47	(0.3537-1)/0.0579^0.5=	**-2.69**
ab	0/0.0016=	0.00	-0.0085/0.0014^0.5=	-0.23	-0.0085/0.0014^0.5=	-0.23
ac	0/0.6021=	0.00	-0.2236/0.0088^0.5=	**-2.38**	-0.2236/0.0088^0.5=	**-2.38**
ad	0/0.301=	0.00	0.0363/0.0382^0.5=	0.19	0.0363/0.0382^0.5=	0.19
ae	0/0.4771=	0.00	0.2423/0.0055^0.5=	**3.27**	0.2423/0.0055^0.5=	**3.27**
af	0/0.0016=	0.00	0.0026/0.0016^0.5=	0.07	0.0026/0.0016^0.5=	0.07
bc	0/0.699=	0.00	0.0898/0.0052^0.5=	1.25	0.0898/0.0052^0.5=	1.25
bd	0/0.0016=	0.00	-0.0164/0.0014^0.5=	-0.44	-0.0164/0.0014^0.5=	-0.44
be	0/0.301=	0.00	-0.1443/0.0166^0.5=	-1.12	-0.1443/0.0166^0.5=	-1.12
bf	0/0.699=	0.00	-0.1447/0.0618^0.5=	-0.58	-0.1447/0.0618^0.5=	-0.58
cd	0/0.301=	0.00	-0.1805/0.0127^0.5=	-1.60	-0.1805/0.0127^0.5=	-1.60
ce	0/0.301=	0.00	-0.1601/0.015^0.5=	-1.31	-0.1601/0.015^0.5=	-1.31
cf	0/0.699=	0.00	-0.2896/0.0144^0.5=	**-2.41**	-0.2896/0.0144^0.5=	**-2.41**
de	0/0.301=	0.00	0.0253/0.0055^0.5=	0.34	0.0253/0.0055^0.5=	0.34
df	0/0.301=	0.00	-0.2288/0.0339^0.5=	-1.24	-0.2288/0.0339^0.5=	-1.24
ef	0/0.301=	0.00	0.1093/0.0721^0.5=	0.41	0.1093/0.0721^0.5=	0.41
abc	0/0.0016=	0.00	0.0059/0.0014^0.5=	0.16	0.0059/0.0014^0.5=	0.16
abd	0/0.0016=	0.00	0.0011/0.0016^0.5=	0.03	0.0011/0.0016^0.5=	0.03
abe	0/0.0016=	0.00	-0.0153/0.0013^0.5=	-0.42	-0.0153/0.0013^0.5=	-0.42
abf	0/0.0016=	0.00	0.0145/0.0014^0.5=	0.39	0.0145/0.0014^0.5=	0.39
acd	0/0.0016=	0.00	0.0165/0.0015^0.5=	0.43	0.0165/0.0015^0.5=	0.43
ace	0/0.0016=	0.00	-0.0154/0.0014^0.5=	-0.41	-0.0154/0.0014^0.5=	-0.41
acf	0/0.0016=	0.00	-0.0080/0.0016^0.5=	-0.20	-0.0080/0.0016^0.5=	-0.20
ade	0/0.0016=	0.00	-0.0298/0.0014^0.5=	-0.80	-0.0298/0.0014^0.5=	-0.80
adf	0/0.0016=	0.00	0.0018/0.0016^0.5=	0.05	0.0018/0.0016^0.5=	0.05
aef	0/0.0016=	0.00	0.0032/0.0016^0.5=	0.08	0.0032/0.0016^0.5=	0.08
bcd	0/0.0016=	0.00	0.0101/0.0014^0.5=	0.27	0.0101/0.0014^0.5=	0.27
bce	0/0.0016=	0.00	0.0259/0.0013^0.5=	0.72	0.0259/0.0013^0.5=	0.72
bcf	0/0.0016=	0.00	0.0085/0.0016^0.5=	0.21	0.0085/0.0016^0.5=	0.21
bde	0/0.0016=	0.00	-0.0225/0.0013^0.5=	-0.63	-0.0225/0.0013^0.5=	-0.63
bdf	0/0.0016=	0.00	0.0104/0.0014^0.5=	0.28	0.0104/0.0014^0.5=	0.28
bef	0/0.0016=	0.00	-0.0031/0.0016^0.5=	-0.08	-0.0031/0.0016^0.5=	-0.08
cde	0/0.0016=	0.00	-0.0287/0.0014^0.5=	-0.77	-0.0287/0.0014^0.5=	-0.77
cdf	0/0.0016=	0.00	0.0095/0.0016^0.5=	0.24	0.0095/0.0016^0.5=	0.24
cef	0/0.0016=	0.00	0.0114/0.0015^0.5=	0.30	0.0114/0.0015^0.5=	0.30
def	0/0.0016=	0.00	-0.0042/0.0016^0.5=	-0.11	-0.0042/0.0016^0.5=	-0.11

Note: Numbers above 1.96 correspond to 5% significance level; Numbers above 1.65 correspond to 10% significance level; Numbers above 1.04 correspond to 30% significance level

Appendix D/2: Quadratic Synthetic Water Models - Prior Data, Parameter Estimates, Covariance and Correlation Matrices, Tests

format short e

a =

[5;0.8;0.7;- 1.5;0.4;0.7;1.0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0;0; 0.5;0.5]

y = [4.799;3.041;3.839;2.973;6.740;2.724;6.903;3.204;4.987;3.519;2.724;4.519;4.724;5.826;2.699;3.114;2.903; 2.919;6.763;5.785;4.663;4.602;4.602;4.568;5.740;5.519]

u1 = [0.6990 1 1 1 1 1 1 0.0016 0.6021 0.3010 0.4771 0.0016 0.6990 0.0016 0.3010 0.6990 0.3010 0.3010 0.6990 0.3010 0.3010 0.3010 0.0016 0.6990 0.6990]

u = diag(u1)

s=0.0189

x=[1 -1 -1 -1 -1 1 -1 1 1 1 -1 1 1
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-1 -1 -1 -1 -1 1 1;
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1 -1 1 -1 1 -1 -1 1 -1 -1 1 -1
1 -1 1 -1 -1 1 -1 1 -1 1 1 -1
1 -1 1 -1 1 1 1;

```
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    -1  -1  -1   1  -1   1   1  -1  -1   1  -1   1
     1   1  -1  -1  -1   1   1;
1   -1   1  -1  -1  -1  -1  -1   1   1   1   1  -1
    -1  -1  -1   1   1   1   1   1   1   1   1   1
     1  -1  -1  -1  -1  -1  -1   1   1   1   1   1
     1  -1  -1  -1  -1   1   1;
1   -1  -1  -1   1  -1  -1   1   1  -1   1   1   1
    -1   1   1  -1   1   1  -1  -1   1  -1   1  -1
    -1   1  -1  -1   1   1  -1   1  -1  -1   1   1
    -1   1   1  -1   1   1   1;
1   -1   1  -1   1   1   1  -1   1  -1  -1  -1  -1
     1   1   1  -1  -1  -1   1   1   1   1  -1  -1
    -1   1   1   1  -1  -1  -1  -1  -1  -1   1   1
     1  -1  -1  -1   1   1   1;
1   -1   1   1  -1   1  -1  -1  -1   1  -1   1   1
    -1   1  -1  -1   1  -1  -1   1  -1  -1   1  -1
     1   1  -1   1   1  -1   1  -1   1  -1  -1   1
    -1  -1   1  -1   1   1   1;
1   1   -1   1  -1  -1   1  -1   1  -1  -1   1  -1
    1   1  -1  -1  -1   1   1  -1  -1  -1   1   1
    -1  -1  -1   1   1  -1  -1   1   1  -1  -1   1
     1   1  -1  -1   1   1   1;
1   -1  -1   1  -1  -1  -1   1  -1   1   1   1  -1
     1   1   1  -1  -1  -1   1   1   1   1  -1  -1
    -1   1   1   1  -1  -1  -1   1   1   1  -1  -1
    -1   1   1   1  -1   1   1;
1   1   1   1   1  -1  -1   1   1   1  -1  -1   1
    1  -1  -1   1  -1  -1  -1  -1   1   1   1  -1
    -1   1  -1  -1  -1  -1   1   1  -1  -1  -1  -1
     1  -1  -1   1   1   1   1;
1   1   -1  -1  -1   1  -1  -1  -1  -1   1  -1   1
    1  -1   1   1  -1   1  -1   1  -1   1   1  -1
    1   1  -1   1  -1   1  -1  -1   1  -1   1  -1
    1   1  -1   1   1   1   1;
1   -1  -1  -1   1   1  -1   1   1  -1  -1   1   1
    -1  -1   1  -1  -1   1   1  -1  -1  -1   1   1
    -1   1   1  -1  -1   1   1   1   1  -1  -1   1
     1  -1   1   1  -1   1   1;
1   -1  -1  -1  -1   0  -1   1   1   1   0   1   1
     1   0   1   1   0   1   0   1   0  -1  -1   0
    -1  -1   0  -1   0  -1   0  -1   0  -1   0  -1
     0   0  -1   0   0   1   0;
1   -1  -1  -1  -1   0  -1   1   1   1   0   1   1
     1   0   1   1   0   1   0   1   0  -1  -1   0
    -1  -1   0  -1   0  -1   0  -1   0  -1   0  -1
     0   0  -1   0   0   1   0;
1   -1  -1  -1  -1   0  -1   1   1   1   0   1   1
     1   0   1   1   0   1   0   1   0  -1  -1   0
    -1  -1   0  -1   0  -1   0  -1   0  -1   0  -1
     0   0  -1   0   0   1   0;
1   -1  -1  -1  -1   0  -1   1   1   1   0   1   1
     1   0   1   1   0   1   0   1   0  -1  -1   0
```

```
        -1      -1      0       -1      0       -1      0       -1      0       -1      0       -1
        0       0       -1      0       0       1       0;
1       -1      -1      -1      0       0       -1      1       1       0       0       1       1
        0       0       1       0       0       1       0       0       0       -1      0       0
        -1      0       0       -1      0       0       0       0       0       -1      0       0
        0       0       0       0       0       0       0;
1       -1      -1      -1      1       0       -1      1       1       -1      0       1       1
        -1      0       1       -1      0       1       0       -1      0       -1      1       0
        -1      1       0       -1      0       1       0       1       0       -1      0       1
        0       0       1       0       0       1       0]
```

```
covmat = inv(inv(u)+(1/s)*x'*x)
parest = inv(inv(u)+(1/s)*x'*x)*(inv(u)*a+(1/s)*x'*y)

wk1write('a',a)
wk1write('y',y)
wk1write('u1',u1)
wk1write('u',u)
wk1write('x',x)
wk1write('covmat',covmat)
wk1write('parest',parest)
```

```
Parameter Estimate:

        1     5.323           abc     0.014
        a     0.364           abd     0.002
        b    -0.229           abe    -0.003
        c    -1.595           abf     0.013
        d    -0.177           acd     0.021
        e     0.515           ace     0.000
        f     0.230           acf    -0.009
       ab    -0.006           ade    -0.017
       ac    -0.233           adf     0.001
       ad    -0.079           aef     0.003
       ae     0.211           bcd     0.017
       af     0.003           bce     0.033
       bc     0.017           bcf     0.010
       bd    -0.016           bde    -0.012
       be    -0.107           bdf     0.009
       bf    -0.262           bef    -0.003
       cd    -0.142           cde    -0.015
       ce    -0.217           cdf     0.011
       cf    -0.342           cef     0.015
       de    -0.009           def    -0.004
       df    -0.300            d2    -0.655
       ef     0.229            e2    -0.199
```

Posterior Covariance Matrix - Quadratic Synthetic Water Models

0.0597	0.0104	0.0231	0.0083	0.0362	-0.0453	0.0419	-0.0004	-0.0138	0.0250	0.0012	-0.0002	0.0034	-0.0008	-0.0177
0.0104	*0.0126*	0.0023	-0.0008	0.0132	-0.0108	0.0143	0.0002	-0.0042	0.0154	-0.0020	0.0015	0.0030	-0.0010	-0.0097
0.0231	0.0023	*0.0295*	0.0127	0.0258	-0.0403	0.0242	0.0003	-0.0087	0.0080	0.0002	-0.0002	0.0018	-0.0002	-0.0076
0.0083	-0.0008	0.0127	*0.0109*	0.0140	-0.0196	0.0106	-0.0003	-0.0035	0.0027	0.0014	-0.0001	0.0028	0.0002	-0.0013
0.0362	0.0132	0.0258	0.0140	*0.0558*	-0.0619	0.0491	-0.0007	-0.0134	0.0304	0.0038	0.0013	0.0103	-0.0006	-0.0179
-0.0453	-0.0108	-0.0403	-0.0196	-0.0619	*0.0810*	-0.0585	-0.0002	0.0165	-0.0313	-0.0035	0.0004	-0.0094	0.0009	0.0210
0.0419	0.0143	0.0242	0.0106	0.0491	-0.0585	*0.0601*	-0.0008	-0.0158	0.0415	0.0029	-0.0003	0.0094	-0.0009	-0.0245
-0.0004	0.0002	0.0003	-0.0003	-0.0007	-0.0002	-0.0008	*0.0014*	0.0004	-0.0015	-0.0002	0.0000	-0.0004	-0.0002	0.0006
-0.0138	-0.0042	-0.0087	-0.0035	-0.0134	0.0165	-0.0158	0.0004	*0.0088*	-0.0113	0.0003	0.0001	-0.0018	-0.0001	0.0072
0.0250	0.0154	0.0080	0.0027	0.0304	-0.0313	0.0415	-0.0015	-0.0113	*0.0405*	-0.0014	-0.0002	0.0072	-0.0006	-0.0210
0.0012	-0.0020	0.0002	0.0014	0.0038	-0.0035	0.0029	-0.0002	0.0003	-0.0014	*0.0057*	0.0000	0.0005	-0.0001	0.0010
-0.0002	0.0015	-0.0002	-0.0001	0.0013	0.0004	-0.0003	0.0000	0.0001	-0.0002	0.0000	*0.0016*	-0.0001	0.0000	0.0001
0.0034	0.0030	0.0018	0.0028	0.0103	-0.0094	0.0094	-0.0004	-0.0018	0.0072	0.0005	-0.0001	*0.0061*	-0.0001	-0.0035
-0.0008	-0.0010	-0.0002	0.0002	-0.0006	0.0009	-0.0009	-0.0002	-0.0001	-0.0006	-0.0001	0.0000	-0.0001	*0.0014*	0.0001
-0.0177	-0.0097	-0.0076	-0.0013	-0.0179	0.0210	-0.0245	0.0006	0.0072	-0.0210	0.0010	0.0001	-0.0035	0.0001	*0.0167*
0.0387	0.0136	0.0343	0.0156	0.0491	-0.0639	0.0552	-0.0008	-0.0169	0.0372	0.0002	-0.0003	0.0085	-0.0007	-0.0233
-0.0086	-0.0088	0.0007	0.0019	-0.0119	0.0106	-0.0142	0.0004	0.0043	-0.0169	0.0024	0.0001	-0.0035	0.0001	0.0105
0.0153	0.0093	0.0085	0.0026	0.0207	-0.0236	0.0229	-0.0005	-0.0068	0.0207	-0.0017	-0.0001	0.0034	0.0001	-0.0128
0.0145	0.0034	0.0156	0.0103	0.0215	-0.0279	0.0200	-0.0006	-0.0068	0.0125	0.0002	-0.0001	0.0033	0.0001	-0.0067
-0.0034	0.0024	-0.0014	-0.0001	-0.0019	0.0028	-0.0002	-0.0002	0.0008	0.0031	-0.0008	0.0000	0.0018	-0.0002	-0.0018
0.0246	0.0097	0.0205	0.0116	0.0404	-0.0457	0.0323	0.0007	-0.0075	0.0163	0.0032	0.0013	0.0075	-0.0013	-0.0093
-0.0419	-0.0088	-0.0385	-0.0197	-0.0581	0.0760	-0.0549	0.0000	0.0147	-0.0288	-0.0041	0.0003	-0.0088	0.0012	0.0180
0.0005	0.0004	-0.0007	0.0001	0.0008	-0.0007	0.0004	-0.0001	0.0002	0.0014	-0.0003	0.0000	0.0003	-0.0001	-0.0004
-0.0003	-0.0001	-0.0002	-0.0001	-0.0004	0.0004	0.0012	0.0000	0.0001	0.0012	0.0000	0.0000	-0.0001	0.0000	0.0002
0.0002	-0.0004	0.0003	-0.0001	0.0004	-0.0005	-0.0003	0.0001	0.0000	-0.0010	0.0004	0.0000	-0.0001	0.0001	0.0005
0.0009	-0.0005	0.0004	-0.0002	0.0009	-0.0012	0.0011	0.0002	0.0000	0.0007	0.0001	0.0000	0.0002	0.0002	-0.0001
0.0011	0.0003	0.0006	0.0004	0.0002	-0.0008	0.0016	0.0001	-0.0002	0.0016	-0.0001	0.0000	-0.0008	0.0001	-0.0012
0.0011	-0.0008	0.0009	0.0006	0.0006	-0.0011	-0.0004	0.0000	-0.0005	-0.0009	0.0002	0.0000	-0.0009	0.0000	0.0006
-0.0003	0.0003	-0.0021	-0.0010	0.0001	0.0010	0.0000	0.0000	0.0011	0.0009	0.0001	0.0000	0.0003	0.0000	-0.0006
0.0006	-0.0001	-0.0002	0.0000	-0.0008	0.0011	-0.0003	0.0000	0.0001	-0.0005	0.0004	0.0000	-0.0004	-0.0001	-0.0007
0.0013	-0.0001	-0.0001	-0.0001	-0.0003	0.0004	0.0012	0.0000	0.0001	0.0013	0.0000	0.0000	-0.0001	0.0000	0.0002
0.0009	-0.0002	-0.0005	-0.0003	0.0007	-0.0005	0.0008	0.0000	0.0002	-0.0005	0.0015	0.0000	-0.0001	0.0000	0.0003
0.0018	0.0004	0.0007	0.0000	0.0005	-0.0003	0.0017	-0.0001	-0.0013	0.0012	-0.0003	0.0000	0.0003	-0.0001	-0.0003
0.0022	0.0012	0.0008	-0.0003	0.0023	-0.0024	0.0021	0.0001	-0.0011	0.0014	-0.0002	0.0000	0.0003	0.0001	-0.0017
0.0002	-0.0004	0.0005	0.0010	0.0014	-0.0009	-0.0001	0.0000	0.0005	-0.0009	-0.0001	0.0000	0.0013	0.0000	0.0006
0.0000	-0.0001	-0.0002	0.0004	-0.0010	0.0005	-0.0003	0.0001	-0.0004	-0.0001	-0.0011	0.0000	0.0002	0.0001	0.0000
0.0003	0.0013	-0.0004	0.0002	0.0005	0.0004	0.0006	0.0002	-0.0003	0.0013	0.0001	0.0000	0.0004	0.0002	-0.0005
-0.0009	0.0002	-0.0011	0.0002	0.0008	0.0005	-0.0007	0.0000	-0.0003	0.0005	0.0000	0.0000	0.0001	0.0000	0.0012
0.0003	0.0013	-0.0003	-0.0016	-0.0011	0.0014	-0.0007	0.0000	0.0003	0.0006	-0.0012	0.0000	-0.0004	-0.0001	-0.0004
0.0004	-0.0003	0.0021	0.0010	0.0000	-0.0011	0.0001	0.0000	0.0004	-0.0008	-0.0001	0.0000	-0.0002	0.0000	0.0005
-0.0002	-0.0005	0.0016	0.0008	0.0007	-0.0017	-0.0008	0.0000	0.0007	-0.0013	-0.0001	0.0000	-0.0004	0.0000	0.0008
-0.0009	0.0002	0.0005	0.0003	-0.0007	0.0005	-0.0008	0.0000	-0.0002	0.0004	0.0001	0.0000	0.0001	0.0000	-0.0003
-0.0164	-0.0001	0.0004	-0.0003	-0.0001	0.0002	-0.0007	0.0000	-0.0004	-0.0011	-0.0003	0.0000	-0.0006	-0.0001	-0.0001
-0.0066	0.0004	-0.0007	0.0011	0.0049	-0.0050	0.0059	-0.0001	0.0009	0.0062	0.0017	0.0000	0.0037	0.0000	-0.0014

Square root of the diagonals:

0.2443	0.1124	0.1718	0.1044	0.2363	0.2847	0.2451	0.0377	0.0939	0.2012	0.0752	0.0399	0.0778	0.0372	0.1294

0.0387	-0.0086	0.0153	0.0145	-0.0034	0.0246	-0.0419	0.0005	-0.0003	0.0002	0.0009	0.0011	0.0011	-0.0003	0.0006
0.0136	-0.0088	0.0093	0.0034	0.0024	0.0097	-0.0088	0.0004	-0.0001	-0.0004	-0.0005	0.0003	-0.0008	0.0003	-0.0001
0.0343	0.0007	0.0085	0.0156	-0.0014	0.0205	-0.0385	-0.0007	-0.0002	0.0003	0.0004	0.0006	0.0009	-0.0021	-0.0002
0.0156	0.0019	0.0026	0.0103	-0.0001	0.0116	-0.0197	0.0001	-0.0001	-0.0001	-0.0002	0.0004	0.0006	-0.0010	0.0000
0.0491	-0.0119	0.0207	0.0215	-0.0019	0.0404	-0.0581	0.0008	-0.0004	0.0004	0.0009	0.0002	0.0006	0.0001	-0.0008
-0.0639	0.0106	-0.0236	-0.0279	0.0028	-0.0457	0.0760	-0.0007	0.0004	-0.0005	-0.0012	-0.0008	-0.0011	0.0010	0.0011
0.0552	-0.0142	0.0229	0.0200	-0.0002	0.0323	-0.0549	0.0004	0.0012	-0.0003	0.0011	0.0016	-0.0004	0.0000	-0.0003
-0.0008	0.0004	-0.0005	-0.0006	-0.0002	0.0007	0.0000	-0.0001	0.0000	0.0001	0.0002	0.0001	0.0000	0.0000	0.0000
-0.0169	0.0043	-0.0068	-0.0068	0.0008	-0.0075	0.0147	0.0002	0.0001	0.0000	0.0000	-0.0002	-0.0005	0.0011	0.0001
0.0372	-0.0169	0.0207	0.0125	0.0031	0.0163	-0.0288	0.0014	0.0012	-0.0010	0.0007	0.0016	-0.0009	0.0009	-0.0005
0.0002	0.0024	-0.0017	0.0002	-0.0008	0.0032	-0.0041	-0.0003	0.0000	0.0004	0.0001	-0.0001	0.0002	0.0001	0.0004
-0.0003	0.0001	-0.0001	-0.0001	0.0000	0.0013	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0085	-0.0035	0.0034	0.0033	0.0018	0.0075	-0.0088	0.0003	-0.0001	-0.0001	0.0002	-0.0008	-0.0009	0.0003	-0.0004
-0.0007	0.0001	0.0001	0.0001	-0.0002	-0.0013	0.0012	-0.0001	0.0000	0.0001	0.0002	0.0001	0.0000	0.0000	-0.0001
-0.0233	0.0105	-0.0128	-0.0067	-0.0018	-0.0093	0.0180	-0.0004	0.0002	0.0005	-0.0001	-0.0012	0.0006	-0.0006	-0.0007
0.0638	-0.0117	0.0234	0.0243	0.0020	0.0333	-0.0602	0.0003	0.0012	-0.0003	0.0009	0.0015	-0.0004	-0.0014	-0.0001
-0.0117	*0.0128*	-0.0105	-0.0027	-0.0018	-0.0063	0.0084	-0.0014	0.0001	0.0000	-0.0001	-0.0004	0.0010	-0.0003	0.0003
0.0234	-0.0105	*0.0154*	0.0086	0.0015	0.0125	-0.0210	0.0004	-0.0002	-0.0004	0.0000	0.0011	-0.0004	0.0006	-0.0006
0.0243	-0.0027	0.0086	*0.0147*	-0.0002	0.0153	-0.0272	0.0005	-0.0002	-0.0005	0.0000	0.0012	0.0010	-0.0008	-0.0007
0.0020	-0.0018	0.0015	-0.0002	*0.0058*	-0.0020	0.0027	-0.0002	0.0000	-0.0011	0.0002	0.0000	-0.0012	-0.0001	0.0002
0.0333	-0.0063	0.0125	0.0153	-0.0020	*0.0347*	-0.0436	0.0005	-0.0002	0.0010	-0.0001	0.0000	0.0001	-0.0003	-0.0006
-0.0602	0.0084	-0.0210	-0.0272	0.0027	-0.0436	*0.0739*	-0.0009	0.0004	-0.0004	-0.0014	-0.0012	-0.0010	0.0011	0.0013
0.0003	-0.0014	0.0004	0.0005	-0.0002	0.0005	-0.0009	*0.0014*	0.0000	0.0001	0.0001	-0.0001	0.0000	0.0000	0.0000
0.0012	0.0001	-0.0002	-0.0002	0.0000	-0.0002	0.0004	0.0000	*0.0016*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0003	0.0000	-0.0004	-0.0005	-0.0011	0.0010	-0.0004	0.0001	0.0000	*0.0014*	-0.0001	0.0000	-0.0001	0.0000	-0.0001
0.0009	-0.0001	0.0000	0.0000	0.0002	-0.0001	-0.0014	0.0001	0.0000	-0.0001	*0.0014*	-0.0001	0.0000	0.0000	0.0001
0.0015	-0.0004	0.0011	0.0012	0.0000	0.0000	-0.0012	-0.0001	0.0000	0.0000	-0.0001	*0.0015*	0.0000	0.0000	0.0001
-0.0004	0.0010	-0.0004	0.0010	-0.0012	0.0001	-0.0010	0.0000	0.0000	-0.0001	0.0000	0.0000	*0.0015*	0.0000	-0.0001
-0.0014	-0.0003	0.0006	-0.0008	-0.0001	-0.0003	0.0011	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0016*	0.0000
-0.0001	0.0003	-0.0006	-0.0007	0.0002	-0.0006	0.0013	0.0000	0.0000	-0.0001	0.0001	0.0001	-0.0001	0.0000	*0.0014*
-0.0004	0.0001	-0.0002	-0.0001	0.0000	-0.0002	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0009	0.0001	-0.0003	-0.0004	0.0000	0.0009	-0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0016	0.0002	0.0003	0.0003	-0.0002	0.0003	-0.0006	-0.0002	0.0000	0.0001	0.0001	-0.0001	0.0000	0.0000	0.0000
0.0018	-0.0013	0.0015	0.0000	0.0001	0.0013	-0.0013	-0.0001	0.0000	0.0000	-0.0001	-0.0002	0.0000	0.0001	0.0001
-0.0002	0.0003	-0.0007	0.0008	0.0001	0.0018	-0.0010	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0002	-0.0003	0.0002	0.0002	0.0004	-0.0009	0.0007	0.0001	0.0000	-0.0002	-0.0001	0.0000	-0.0001	0.0000	-0.0001
0.0006	-0.0003	0.0005	0.0006	0.0002	0.0007	0.0003	0.0001	0.0000	-0.0001	-0.0002	-0.0001	0.0000	0.0000	0.0001
-0.0007	-0.0002	0.0004	0.0004	0.0000	0.0006	0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0005	-0.0010	0.0007	-0.0008	0.0002	-0.0008	0.0016	0.0000	0.0000	-0.0001	0.0001	0.0000	-0.0001	0.0000	-0.0001
0.0015	0.0018	-0.0006	0.0008	0.0001	0.0004	-0.0012	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0006	0.0004	0.0006	0.0005	0.0001	0.0013	-0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0008	-0.0001	0.0003	0.0004	0.0015	-0.0009	0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0007	-0.0002	-0.0003	-0.0002	-0.0007	-0.0004	0.0005	0.0002	0.0000	0.0002	0.0001	0.0002	0.0003	0.0000	0.0002
0.0057	-0.0012	0.0026	0.0024	0.0023	0.0034	-0.0054	-0.0005	-0.0001	-0.0007	0.0000	-0.0004	-0.0010	0.0001	-0.0007
0.2525	0.1131	0.1242	0.1214	0.0762	0.1862	0.2719	0.0372	0.0399	0.0371	0.0373	0.0384	0.0381	0.0395	0.0380

0.0013	0.0009	0.0018	0.0022	0.0002	0.0000	0.0003	-0.0009	0.0003	0.0004	-0.0002	-0.0009	-0.0164	-0.0066
-0.0001	-0.0002	0.0004	0.0012	-0.0004	-0.0001	0.0013	0.0002	0.0013	-0.0003	-0.0005	0.0002	-0.0001	0.0004
-0.0001	-0.0005	0.0007	0.0008	0.0005	-0.0002	-0.0004	-0.0011	-0.0003	0.0021	0.0016	0.0005	0.0004	-0.0007
-0.0001	-0.0003	0.0000	-0.0003	0.0010	0.0004	0.0002	0.0002	-0.0016	0.0010	0.0008	0.0003	-0.0003	0.0011
-0.0003	0.0007	0.0005	0.0023	0.0014	-0.0010	0.0005	0.0008	-0.0011	0.0000	0.0007	-0.0007	-0.0001	0.0049
0.0004	-0.0005	-0.0003	-0.0024	-0.0009	0.0005	0.0004	0.0005	0.0014	-0.0011	-0.0017	0.0005	0.0002	-0.0050
0.0012	0.0008	0.0017	0.0021	-0.0001	-0.0003	0.0006	-0.0007	-0.0007	0.0001	-0.0008	-0.0008	-0.0007	0.0059
0.0000	0.0000	-0.0001	0.0001	0.0000	0.0001	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	-0.0001
0.0001	0.0002	-0.0013	-0.0011	0.0005	-0.0004	-0.0003	-0.0003	0.0003	0.0004	0.0007	-0.0002	-0.0004	0.0009
0.0013	-0.0005	0.0012	0.0014	-0.0009	-0.0001	0.0013	0.0005	0.0006	-0.0008	-0.0013	0.0004	-0.0011	0.0062
0.0000	0.0015	-0.0003	-0.0002	-0.0001	-0.0011	0.0001	0.0000	-0.0012	-0.0001	-0.0001	0.0001	-0.0003	0.0017
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-0.0001	-0.0001	0.0003	0.0003	0.0013	0.0002	0.0004	0.0001	-0.0004	-0.0002	-0.0004	0.0001	-0.0006	0.0037
0.0000	0.0000	-0.0001	0.0001	0.0000	0.0001	0.0002	0.0000	-0.0001	0.0000	0.0000	0.0000	-0.0001	0.0000
0.0002	0.0003	-0.0003	-0.0017	0.0006	0.0000	-0.0005	0.0012	-0.0004	0.0005	0.0008	-0.0003	-0.0001	-0.0014
-0.0004	-0.0009	0.0016	0.0018	-0.0002	-0.0002	0.0006	-0.0007	-0.0005	0.0015	0.0006	0.0008	-0.0007	0.0057
0.0001	0.0001	0.0002	-0.0013	0.0003	-0.0003	-0.0003	-0.0002	-0.0010	0.0018	0.0004	-0.0001	-0.0002	-0.0012
-0.0002	-0.0003	0.0003	0.0015	-0.0007	0.0002	0.0005	0.0004	0.0007	-0.0006	0.0006	0.0003	-0.0003	0.0026
-0.0001	-0.0004	0.0003	0.0000	0.0008	0.0002	0.0006	0.0004	-0.0008	0.0008	0.0005	0.0004	-0.0002	0.0024
0.0000	0.0000	-0.0002	0.0001	0.0001	0.0004	0.0002	0.0000	0.0002	0.0001	0.0001	0.0015	-0.0007	0.0023
-0.0002	0.0009	0.0003	0.0013	0.0018	-0.0009	0.0007	0.0006	-0.0008	0.0004	0.0013	-0.0009	-0.0004	0.0034
0.0003	-0.0006	-0.0006	-0.0013	-0.0010	0.0007	0.0003	0.0006	0.0016	-0.0012	-0.0018	0.0006	0.0005	-0.0054
0.0000	0.0000	-0.0002	-0.0001	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	-0.0005
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	-0.0001
0.0000	0.0000	0.0001	0.0000	0.0000	-0.0002	-0.0001	0.0000	-0.0001	0.0000	0.0000	0.0000	0.0002	-0.0007
0.0000	0.0000	0.0001	-0.0001	0.0000	-0.0001	-0.0002	0.0000	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000
0.0000	0.0000	-0.0001	-0.0002	0.0000	0.0000	-0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	-0.0004
0.0000	0.0000	0.0000	0.0000	0.0000	-0.0001	0.0000	0.0000	-0.0001	0.0000	0.0000	0.0000	0.0003	-0.0010
0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
0.0000	0.0000	0.0000	0.0001	0.0000	-0.0001	0.0001	0.0000	-0.0001	0.0000	0.0000	0.0000	0.0002	-0.0007
0.0016	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	*0.0016*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	-0.0001
0.0000	0.0000	*0.0014*	-0.0001	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	-0.0005
0.0000	0.0000	-0.0001	*0.0013*	-0.0001	0.0001	-0.0001	0.0000	0.0001	0.0000	-0.0001	0.0000	0.0003	-0.0006
0.0000	0.0000	0.0000	-0.0001	*0.0016*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	-0.0001
0.0000	0.0000	0.0001	0.0001	0.0000	*0.0014*	-0.0001	0.0000	-0.0001	0.0000	0.0000	0.0000	0.0001	-0.0006
0.0000	0.0000	0.0001	-0.0001	0.0000	-0.0001	*0.0014*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0016*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
0.0000	0.0000	0.0000	0.0001	0.0000	-0.0001	0.0000	0.0000	*0.0014*	0.0000	0.0000	0.0000	0.0002	-0.0008
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0016*	0.0000	0.0000	0.0001	-0.0001
0.0000	0.0000	0.0000	-0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0015*	0.0000	0.0001	-0.0002
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0016*	0.0000	0.0000
0.0000	0.0000	0.0002	0.0003	0.0001	0.0001	0.0000	0.0000	0.0002	0.0001	0.0001	0.0000	*0.0228*	-0.0064
0.0000	-0.0001	-0.0005	-0.0006	-0.0001	-0.0006	0.0001	0.0001	-0.0008	-0.0001	-0.0002	0.0000	-0.0064	*0.0170*
0.0399	0.0398	0.0370	0.0361	0.0394	0.0370	0.0376	0.0398	0.0378	0.0394	0.0392	0.0397	0.1510	0.1305

Posterior Correlation Matrix - Quadratic Synthetic Water Models

1.0001	0.3770	0.5502	0.3249	0.6279	-0.6516	0.7003	-0.0465	-0.6018	0.5085	0.0639	-0.0211	0.1803	-0.0850	-0.5601
0.3770	0.9995	0.1200	-0.0703	0.4967	-0.3380	0.5183	0.0491	-0.3987	0.6794	-0.2396	0.3360	0.3477	-0.2481	-0.6662
0.5502	0.1200	1.0001	0.7071	0.6360	-0.8233	0.5758	0.0515	-0.5416	0.2326	0.0163	-0.0224	0.1367	-0.0388	-0.3410
0.3249	-0.0703	0.7071	0.9997	0.5674	-0.6595	0.4161	-0.0735	-0.3563	0.1297	0.1837	-0.0199	0.3408	0.0565	-0.0975
0.6279	0.4967	0.6360	0.5674	0.9997	-0.9208	0.9484	-0.0799	-0.6048	0.6388	0.2158	0.1352	0.5593	-0.0728	-0.5861
-0.6516	-0.3380	-0.8233	-0.6595	-0.9208	0.9999	-0.8377	-0.0174	0.6163	-0.5472	-0.1633	0.0316	-0.4222	0.0884	0.5707
0.7003	0.5183	0.5758	0.4161	0.9484	-0.8377	1.0000	-0.0908	-0.6885	0.8414	0.1555	-0.0279	0.4927	-0.0950	-0.7735
-0.0465	0.0491	0.0515	-0.0735	-0.0799	-0.0174	-0.0908	0.9979	0.1007	-0.1920	-0.0568	-0.0018	-0.1407	-0.1271	0.1238
-0.6018	-0.3987	-0.5416	-0.3563	-0.6048	0.6163	-0.6885	0.1007	0.9990	-0.5979	0.0475	0.0182	-0.2526	-0.0200	0.5956
0.5085	0.6794	0.2326	0.1297	0.6388	-0.5472	0.8414	-0.1920	-0.5979	0.9996	-0.0941	-0.0199	0.4570	-0.0778	-0.8061
0.0639	-0.2396	0.0163	0.1837	0.2158	-0.1633	0.1555	-0.0568	0.0475	-0.0941	1.0003	-0.0067	0.0813	-0.0218	0.1051
-0.0211	0.3360	-0.0224	-0.0199	0.1352	0.0316	-0.0279	-0.0018	0.0182	-0.0199	-0.0067	0.9978	-0.0197	0.0063	0.0181
0.1803	0.3477	0.1367	0.3408	0.5593	-0.4222	0.4927	-0.1407	-0.2526	0.4570	0.0813	-0.0197	1.0009	-0.0493	-0.3438
-0.0850	-0.2481	-0.0388	0.0565	-0.0728	0.0884	-0.0950	-0.1271	-0.0200	-0.0778	-0.0218	0.0063	-0.0493	1.0008	0.0120
-0.5601	-0.6662	-0.3410	-0.0975	-0.5861	0.5707	-0.7735	0.1238	0.5956	-0.8063	0.1051	0.0181	-0.3438	0.0120	0.9995
0.6279	0.4809	0.7909	0.5926	0.8234	-0.8883	0.8914	-0.0848	-0.7129	0.7325	0.0131	-0.0276	0.4338	-0.0777	-0.7118
-0.3117	-0.6922	0.0349	0.1573	-0.4443	0.3295	-0.5134	0.0889	0.4055	-0.7409	0.2768	0.0148	-0.4002	0.0209	0.7172
0.5052	0.6657	0.3999	0.2014	0.7047	-0.6675	0.7531	-0.1142	-0.5858	0.8279	-0.1841	-0.0231	0.3556	0.0182	-0.7976
0.4887	0.2524	0.7469	0.8113	0.7507	-0.8059	0.6708	-0.1399	-0.5946	0.5116	0.0193	-0.0251	0.3500	0.0192	-0.4259
-0.1833	0.2764	-0.1036	-0.0124	-0.1076	0.1273	-0.0108	-0.0574	0.1077	0.2006	-0.1444	0.0032	0.3090	-0.0803	-0.1819
0.5410	0.4640	0.6400	0.5968	0.9190	-0.8612	0.7084	0.0943	-0.4291	0.4344	0.2312	0.1798	0.5191	-0.1823	-0.3861
-0.6304	-0.2892	-0.8245	-0.6938	-0.9049	0.9820	-0.8235	-0.0037	0.5748	-0.5259	-0.1994	0.0312	-0.4157	0.1166	0.5109
0.0565	0.0950	-0.1031	0.0274	0.0944	-0.0699	0.0452	-0.0849	0.0604	0.1815	-0.0978	-0.0032	0.1119	-0.0706	-0.0861
-0.0296	-0.0303	-0.0234	-0.0162	-0.0374	0.0358	0.1183	0.0073	0.0331	0.1536	0.0008	0.0012	-0.0234	0.0041	0.0395
0.0267	-0.1043	0.0458	-0.0265	0.0491	-0.0456	-0.0355	0.0814	0.0062	-0.1282	0.1468	-0.0034	-0.0394	0.0940	0.1088
0.1010	-0.1171	0.0563	-0.0408	0.0994	-0.1126	0.1160	0.1295	0.0067	0.0924	0.0267	0.0001	0.0647	0.1490	-0.0254
0.1225	0.0792	0.0978	0.1020	0.0262	-0.0762	0.1710	0.0348	-0.0666	0.2033	-0.0436	-0.0007	-0.2608	0.0533	-0.2436
0.1213	-0.1977	0.1422	0.1518	0.0722	-0.0994	-0.0438	-0.0230	-0.1426	-0.1161	0.0675	-0.0001	-0.3063	-0.0111	0.1121
-0.0293	0.0756	-0.3079	-0.2535	0.0104	0.0919	0.0050	-0.0030	0.3028	0.1163	0.0299	0.0007	0.0861	-0.0203	-0.1142
0.0595	-0.0272	-0.0315	0.0063	-0.0836	0.0997	-0.0301	-0.0340	0.0416	-0.0674	0.1265	0.0031	-0.1252	-0.0526	-0.1345
0.1296	-0.0286	-0.0196	-0.0121	-0.0343	0.0320	0.1214	0.0067	0.0312	0.1571	0.0001	0.0011	-0.0196	0.0042	0.0371
0.0934	-0.0395	-0.0748	-0.0628	0.0758	-0.0455	0.0782	0.0000	0.0593	-0.0571	0.5089	-0.0036	-0.0461	0.0127	0.0551
0.1983	0.0845	0.1096	-0.0012	0.0620	-0.0328	0.1862	-0.0836	-0.3681	0.1607	-0.1054	-0.0021	0.1016	-0.0676	-0.0639
0.2510	0.2964	0.1221	-0.0766	0.2726	-0.2295	0.2322	0.0519	-0.3137	0.1884	-0.0800	-0.0087	0.1241	0.0930	-0.3614
0.0258	-0.0833	0.0715	0.2481	0.1466	-0.0824	-0.0117	0.0005	0.1261	-0.1195	-0.0382	-0.0071	0.4217	0.0246	0.1148
-0.0043	-0.0238	-0.0384	0.1126	-0.1151	0.0506	-0.0313	0.0834	-0.1216	-0.0108	-0.3801	0.0044	0.0554	0.0687	-0.0051
0.0306	0.3124	-0.0690	0.0583	0.0536	0.0417	0.0699	0.1256	-0.0877	0.1778	0.0520	-0.0045	0.1258	0.1334	-0.1108
-0.0907	0.0441	-0.1569	0.0566	0.0871	0.0482	-0.0745	-0.0042	-0.0676	0.0670	0.0100	-0.0029	0.0405	-0.0090	0.2401
0.0317	0.3008	-0.0420	-0.3978	-0.1199	0.1302	-0.0750	-0.0263	0.0760	0.0848	-0.4168	0.0026	-0.1509	-0.0493	-0.0836
0.0380	-0.0627	0.3115	0.2540	0.0002	-0.1011	0.0072	0.0010	0.1164	-0.1015	-0.0345	-0.0011	-0.0786	0.0202	0.0998
-0.0257	-0.1159	0.2447	0.2006	0.0773	-0.1502	-0.0795	0.0023	0.1781	-0.1702	-0.0445	-0.0048	-0.1298	0.0309	0.1656
-0.0922	0.0383	0.0760	0.0619	-0.0763	0.0453	-0.0792	0.0010	-0.0608	0.0558	0.0209	0.0037	0.0425	-0.0118	-0.0540
-0.4456	-0.0036	0.0139	-0.0171	-0.0034	0.0044	-0.0202	-0.0004	-0.0258	-0.0371	-0.0295	0.0004	-0.0511	-0.0094	-0.0042
-0.2056	0.0240	-0.0331	0.0779	0.1589	-0.1340	0.1857	-0.0166	0.0714	0.2343	0.1748	-0.0051	0.3687	0.0088	-0.0804

0.6279	-0.3117	0.5052	0.4887	-0.1833	0.5410	-0.6304	0.0565	-0.0296	0.0267	0.1010	0.1225	0.1213	-0.0293	0.0595
0.4809	-0.6922	0.6657	0.2524	0.2764	0.4640	-0.2892	0.0950	-0.0303	-0.1043	-0.1171	0.0792	-0.1977	0.0756	-0.0272
0.7909	0.0349	0.3999	0.7469	-0.1036	0.6400	-0.8245	-0.1031	-0.0234	0.0458	0.0563	0.0978	0.1422	-0.3079	-0.0315
0.5926	0.1573	0.2014	0.8113	-0.0124	0.5968	-0.6938	0.0274	-0.0162	-0.0265	-0.0408	0.1020	0.1518	-0.2535	0.0063
0.8234	-0.4443	0.7047	0.7507	-0.1076	0.9190	-0.9049	0.0944	-0.0374	0.0491	0.0994	0.0262	0.0722	0.0104	-0.0836
-0.8883	0.3295	-0.6675	-0.8059	0.1273	-0.8612	0.9820	-0.0699	0.0358	-0.0456	-0.1126	-0.0762	-0.0994	0.0919	0.0997
0.8914	-0.5134	0.7531	0.6708	-0.0108	0.7084	-0.8235	0.0452	0.1183	-0.0355	0.1160	0.1710	-0.0438	0.0050	-0.0301
-0.0848	0.0889	-0.1142	-0.1399	-0.0574	0.0943	-0.0037	-0.0849	0.0073	0.0814	0.1295	0.0348	-0.0230	-0.0030	-0.0340
-0.7129	0.4055	-0.5858	-0.5946	0.1077	-0.4291	0.5748	0.0604	0.0331	0.0062	0.0067	-0.0666	-0.1426	0.3028	0.0416
0.7325	-0.7409	0.8279	0.5116	0.2006	0.4344	-0.5259	0.1815	0.1536	-0.1282	0.0924	0.2033	-0.1161	0.1163	-0.0674
0.0131	0.2768	-0.1841	0.0193	-0.1444	0.2312	-0.1994	-0.0978	0.0008	0.1468	0.0267	-0.0436	0.0675	0.0299	0.1265
-0.0276	0.0148	-0.0231	-0.0251	0.0032	0.1798	0.0312	-0.0032	0.0012	-0.0034	0.0001	-0.0007	-0.0001	0.0007	0.0031
0.4338	-0.4002	0.3556	0.3500	0.3090	0.5191	-0.4157	0.1119	-0.0234	-0.0394	0.0647	-0.2608	-0.3063	0.0861	-0.1252
-0.0777	0.0209	0.0182	0.0192	-0.0803	-0.1823	0.1166	-0.0706	0.0041	0.0940	0.1490	0.0533	-0.0111	-0.0203	-0.0526
-0.7118	0.7172	-0.7976	-0.4259	-0.1819	-0.3861	0.5109	-0.0861	0.0395	0.1088	-0.0254	-0.2436	0.1121	-0.1142	-0.1345
1.0000	-0.4099	0.7476	0.7928	0.1061	0.7090	-0.8763	0.0366	0.1159	-0.0337	0.0987	0.1500	-0.0467	-0.1400	-0.0128
-0.4099	*0.9997*	-0.7499	-0.1973	-0.2037	-0.2993	0.2719	-0.3363	0.0308	0.0026	-0.0321	-0.0874	0.2320	-0.0636	0.0783
0.7476	-0.7499	*1.0001*	0.5687	0.1533	0.5401	-0.6217	0.0835	-0.0404	-0.0862	-0.0006	0.2335	-0.0830	0.1318	-0.1355
0.7928	-0.1973	0.5687	*0.9997*	-0.0253	0.6775	-0.8240	0.1010	-0.0318	-0.1152	0.0001	0.2562	0.2187	-0.1692	-0.1429
0.1061	-0.2037	0.1533	-0.0253	*0.9989*	-0.1377	0.1282	-0.0608	-0.0068	-0.3837	0.0777	0.0073	-0.4284	-0.0188	0.0797
0.7090	-0.2993	0.5401	0.6775	-0.1377	*1.0000*	-0.8609	0.0745	-0.0289	0.1381	-0.0198	0.0032	0.0111	-0.0465	-0.0895
-0.8763	0.2719	-0.6217	-0.8240	0.1282	-0.8609	*1.0002*	-0.0938	0.0349	-0.0413	-0.1405	-0.1110	-0.0992	0.1053	0.1289
0.0366	-0.3363	0.0835	0.1010	-0.0608	0.0745	-0.0938	*0.9982*	-0.0058	0.0910	0.0728	-0.0571	-0.0234	0.0126	0.0114
0.1159	0.0308	-0.0404	-0.0318	-0.0068	-0.0289	0.0349	-0.0058	*0.9981*	0.0043	-0.0050	-0.0093	0.0042	-0.0011	0.0023
-0.0337	0.0026	-0.0862	-0.1152	-0.3837	0.1381	-0.0413	0.0910	0.0043	*0.9974*	-0.0906	0.0046	-0.0722	0.0067	-0.0585
0.0987	-0.0321	-0.0006	0.0001	0.0777	-0.0198	-0.1405	0.0728	-0.0050	-0.0906	*1.0003*	-0.0526	0.0104	0.0194	0.0499
0.1500	-0.0874	0.2335	0.2562	0.0073	0.0032	-0.1110	-0.0571	-0.0093	0.0046	-0.0526	*1.0010*	-0.0087	0.0220	0.0402
-0.0467	0.2320	-0.0830	0.2187	-0.4284	0.0111	-0.0992	-0.0234	0.0042	-0.0722	0.0104	-0.0087	*1.0007*	0.0155	-0.0713
-0.1400	-0.0636	0.1318	-0.1692	-0.0188	-0.0465	0.1053	0.0126	-0.0011	0.0067	0.0194	0.0220	0.0155	*1.0017*	-0.0163
-0.0128	0.0783	-0.1355	-0.1429	0.0797	-0.0895	0.1289	0.0114	0.0023	-0.0585	0.0499	0.0402	-0.0713	-0.0163	*0.9979*
-0.0372	0.0293	-0.0367	-0.0272	-0.0027	-0.0262	0.0310	-0.0061	-0.0049	0.0034	-0.0050	-0.0088	0.0018	-0.0028	0.0013
-0.0851	0.0330	-0.0637	-0.0767	0.0142	0.1251	-0.0520	-0.0076	0.0020	-0.0069	-0.0096	-0.0084	-0.0074	0.0061	0.0075
0.1695	0.0470	0.0620	0.0683	-0.0551	0.0453	-0.0567	-0.1541	-0.0086	0.0916	0.0691	-0.0632	-0.0300	0.0206	0.0104
0.1999	-0.3124	0.3398	0.0104	0.0193	0.1916	-0.1308	-0.0958	-0.0102	-0.0003	-0.0862	-0.1312	-0.0138	0.0374	0.0681
-0.0235	0.0690	-0.1366	0.1644	0.0220	0.2460	-0.0956	-0.0165	0.0037	-0.0107	-0.0184	-0.0180	-0.0139	0.0184	0.0193
-0.0206	-0.0640	0.0492	0.0532	0.1461	-0.1306	0.0689	0.1073	0.0009	-0.1477	-0.0721	0.0222	-0.0533	-0.0105	-0.0775
0.0640	-0.0779	0.0971	0.1212	0.0598	0.1054	0.0278	0.0827	-0.0064	-0.0847	-0.1288	-0.0353	0.0237	0.0039	0.0366
-0.0673	-0.0412	0.0721	0.0767	-0.0048	0.0771	0.0561	0.0068	-0.0001	0.0000	0.0112	0.0154	0.0044	-0.0074	-0.0034
-0.0528	-0.2397	0.1483	-0.1686	0.0662	-0.1145	0.1566	-0.0014	-0.0008	-0.0522	0.0476	0.0290	-0.0611	-0.0243	-0.1042
0.1511	0.4049	-0.1170	0.1764	0.0212	0.0539	-0.1133	-0.0079	0.0005	-0.0068	-0.0190	-0.0203	-0.0182	0.0229	0.0149
0.0645	0.0998	0.1316	0.1006	0.0263	0.1838	-0.1706	-0.0215	0.0044	-0.0114	-0.0265	-0.0310	-0.0191	0.0259	0.0274
0.0842	-0.0328	0.0635	0.0771	0.5054	-0.1255	0.0521	0.0088	-0.0019	0.0099	0.0086	0.0086	0.0114	-0.0061	-0.0091
-0.0190	-0.0145	-0.0135	-0.0116	-0.0574	-0.0154	0.0112	0.0290	0.0015	0.0325	0.0090	0.0273	0.0485	-0.0082	0.0311
0.1725	-0.0785	0.1608	0.1503	0.2324	0.1405	-0.1515	-0.1120	-0.0106	-0.1509	-0.0049	-0.0810	-0.2034	0.0237	-0.1509

0.1296	0.0934	0.1983	0.2510	0.0258	-0.0043	0.0306	-0.0907	0.0317	0.0380	-0.0257	-0.0922	-0.4456	-0.2056
-0.0286	-0.0395	0.0845	0.2964	-0.0833	-0.0238	0.3124	0.0441	0.3008	-0.0627	-0.1159	0.0383	-0.0036	0.0240
-0.0196	-0.0748	0.1096	0.1221	0.0715	-0.0384	-0.0690	-0.1569	-0.0420	0.3115	0.2447	0.0760	0.0139	-0.0331
-0.0121	-0.0628	-0.0012	-0.0766	0.2481	0.1126	0.0583	0.0566	-0.3978	0.2540	0.2006	0.0619	-0.0171	0.0779
-0.0343	0.0758	0.0620	0.2726	0.1466	-0.1151	0.0536	0.0871	-0.1199	0.0002	0.0773	-0.0763	-0.0034	0.1589
0.0320	-0.0455	-0.0328	-0.2295	-0.0824	0.0506	0.0417	0.0482	0.1302	-0.1011	-0.1502	0.0453	0.0044	-0.1340
0.1214	0.0782	0.1862	0.2322	-0.0117	-0.0313	0.0699	-0.0745	-0.0750	0.0072	-0.0795	-0.0792	-0.0202	0.1857
0.0067	0.0000	-0.0836	0.0519	0.0005	0.0834	0.1256	-0.0042	-0.0263	0.0010	0.0023	0.0010	-0.0004	-0.0166
0.0312	0.0593	-0.3681	-0.3137	0.1261	-0.1216	-0.0877	-0.0676	0.0760	0.1164	0.1781	-0.0608	-0.0258	0.0714
0.1571	-0.0571	0.1607	0.1884	-0.1195	-0.0108	0.1778	0.0670	0.0848	-0.1015	-0.1702	0.0558	-0.0371	0.2343
0.0001	0.5089	-0.1054	-0.0800	-0.0382	-0.3801	0.0520	0.0100	-0.4168	-0.0345	-0.0445	0.0209	-0.0295	0.1748
0.0011	-0.0036	-0.0021	-0.0087	-0.0071	0.0044	-0.0045	-0.0029	0.0026	-0.0011	-0.0048	0.0037	0.0004	-0.0051
-0.0196	-0.0461	0.1016	0.1241	0.4217	0.0554	0.1258	0.0405	-0.1509	-0.0786	-0.1298	0.0425	-0.0511	0.3687
0.0042	0.0127	-0.0676	0.0930	0.0246	0.0687	0.1334	-0.0090	-0.0493	0.0202	0.0309	-0.0118	-0.0094	0.0088
0.0371	0.0551	-0.0639	-0.3614	0.1148	-0.0051	-0.1108	0.2401	-0.0836	0.0998	0.1656	-0.0540	-0.0042	-0.0804
-0.0372	-0.0851	0.1695	0.1999	-0.0235	-0.0206	0.0640	-0.0673	-0.0528	0.1511	0.0645	0.0842	-0.0190	0.1725
0.0293	0.0330	0.0470	-0.3124	0.0690	-0.0640	-0.0779	-0.0412	-0.2397	0.4049	0.0998	-0.0328	-0.0145	-0.0785
-0.0367	-0.0637	0.0620	0.3398	-0.1366	0.0492	0.0971	0.0721	0.1483	-0.1170	0.1316	0.0635	-0.0135	0.1608
-0.0272	-0.0767	0.0683	0.0104	0.1644	0.0532	0.1212	0.0767	-0.1686	0.1764	0.1006	0.0771	-0.0116	0.1503
-0.0027	0.0142	-0.0551	0.0193	0.0220	0.1461	0.0598	-0.0048	0.0662	0.0212	0.0263	0.5054	-0.0574	0.2324
-0.0262	0.1251	0.0453	0.1916	0.2460	-0.1306	0.1054	0.0771	-0.1145	0.0539	0.1838	-0.1255	-0.0154	0.1405
0.0310	-0.0520	-0.0567	-0.1308	-0.0956	0.0689	0.0278	0.0561	0.1566	-0.1133	-0.1706	0.0521	0.0112	-0.1515
-0.0061	-0.0076	-0.1541	-0.0958	-0.0165	0.1073	0.0827	0.0068	-0.0014	-0.0079	-0.0215	0.0088	0.0290	-0.1120
-0.0049	0.0020	-0.0086	-0.0102	0.0037	0.0009	-0.0064	-0.0001	-0.0008	0.0005	0.0044	-0.0019	0.0015	-0.0106
0.0034	-0.0069	0.0916	-0.0003	-0.0107	-0.1477	-0.0847	0.0000	-0.0522	-0.0068	-0.0114	0.0099	0.0325	-0.1509
-0.0050	-0.0096	0.0691	-0.0862	-0.0184	-0.0721	-0.1288	0.0112	0.0476	-0.0190	-0.0265	0.0086	0.0090	-0.0049
-0.0088	-0.0084	-0.0632	-0.1312	-0.0180	0.0222	-0.0353	0.0154	0.0290	-0.0203	-0.0310	0.0086	0.0273	-0.0810
0.0018	-0.0074	-0.0300	-0.0138	-0.0139	-0.0533	0.0237	0.0044	-0.0611	-0.0182	-0.0191	0.0114	0.0485	-0.2034
-0.0028	0.0061	0.0206	0.0374	0.0184	-0.0105	0.0039	-0.0074	-0.0243	0.0229	0.0259	-0.0061	-0.0082	0.0237
0.0013	0.0075	0.0104	0.0681	0.0193	-0.0775	0.0366	-0.0034	-0.1042	0.0149	0.0274	-0.0091	0.0311	-0.1509
0.9978	-0.0005	-0.0089	-0.0108	0.0030	0.0007	-0.0059	0.0002	-0.0020	0.0021	0.0057	0.0006	0.0074	-0.0052
-0.0005	*0.9985*	-0.0081	-0.0200	-0.0119	0.0102	-0.0031	0.0018	0.0141	-0.0070	-0.0118	0.0082	0.0075	-0.0121
-0.0089	-0.0081	*1.0021*	-0.1037	-0.0172	0.1091	0.0822	0.0114	0.0044	-0.0190	-0.0237	0.0094	0.0363	-0.1122
-0.0108	-0.0200	-0.1037	*1.0016*	-0.0397	0.0409	-0.0585	0.0232	0.0500	-0.0308	-0.0564	0.0204	0.0469	-0.1332
0.0030	-0.0119	-0.0172	-0.0397	*1.0021*	0.0148	-0.0066	0.0028	0.0275	-0.0203	-0.0292	0.0120	0.0089	-0.0286
0.0007	0.0102	0.1091	0.0409	0.0148	*0.9995*	-0.0800	-0.0039	-0.0814	0.0122	0.0180	-0.0093	0.0240	-0.1254
-0.0059	-0.0031	0.0822	-0.0585	-0.0066	-0.0800	*1.0016*	0.0020	0.0280	-0.0021	-0.0066	0.0022	0.0009	0.0128
0.0002	0.0018	0.0114	0.0232	0.0028	-0.0039	0.0020	*0.9993*	-0.0071	0.0085	0.0065	-0.0018	-0.0070	0.0096
-0.0020	0.0141	0.0044	0.0500	0.0275	-0.0814	0.0280	-0.0071	*1.0003*	0.0275	0.0328	-0.0122	0.0321	-0.1610
0.0021	-0.0070	-0.0190	-0.0308	-0.0203	0.0122	-0.0021	0.0085	0.0275	*1.0008*	-0.0286	0.0070	0.0086	-0.0230
0.0057	-0.0118	-0.0237	-0.0564	-0.0292	0.0180	-0.0066	0.0065	0.0328	-0.0286	*0.9984*	0.0118	0.0095	-0.0392
0.0006	0.0082	0.0094	0.0204	0.0120	-0.0093	0.0022	-0.0018	-0.0122	0.0070	0.0118	*1.0017*	-0.0068	0.0086
0.0074	0.0075	0.0363	0.0469	0.0089	0.0240	0.0009	-0.0070	0.0321	0.0086	0.0095	-0.0068	*0.9994*	-0.3267
-0.0052	-0.0121	-0.1122	-0.1332	-0.0286	-0.1254	0.0128	0.0096	-0.1610	-0.0230	-0.0392	0.0086	-0.3267	*1.0000*

Tests - Quadratic Synthetic Water Models

test #1: (prior mean)/(prior standard deviation)

test #2: (posterior mean)/(posterior standard deviation)

test #3: (posterior mean - prior mean)/(posterior standard deviation)

	test #1		test #2		test #3	
	formula	ratio	formula	ratio	formula	ratio
1	5/0.699=	7.15	5.3226/0.0597=	**89.16**	(5.3226-5)/0.0597=	**5.40**
a	0.8/1=	0.8	0.3642/0.0126^0.5=	**3.24**	(0.3642-0.8)/0.0126^0.5=	**-3.88**
b	0.7/1=	0.7	-0.2288/0.0295^0.5=	-1.33	(-0.2288-0.7)/0.0295^0.5=	**-5.41**
c	-1.5/1=	-1.5	-1.595/0.0109^0.5=	**-15.28**	(-1.595+1.5)/0.0109^0.5=	-0.91
d	0.4/1=	0.4	-0.1765/0.0558^0.5=	-0.75	(-0.1765-0.4)/0.0558^0.5=	**-2.44**
e	0.7/1=	0.7	0.5146/0.081^0.5=	1.81	(0.5146-0.7)/0.081^0.5=	-0.65
f	1/1=	1	0.2304/0.0601^0.5=	0.94	(0.2304-1)/0.0601^0.5=	**-3.14**
ab	0/0.0016=	0	-0.0064/0.0014^0.5=	-0.17	-0.0064/0.0014^0.5=	-0.17
ac	0/0.6021=	0	-0.2326/0.0939=	**-2.48**	-0.2326/0.0939=	**-2.48**
ad	0/0.301=	0	-0.0786/0.2012=	-0.39	-0.0786/0.2012=	-0.39
ae	0/0.4771=	0	0.2109/0.0752=	**2.80**	0.2109/0.0752=	**2.80**
af	0/0.0016=	0	0.0032/0.0399=	0.08	0.0032/0.0399=	0.08
bc	0/0.699=	0	0.0168/0.0778=	0.22	0.0168/0.0778=	0.22
bd	0/0.0016=	0	-0.0156/0.0372=	-0.42	-0.0156/0.0372=	-0.42
be	0/0.301=	0	-0.107/0.1294=	-0.83	-0.107/0.1294=	-0.83
bf	0/0.699=	0	-0.2623/0.2525=	-1.04	-0.2623/0.2525=	-1.04
cd	0/0.301=	0	-0.1424/0.1131=	-1.26	-0.1424/0.1131=	-1.26
ce	0/0.301=	0	-0.2169/0.1242=	-1.75	-0.2169/0.1242=	-1.75
cf	0/0.699=	0	-0.3421/0.1214=	**-2.82**	-0.3421/0.1214=	**-2.82**
de	0/0.301=	0	-0.0093/0.0762=	-0.12	-0.0093/0.0762=	-0.12
df	0/0.301=	0	-0.2995/0.1862=	-1.61	-0.2995/0.1862=	-1.61
ef	0/0.301=	0	0.2286/0.2719=	0.84	0.2286/0.2719=	0.84
abc	0/0.0016=	0	0.0138/0.0372=	0.37	0.0138/0.0372=	0.37
abd	0/0.0016=	0	0.0022/0.0399=	0.06	0.0022/0.0399=	0.06
abe	0/0.0016=	0	-0.0033/0.0371=	-0.09	-0.0033/0.0371=	-0.09
abf	0/0.0016=	0	0.0133/0.0373=	0.36	0.0133/0.0373=	0.36
acd	0/0.0016=	0	0.0211/0.0384=	0.55	0.0211/0.0384=	0.55
ace	0/0.0016=	0	0.00007/0.0381=	0.00	0.00007/0.0381=	0.00
acf	0/0.0016=	0	-0.0093/0.0395=	-0.24	-0.0093/0.0395=	-0.24
ade	0/0.0016=	0	-0.0173/0.038=	-0.46	-0.0173/0.038=	-0.46
adf	0/0.0016=	0	0.0009/0.0399=	0.02	0.0009/0.0399=	0.02
aef	0/0.0016=	0	0.0032/0.0398=	0.08	0.0032/0.0398=	0.08
bcd	0/0.0016=	0	0.0165/0.037=	0.45	0.0165/0.037=	0.45
bce	0/0.0016=	0	0.0326/0.0361=	0.90	0.0326/0.0361=	0.90
bcf	0/0.0016=	0	0.0103/0.0394=	0.26	0.0103/0.0394=	0.26
bde	0/0.0016=	0	-0.012/0.037=	-0.32	-0.012/0.037=	-0.32
bdf	0/0.0016=	0	0.0087/0.0376=	0.23	0.0087/0.0376=	0.23
bef	0/0.0016=	0	-0.0029/0.0398=	-0.07	-0.0029/0.0398=	-0.07
cde	0/0.0016=	0	-0.0152/0.0378=	-0.40	-0.0152/0.0378=	-0.40
cdf	0/0.0016=	0	0.0107/0.0394=	0.27	0.0107/0.0394=	0.27
cef	0/0.0016=	0	0.0145/0.0392=	0.37	0.0145/0.0392=	0.37
def	0/0.0016=	0	-0.0038/0.0397=	-0.10	-0.0038/0.0397=	-0.10
d^2	0.5/0.699=	0.72	-0.6547/0.151=	**-4.34**	(-0.6547-0.5)/0.151=	**-7.65**
e^2	0.5/0.699=	0.72	-0.1993/0.1305=	-1.53	(-0.1993-0.5)/0.1305=	**-5.36**

Note: Numbers above 1.96 correspond to 5% significance level

Numbers above 1.65 correspond to 10% significance level

Numbers above 1.04 correspond to 30% significance level

Appendix D/3: Residual Plots - Synthetic Water Models

Fit of Linear Synthetic Water Models to Synthetic Water Data

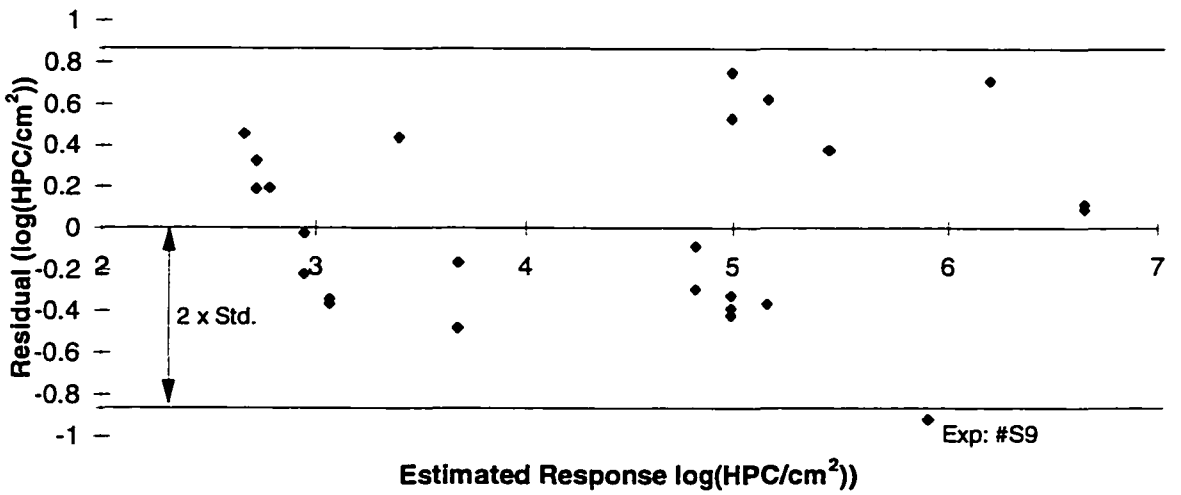


Figure D/3.1: Residual plot - linear synthetic water model fitted to synthetic water data
5% confidence, Var: 0.188, Std.: 0.433
7 reestimated parameters: l=4.369; a=0.428; c=-1.408; e=0.273; ac=-0.216; ae=0.098; cf=-0.143

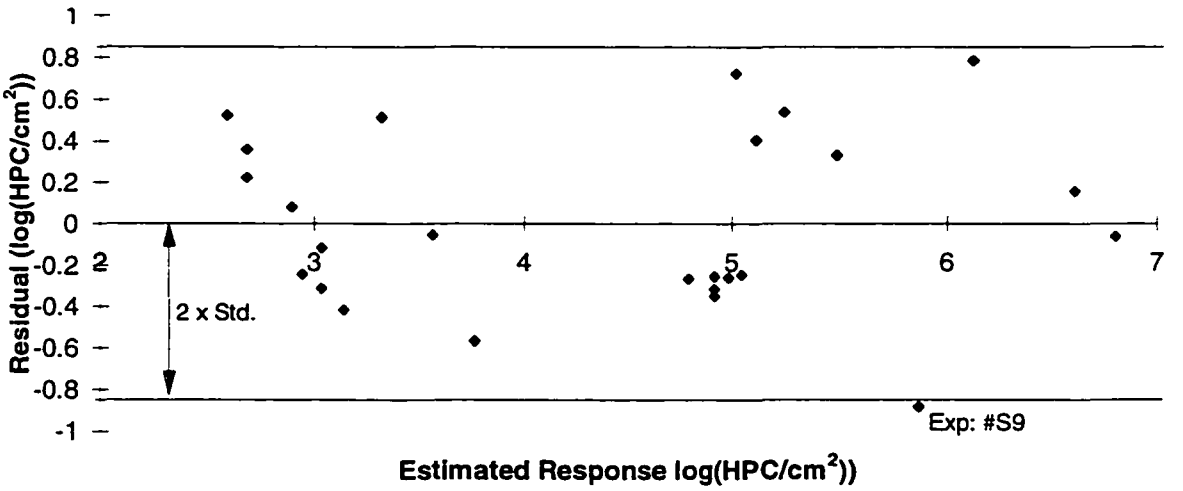


Figure D/3.2: Residual plot - linear synthetic water model fitted to synthetic water data
5% confidence + 'd' (shear), Var: 0.188, Std.: 0.433
8 reestimated parameters: l=4.392; a=0.428; c=-1.408; d=0.099; e=0.249; ac=-0.232; ae=0.118; cf=-0.124

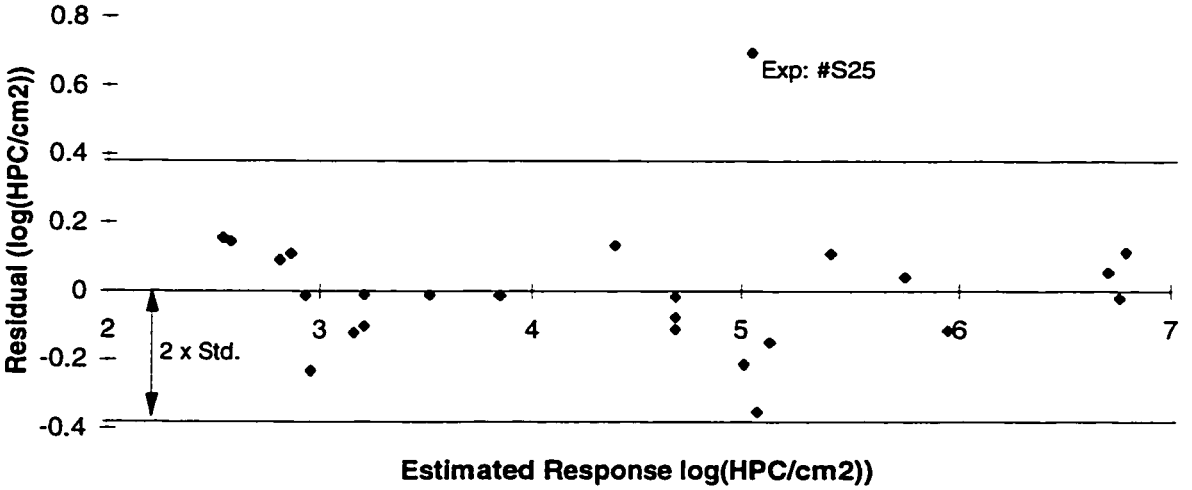


Figure D/3.3: Residual plot - linear synthetic water model fitted to synthetic water data
30% confidence; Var: 0.036, Std.: 0.190
14 reestimated parameters: l=4.627; a=0.353; b=-0.081; c=-1.512; e=0.238; f=0.390; ac=-0.268; ae=0.226; bc=0.078; be=-0.179; cd=-0.170; ce=-0.143; cf=-0.242; df=-0.195

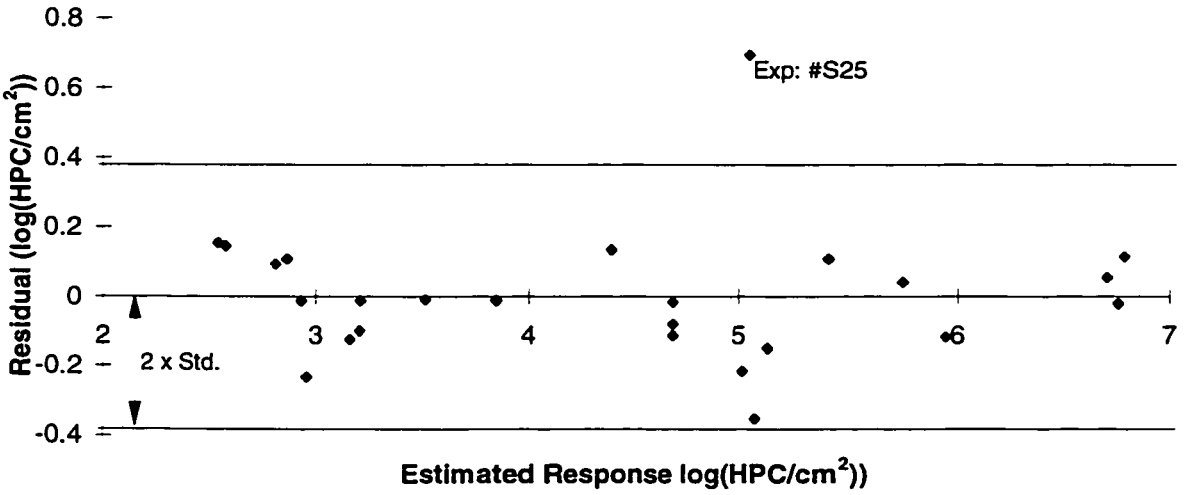


Figure D/3.4: Residual plot - linear synthetic water model fitted to synthetic water data
30% confidence + 'd' (shear); Var: 0.036, Std.: 0.190
15 reestimated parameters: l=4.627; a=0.353; b=-0.081; c=-1.512; d=0.001; e=0.238; f=0.390; ac=-0.268; ae=0.226; bc=0.079; be=-0.179; cd=-0.170; ce=-0.143; cf=-0.242; df=-0.194

Fit of Quadratic Synthetic Water Models to Synthetic Water Data

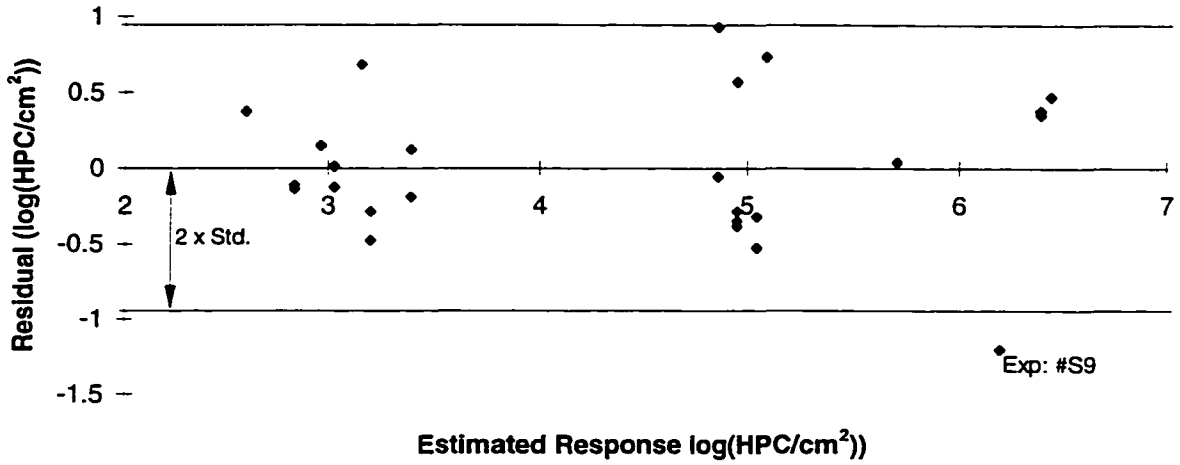


Figure D/3.5: Residual plot - quadratic synthetic water model fitted to synthetic water data
5% confidence; Var: 0.223, Std.: 0.472
7 reestimated parameters: l=5.121; a=0.426; c=-1.371; ac=-0.243; ae=0.096; cf=-0.119; d^2=-0.753

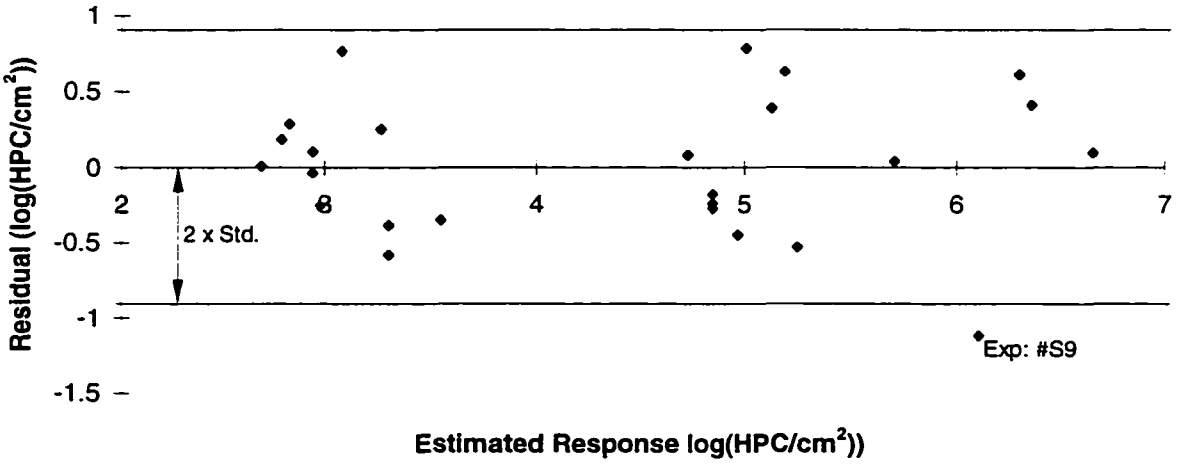


Figure D/3.6: Residual plot - quadratic synthetic water model fitted to synthetic water data
5% confidence + 'd" (shear); Var: 0.205, Std.: 0.452
8 reestimated parameters: l=5.118; a=0.428; c=-1.374; d=0.144; ac=-0.265; ae=0.125; cf=-0.095; d^2=-0.717

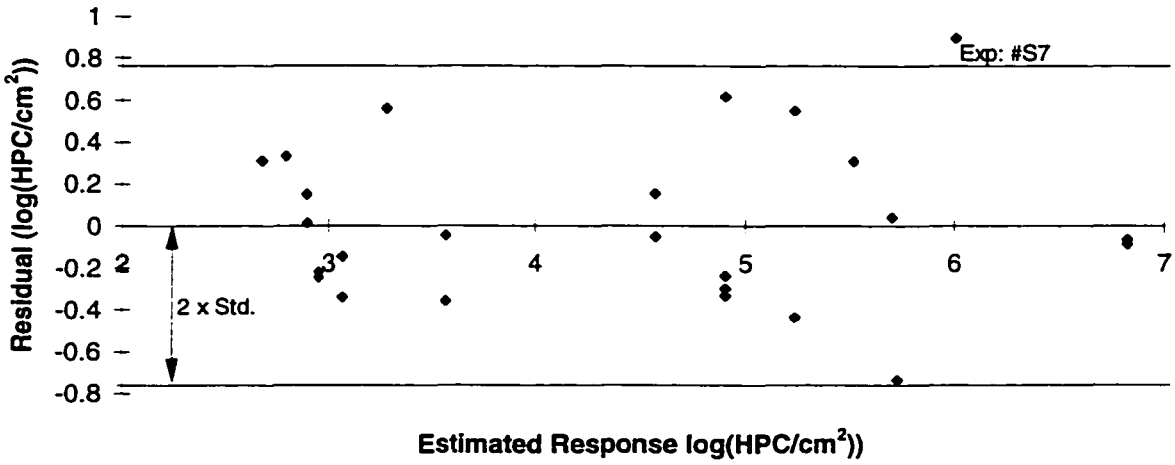


Figure D/3.7: Residual plot - quadratic synthetic water model fitted to synthetic water data
10% confidence; Var: 0.145, Std.: 0.381
9 reestimated parameters: l=5.151; a=0.443; c=-1.379; e=0.291; ac=-0.244; ae=0.110; ce=-0.152; cf=-0.142; d^2=-0.798

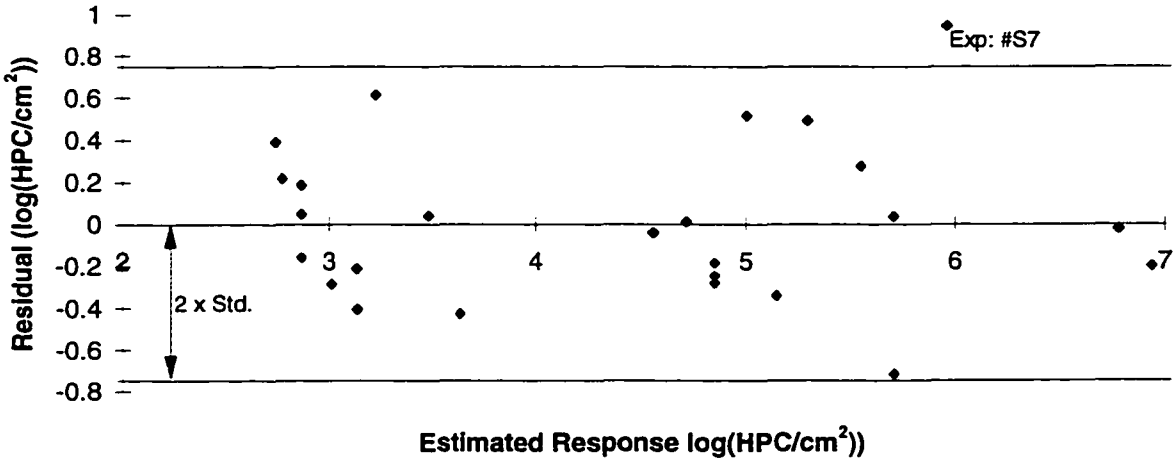


Figure D/3.8: Residual plot - quadratic synthetic water model fitted to synthetic water data
10% confidence + 'd' (shear); Var: 0.140, Std.: 0.374
10 reestimated parameters: l=5.148; a=0.442; c=-1.380; d=0.077; e=0.271; ac=-0.256; ae=0.125; ce=-0.145; cf=-0.127; d^2=-0.776

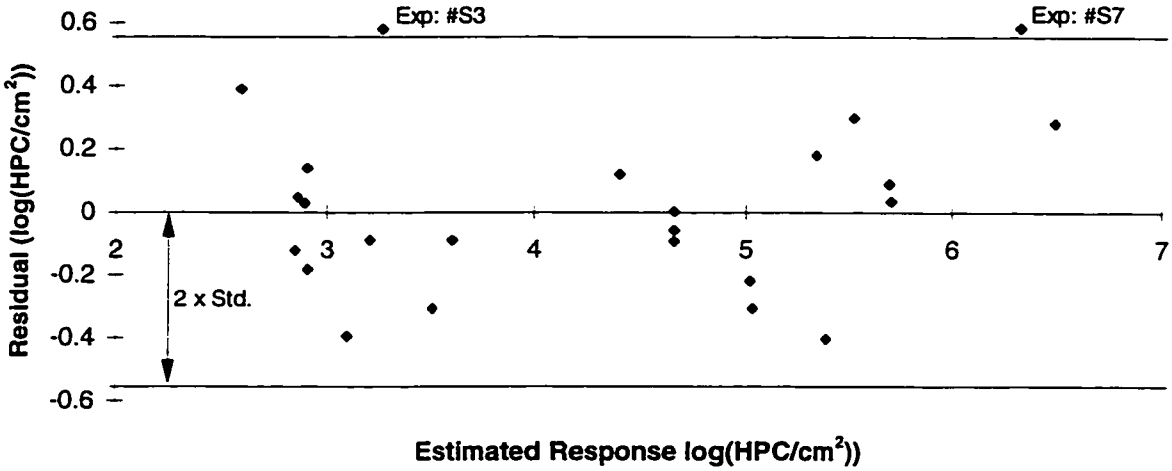


Figure D/3.9: Residual plot - quadratic synthetic water model fitted to synthetic water data
30% confidence; Var: 0.077, Std.: 0.277
14 reestimated parameters: l=5.057; a=0.399; b=-0.129; c=-1.477; e=0.321; ac=-0.226; ae=0.107; bf=-0.152; cd=-0.205; ce=-0.118; cf=-0.179; df=-0.134; d^2=-0.707; e^2=0.025

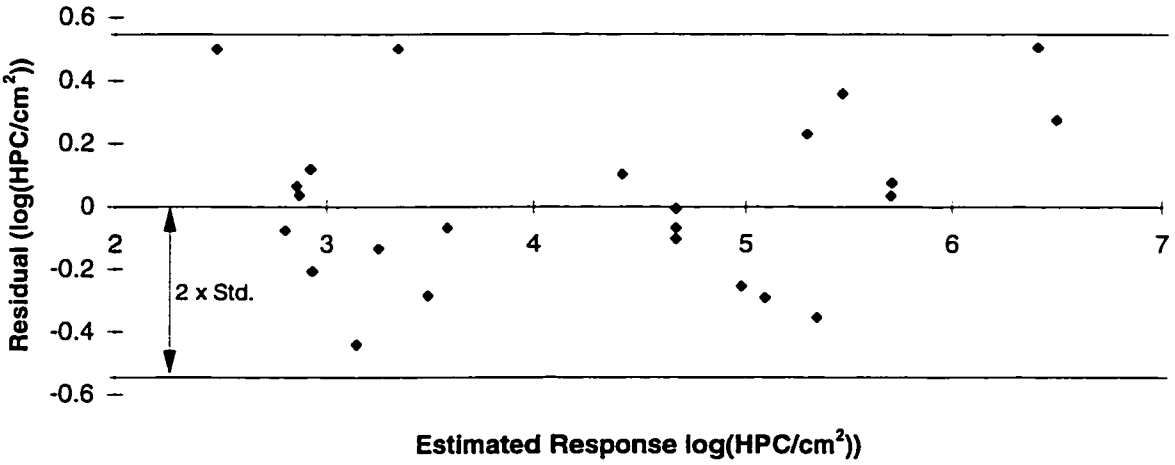


Figure D/3.10: Residual plot - quadratic synthetic water model fitted to synthetic water data
30% confidence + 'd' (shear); Var: 0.075, Std.: 0.273
15 reestimated parameters: l=5.036; a=0.371; b=-0.122; c=-1.482; d=-0.072; e=0.341; ac=-0.224; ae=0.104; bf=-0.150; cd=-0.193; ce=-0.128; cf=-0.190; df=-0.188; d^2=-0.725; e^2=0.055

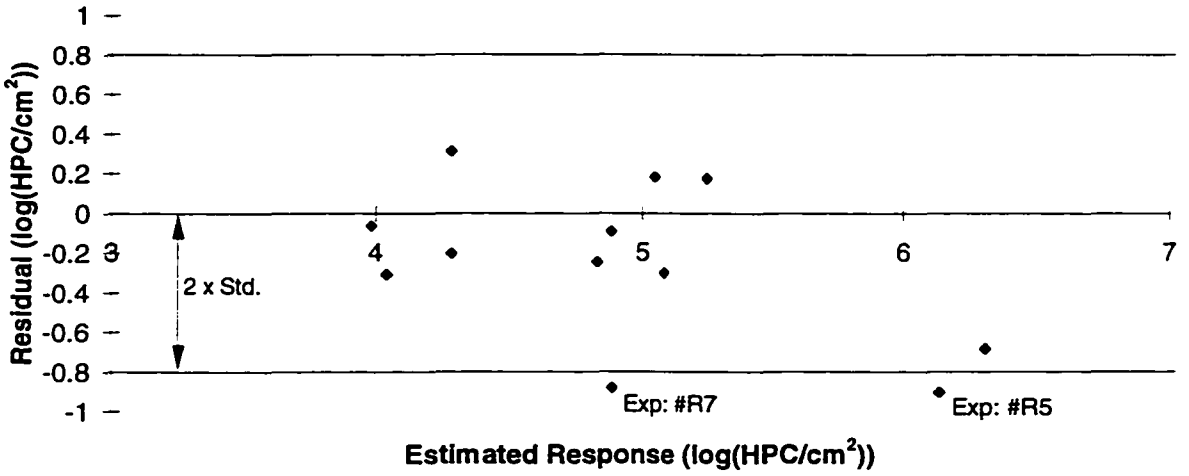


Figure D/3.11: Residual plot - linear synthetic water model fitted to real water data
5% confidence, Var: 0.160, Std.: 0-400
7 reestimated parameters: l=4.369; a=0.428; c=-1.408; e=0.273; ac=-0.216; ae=0.098; cf=-0.143

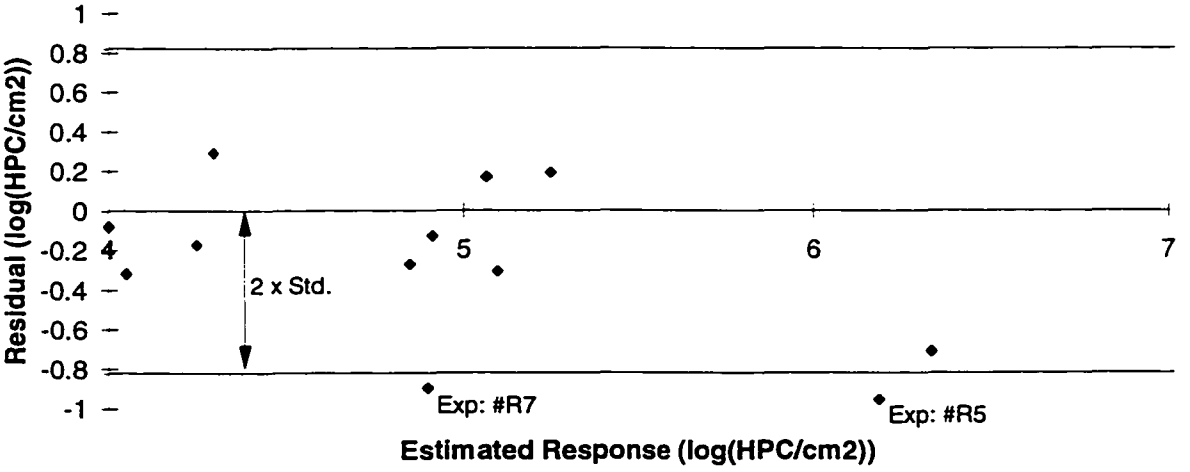


Figure D/3.12: Residual plot - linear synthetic water model fitted to real water data
5% confidence + 'd' (shear), Var: 0.168, Std.: 0.410
8 reestimated parameters: l=4.392; a=0.428; c=-1.408; d=0.099; e=0.249; ac=-0.232; ae=0.118; cf=-0.124

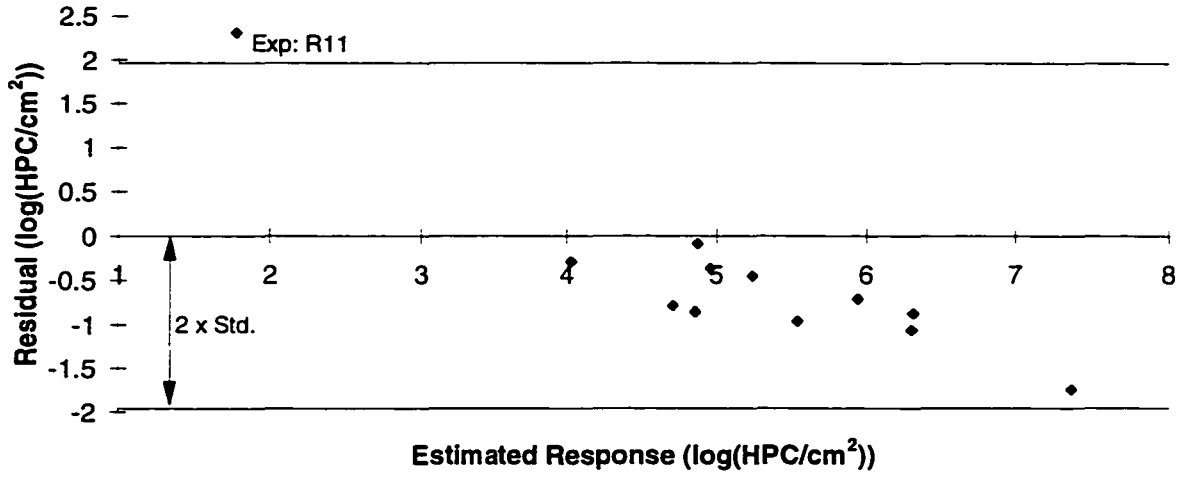


Figure D/3.13: Residual plot - linear synthetic water model fitted to real water data
30% confidence; Var: 0.964, Std.: 0.982
14 reestimated parameters: l=4.627; a=0.353; b=-0.081; c=-1.512; e=0.238; f=0.390; ac=-0.268; ae=0.226; bc=0.078; be=-0.179; cd=-0.170; ce=-0.143; cf=-0.242; df=-0.195

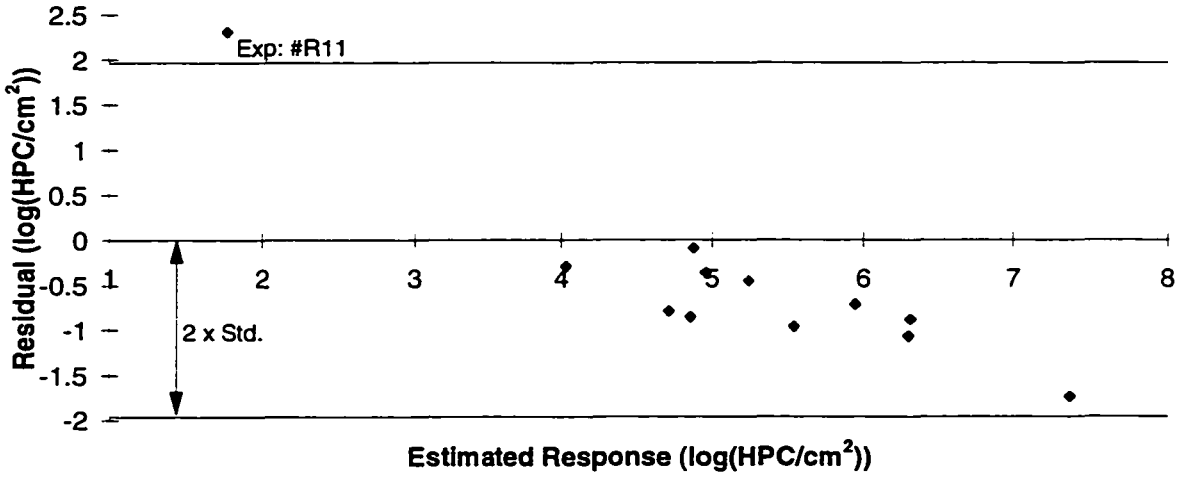


Figure D/3.14: Residual plot - linear synthetic water model fitted to real water data
30% confidence + 'd' (shear); Var: 0.964, Std.: 0.982
15 reestimated parameters: l=4.627; a=0.353; b=-0.081; c=-1.512; d=0.001; e=0.238; f=0.390; ac=-0.268; ae=0.226; bc=0.079; be=-0.179; cd=-0.170; ce=-0.143; cf=-0.242; df=-0.194

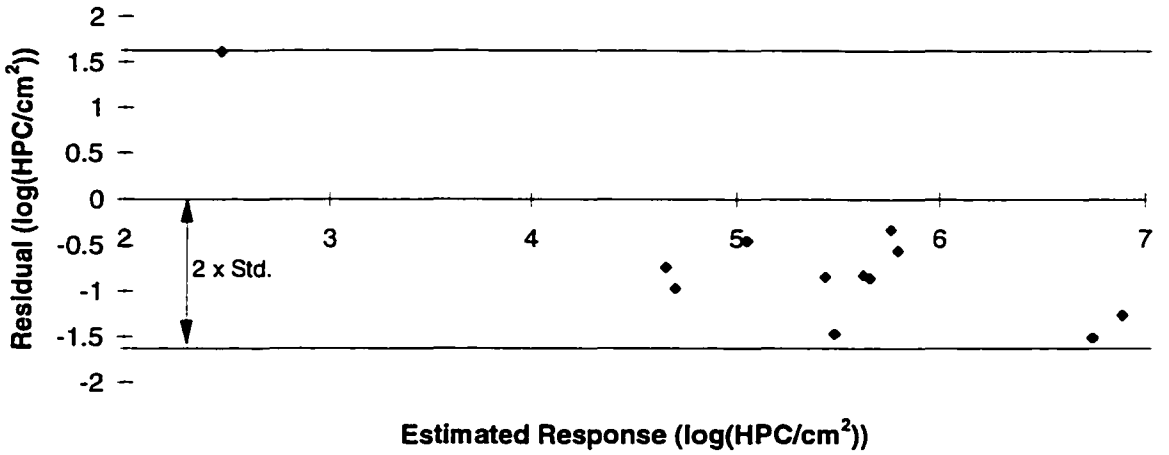


Figure D/3.15: Residual plot - quadratic synthetic water model fitted to real water data
5% confidence; Var: 0.0.665, Std.: 0.815
7 reestimated parameters: l=5.121; a=0.426; c=-1.371; ac=-0.243; ae=0.096; cf=-0.119; d^2=-0.753

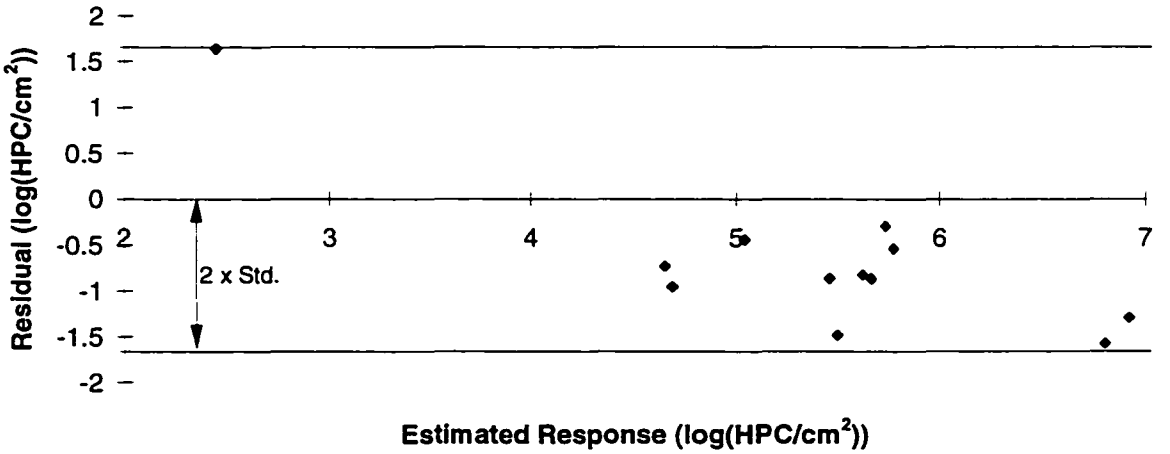


Figure D/3.16: Residual plot - quadratic synthetic water model fitted to real water data
5% confidence + 'd" (shear); Var: 0.691, Std.: 0.831
8 reestimated parameters: l=5.118; a=0.428; c=-1.374; d=0.144; ac=-0.265; ae=0.125; cf=-0.095; d^2=-0.717

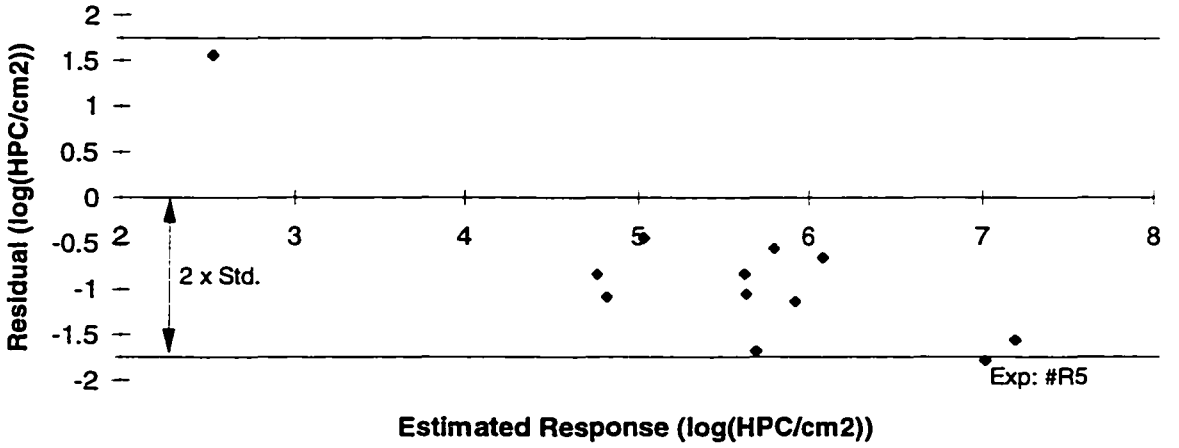


Figure D/3.17: Residual plot - quadratic synthetic water model fitted to real water data
10% confidence; Var: 0.759, Std.: 0.871
9 reestimated parameters: l=5.151; a=0.443; c=-1.379; e=0.291; ac=-0.244; ae=0.110; ce=-0.152; cf=-0.142; d^2=-0.798

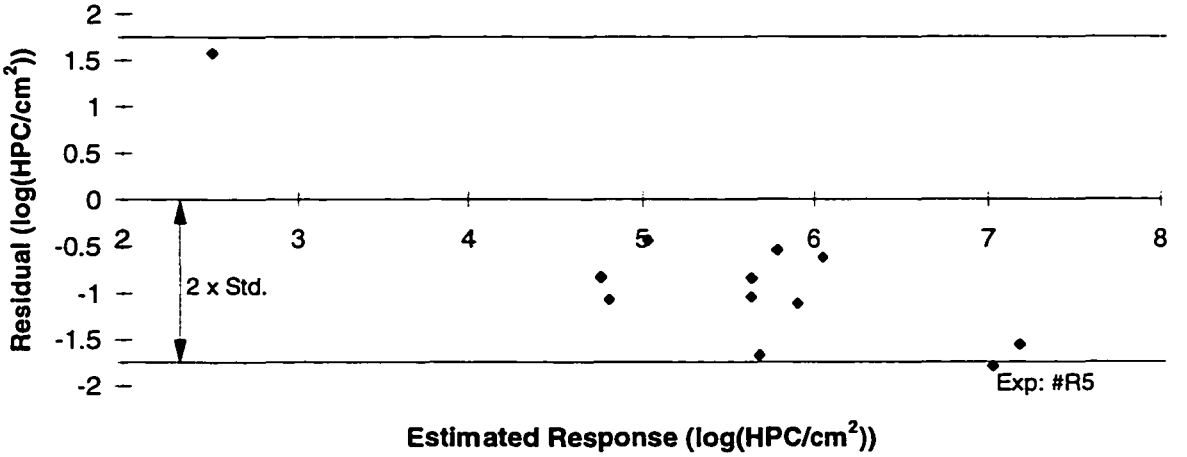


Figure D/3.18: Residual plot - quadratic synthetic water model fitted to real water data
10% confidence + 'd' (shear); Var: 0.766, Std.: 0.875
10 reestimated parameters: l=5.148; a=0.442; c=-1.380; d=0.077; e=0.271; ac=-0.256; ae=0.125; ce=-0.145; cf=-0.127; d^2=-0.776

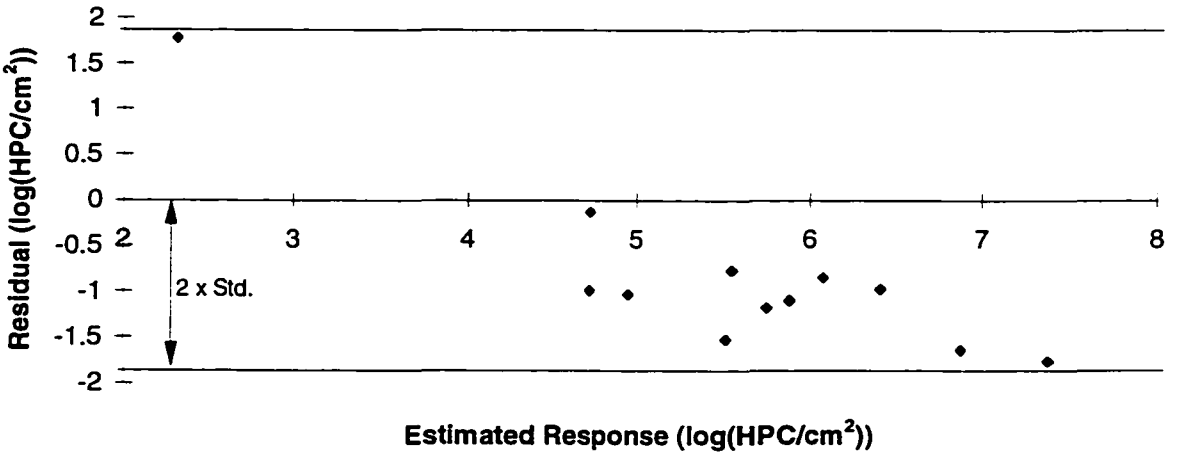


Figure D/3.19: Residual plot - quadratic synthetic water model fitted to real water data
30% confidence; Var: 0.869, Std.: 0.932
14 reestimated parameters: l=5.057; a=0.399; b=-0.129; c=-1.477; e=0.321; ac=-0.226; ae=0.107; bf=-0.152; cd=-0.205; ce=-0.118; cf=-0.179; df=-0.134; d^2=-0.707; e^2=0.025

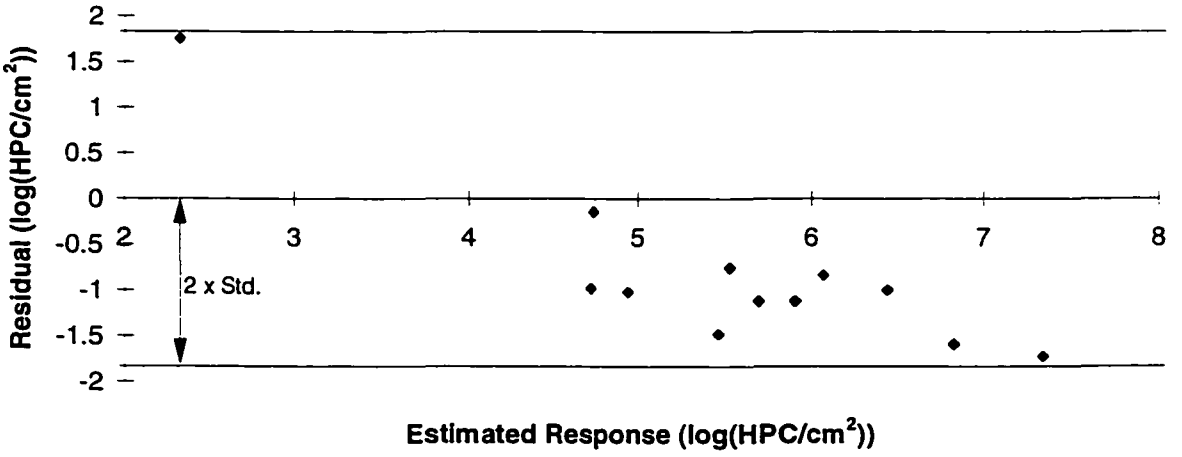


Figure D/3.20: Residual plot - quadratic synthetic water model fitted to real water data
30% confidence + 'd' (shear); Var: 0.839, Std.: 0.916
15 reestimated parameters: l=5.036; a=0.371; b=-0.122; c=-1.482; d=-0.072; e=0.341; ac=-0.224; ae=0.104; bf=-0.150; cd=-0.193; ce=-0.128; cf=-0.190; df=-0.188; d^2=-0.725; e^2=0.055

Appendix E: Real Water Models

E/1 Tests - Linear Real Water Models

E/2 Tests - Quadratic Real Water Models

E/3 Residual Plots - Real Water Models

Appendix E/1: Tests - Linear Real Water Models

Prior Covariance Matrix = 2 x Posterior Covariance Matrix of Linear Synthetic Water Model

test #1: (prior mean)/(prior standard deviation)

test #2: (posterior mean)/(posterior standard deviation)

test #3: (posterior mean - prior mean)/(posterior standard deviation)

	test #1		test #2		test #3	
	formula	ratio	formula	ratio	formula	ratio
1	4.567/0.0793^0.5=	**16.23**	4.294/0.0036^0.5=	**71.57**	(4.294-4.567)/0.0036^0.5=	**-4.55**
a	0.371/0.0252^0.5=	**2.34**	0.173/0.0055^0.5=	**2.33**	(0.173-0.371)/0.0055^0.5=	**-2.67**
b	-0.235/0.059^0.5=	-0.97	-0.084/0.0031^0.5=	-1.51	(-0.084+0.235)/0.0031^0.5=	**2.71**
c	-1.578/0.0217^0.5=	**-10.71**	-1.342/0.0071^0.5=	**-15.93**	(-1.342+1.578)/0.0071^0.5=	**2.80**
d	-0.057/0.1085^0.5=	-0.17	-0.178/0.0151^0.5=	-1.45	(-0.178+0.057)/0.0151^0.5=	-0.98
e	0.396/0.1589^0.5=	0.99	0.245/0.0095795=	**2.51**	(0.245-0.396)/0.0095795=	-1.55
f	0.354/0.1158^0.5=	1.04	0.138/0.0035^0.5=	**2.33**	(0.138-0.354)/0.0035^0.5=	**-3.65**
ab	-0.009/0.0028^0.5=	-0.17	0.051/0.0022^0.5=	1.09	(0.051+0.009)/0.0022^0.5=	1.28
ac	-0.224/0.0175^0.5=	-1.69	-0.1/0.0056^0.5=	-1.34	(-0.1+0.224)/0.0056^0.5=	1.66
ad	0.036/0.0763^0.5=	0.13	-0.319/0.0096^0.5=	**-3.26**	(-0.319-0.036)/0.0096^0.5=	**-3.62**
ae	0.242/0.011^0.5=	**2.31**	0.197/0.0082^0.5=	**2.18**	(0.197-0.242)/0.0082^0.5=	-0.50
af	0.003/0.0032^0.5=	0.05	-0.01/0.002^0.5=	-0.22	(-0.01-0.003)/0.002^0.5=	-0.29
bc	0.090/0.0104^0.5=	0.88	0.021/0.006^0.5=	0.27	(0.021-0.09)/0.006^0.5=	-0.89
bd	-0.016/0.0028^0.5=	-0.30	-0.009/0.0026^0.5=	-0.18	(-0.009+0.016)/0.0026^0.5=	0.14
be	-0.144/0.0332^0.5=	-0.79	0.107/0.0068^0.5=	1.30	(0.107+0.144)/0.0068^0.5=	**3.04**
bf	-0.145/0.1235^0.5=	-0.41	-0.052/0.003^0.5=	-0.95	(-0.052+0.145)/0.003^0.5=	1.70
cd	-0.181/0.0254^0.5=	-1.14	0.095/0.0101^0.5=	0.95	(0.095+0.181)/0.0101^0.5=	**2.75**
ce	-0.160/0.03^0.5=	-0.92	-0.249/0.0087^0.5=	**-2.67**	(-0.249+0.160)/0.0087^0.5=	-0.95
cf	-0.290/0.0288^0.5=	-0.29	-0.168/0.0057^0.5=	**-2.23**	(-0.168+0.290)/0.0057^0.5=	1.62
de	0.025/0.011^0.5=	0.24	0.023/0.0076^0.5=	0.26	(0.023-0.025)/0.0076^0.5=	-0.02
df	-0.229/0.0679^0.5=	-0.88	-0.149/0.0158^0.5=	-1.19	(-0.149+0.229)/0.0158^0.5=	0.64
ef	0.109/0.1443^0.5=	0.29	-0.052/0.0077^0.5=	-0.59	(-0.052-0.109)/0.0077^0.5=	-1.83
abc	0.006/0.0027^0.5=	0.12	-0.035/0.0023^0.5=	-0.73	(-0.035-0.006)/0.0023^0.5=	-0.85
abd	0.001/0.0032^0.5=	0.02	0.069/0.0026^0.5=	1.35	(0.069-0.001)/0.0026^0.5=	1.33
abe	-0.015/0.0027^0.5=	-0.29	0.02/0.0024^0.5=	0.41	(0.02+0.015)/0.0024^0.5=	0.71
abf	0.015/0.0028^0.5=	0.28	-0.034/0.0017^0.5=	-0.82	(-0.034-0.015)/0.0C17^0.5=	-1.19
acd	0.017/0.0029^0.5=	0.32	0.043/0.0023^0.5=	0.90	(0.043-0.017)/0.0023^0.5=	0.54
ace	-0.015/0.0028^0.5=	-0.28	0/0.0023^0.5=	0.00	(0+0.015)/0.0023^0.5=	0.31
acf	-0.008/0.0031^0.5=	-0.14	-0.003/0.002^0.5=	-0.07	(-0.003+0.008)/0.002^0.5=	0.11
ade	-0.030/0.0028^0.5=	-0.57	-0.065/0.0025^0.5=	-1.30	(-0.065+0.030)/0.0025^0.5=	-0.70
adf	0.002/0.0032^0.5=	0.04	-0.021/0.0027^0.5=	-0.40	(-0.021-0.002)/0.0027^0.5=	-0.44
aef	0.003/0.0032^0.5=	0.05	-0.032/0.0023^0.5=	-0.67	(-0.032-0.003)/0.0023^0.5=	-0.73
bcd	0.010/0.0027^0.5=	0.19	-0.012/0.0024^0.5=	-0.24	(-0.012-0.010)/0.0024^0.5=	-0.45
bce	0.026/0.0026^0.5=	0.51	-0.015/0.0022^0.5=	-0.32	(-0.015-0.026)/0.0022^0.5=	-0.87
bcf	0.009/0.0031^0.5=	0.16	0.017/0.0028^0.5=	0.32	(0.017-0.009)/0.0028^0.5=	0.15
bde	-0.023/0.0027^0.5=	-0.44	0.004/0.0024^0.5=	0.08	(0.004+0.023)/0.0024^0.5=	0.55
bdf	0.010/0.0028^0.5=	0.19	-0.006/0.0024^0.5=	-0.12	(-0.006-0.010)/0.0024^0.5=	-0.33
bef	-0.003/0.0032^0.5=	-0.05	-0.015/0.0025^0.5=	-0.30	(-0.015+0.003)/0.0025^0.5=	-0.24
cde	-0.029/0.0028^0.5=	-0.55	-0.064/0.0025^0.5=	-1.28	(-0.064+0.029)/0.0025^0.5=	-0.70
cdf	0.010/0.0031^0.5=	0.18	0.078/0.0026^0.5=	1.53	(0.078-0.010)/0.0026^0.5=	1.33
cef	0.011/0.0031^0.5=	0.20	0.09/0.0023^0.5=	1.88	(0.09-0.011)/0.0023^0.5=	1.65
def	-0.004/0.0032^0.5=	-0.07	0.022/0.0028^0.5=	0.42	(0.022+0.004)/0.0028^0.5=	0.49

Note: Numbers above 1.96 correspond to 5% significance level
Numbers above 1.65 correspond to 10% significance level
Numbers above 1.04 correspond to 30% significance level

Prior Covariance Matrix = 3 x Posterior Covariance Matrix of Linear Synthetic Water Model

test #1: (prior mean)/(prior standard deviation)

test #2: (posterior mean)/(posterior standard deviation)

test #3: (posterior mean - prior mean)/(posterior standard deviation)

	test #1		test #2		test #3	
	formula	ratio	formula	ratio	formula	ratio
1	4.567/0.1188^0.5=	**13.25**	4.284/0.0047^0.5=	**62.49**	(4.284-4.567)/0.0047^0.5=	**-4.13**
a	0.371/0.0379^0.5=	1.91	0.179/0.0077^0.5=	**2.04**	(0.179-0.371)/0.0077^0.5=	**-2.19**
b	-0.235/0.0885^0.5=	-0.79	-0.082/0.004^0.5=	1.30	(-0.082+0.235)/0.004^0.5=	**5.01**
c	-1.578/0.0325^0.5=	**-8.75**	-1.343/0.0103^0.5=	**-13.23**	(-1.343+1.578)/0.0103^0.5=	**2.32**
d	-0.057/0.1628^0.5=	-0.14	-0.18/0.02^0.5=	-1.27	(-0.18+0.057)/0.02^0.5=	-0.87
e	0.396/0.2384^0.5=	0.81	0.248/0.0125795=	**2.22**	(0.248-0.396)/0.0125795=	-1.32
f	0.354/0.1737^0.5=	0.85	0.144/0.0043^0.5=	**2.20**	(0.144-0.354)/0.0043^0.5=	**-3.20**
ab	-0.009/0.0043^0.5=	-0.14	0.058/0.0033^0.5=	1.01	(0.058+0.009)/0.0033^0.5=	1.17
ac	-0.224/0.0263^0.5=	-1.38	-0.082/0.0076^0.5=	-0.94	(-0.082+0.224)/0.0076^0.5=	1.63
ad	0.036/0.1145^0.5=	0.11	-0.315/0.0136^0.5=	**-2.70**	(-0.315-0.036)/0.0136^0.5=	**-3.01**
ae	0.242/0.0164^0.5=	1.89	0.196/0.012^0.5=	1.79	(0.196-0.242)/0.012^0.5=	-0.42
af	0.003/0.0048^0.5=	0.04	-0.008/0.0028^0.5=	-0.15	(-0.008-0.003)/0.0028^0.5=	-0.21
bc	0.090/0.0156^0.5=	0.72	0.025/0.0086^0.5=	0.27	(0.025-0.090)/0.0086^0.5=	0.27
bd	-0.016/0.0042^0.5=	-0.25	-0.006/0.0039^0.5=	-0.10	(-0.006+0.016)/0.0039^0.5=	0.16
be	-0.144/0.0498^0.5=	-0/65	0.107/0.0096^0.5=	1.09	(0.107+0.144)/0.0096^0.5=	**2.56**
bf	-0.145/0.1853^0.5=	-0.34	-0.042/0.0036^0.5=	-0.70	(-0.042+0.145)/0.0036^0.5=	1.72
cd	-0.181/0.0381^0.5=	-0.93	0.104/0.0147^0.5=	0.86	(0.104+0.181)/0.0147^0.5=	**2.35**
ce	-0.160/0.045^0.5=	-0.75	-0.241/0.0126^0.5=	**-2.15**	(-0.241+0.160)/0.0126^0.5=	-0.72
cf	-0.290/1.0431^0.5=	-0.28	-0.168/0.0078^0.5=	-1.90	(-0.168+0.290)/0.0078^0.5=	1.38
de	0.025/0.0165^0.5=	0.19	0.025/0.0109^0.5=	0.24	(0.025-0.025)/0.0109^0.5=	0.00
df	-0.229/0.1018^0.5=	-0.72	-0.149/0.021^0.5=	-1.02	(-0.149+0.229)/0.021^0.5=	0.56
ef	0.109/0.2164^0.5=	0.23	-0.043/0.0098^0.5=	-0.43	(-0.043-0.109)/0.0098^0.5=	-1.54
abc	0.006/0.0041^0.5=	0.09	-0.044/0.0034^0.5=	-0.75	(-0.044-0.006)/0.0034^0.5=	-0.86
abd	0.001/0.0048^0.5=	0.01	0.079/0.0038^0.5=	1.28	(0.079-0.001)/0.0038^0.5=	1.27
abe	-0.015/0.004^0.5=	-0.24	0.02/0.0034^0.5=	0.34	(0.02+0.015)/0.0034^0.5=	0.60
abf	0.015/0.0042^0.5=	0.23	-0.042/0.0025^0.5=	-0.84	(-0.042-0.015)/0.0025^0.5=	-1.14
acd	0.017/0.0044^0.5=	0.26	0.04/0.0034^0.5=	0.69	(0.04-0.017)/0.0034^0.5=	0.39
ace	-0.015/0.0042^0.5=	-0.23	0.001/0.0033^0.5=	0.02	(0.001+0.015)/0.0033^0.5=	0.28
acf	-0.008/0.0047^0.5=	-0.12	0.014/0.0027^0.5=	0.27	(0.014+0.008)/0.0027^0.5=	0.42
ade	-0.030/0.0042^0.5=	-0.46	-0.074/0.0037^0.5=	-1.22	(-0.074+0.030)/0.0037^0.5=	-0.72
adf	0.002/0.0048^0.5=	0.03	-0.02/0.004^0.5=	-0.32	(-0.02-0.002)/0.004^0.5=	-0.35
aef	0.003/0.0047^0.5=	0.04	-0.035/0.0033^0.5=	-0.61	(-0.035-0.003)/0.0033^0.5=	-0.66
bcd	0.010/0.0041^0.5=	0.16	-0.016/0.0037^0.5=	-0.26	(-0.016-0.010)/0.0037^0.5=	-0.43
bce	0.026/0.0038^0.5=	0.42	-0.01/0.0032^0.5=	-0.18	(-0.01-0.026)/0.0032^0.5=	-0.64
bcf	0.009/0.0047^0.5=	0.13	0.013/0.0041^0.5=	0.20	(0.013-0.009)/0.0041^0.5=	0.06
bde	-0.023/0.004^0.5=	-0.36	0.009/0.0035^0.5=	0.15	(0.009+0.023)/0.0035^0.5=	0.54
bdf	0.010/0.0042^0.5=	0.15	-0.007/0.0036^0.5=	-0.12	(-0.007-0.010)/0.0036^0.5=	-0.28
bef	-0.003/0.0047^0.5=	-0.04	-0.02/0.0035^0.5=	-0.34	(-0.02+0.003)/0.0035^0.5=	-0.29
cde	-0.029/0.0042^0.5=	-0.45	-0.061/0.0037^0.5=	-1.00	(-0.061+0.029)/0.0037^0.5=	-0.53
cdf	0.010/0.0047^0.5=	0.15	0.081/0.0039^0.5=	1.30	(0.081-0.010)/0.0039^0.5=	1.14
cef	0.011/0.0046^0.5=	0.16	0.092/0.0034^0.5=	1.58	(0.092-0.011)/0.0034^0.5=	1.39
def	-0.004/0.0047^0.5=	-0.06	0.023/0.004^0.5=	0.36	(0.023+0.004)/0.004^0.5=	0.43

Note: Numbers above 1.96 correspond to 5% significance level
Numbers above 1.65 correspond to 10% significance level
Numbers above 1.04 correspond to 30% significance level

Appendix E/2: Tests - Quadratic Real Water Models - Test Summary

Prior Covariance Matrix = 2 x Posterior Covariance Matrix of Quadratic Synthetic Water Model

test #1: (prior mean)/(prior standard deviation)

test #2: (posterior mean)/(posterior standard deviation)

test #3: (posterior mean - prior mean)/(posterior standard deviation)

	test #1		test #2		test #3	
	formula	ratio	formula	ratio	formula	ratio
1	5.323/0.1194^0.5=	**15.40**	4.458/0.0036^0.5=	74.30	(4.458-5.323)/0.0036^0.5=	**-14.42**
a	0.364/0.0253^0.5=	**2.29**	0.315/0.0055^0.5=	4.25	(0.315-0.364)/0.0055^0.5=	-0.66
b	-0.229/0.059^0.5=	-0.94	0.0992/0.0031^0.5=	1.78	(0.0992+0.229)/0.0031^0.5=	**5.89**
c	-1.595/0.021^0.5=	**-10.83**	-1.376/0.007^0.5=	-16.33	(-1.376+1.595)/0.007^0.5=	**2.60**
d	-0.177/0.1116^0.5=	-0.53	-0.067/0.0151^0.5=	-0.55	(-0.067+0.177)/0.0151^0.5=	0.90
e	0.515/0.1621^0.5=	1.28	0.224/0.0095^0.5=	2.30	(0.224-0.515)/0.0095^0.5=	**-2.99**
f	0.23/0.1201^0.5=	0.66	0.181/0.0035^0.5=	3.06	(0.181-0.23)/0.0035^0.5=	-0.83
ab	-0.006/0.0028^0.5=	-0.11	0.004/0.0022^0.5=	0.09	(0.004+0.006)/0.0022^0.5=	0.21
ac	-0.233/0.0175^0.5=	-1.76	-0.128/0.0056^0.5=	-1.71	(-0.128+0.233)/0.0056^0.5=	1.40
ad	-0.079/0.0809^0.5=	-0.28	-0.235/0.0096^0.5=	-2.40	(-0.235+0.079)/0.0096^0.5=	-1.59
ae	0.211/0.0113^0.5=	**1.98**	0.225/0.0082^0.5=	2.48	(0.225-0.211)/0.0082^0.5=	0.15
af	0.003/0.0032^0.5=	0.05	0.023/0.002^0.5=	0.51	(0.023-0.003)/0.002^0.5=	0.45
bc	0.017/0.0121^0.5=	0.15	0.013/0.006^0.5=	0.17	(0.013-0.017)/0.006^0.5=	-0.05
bd	-0.016/0.0028^0.5=	-0.30	-0.019/0.0026^0.5=	-0.37	(-0.019+0.016)/0.0026^0.5=	-0.06
be	-0.107/0.0335^0.5=	-0.58	0.002/0.0068^0.5=	0.02	(0.002+0.107)/0.0068^0.5=	1.32
bf	-0.262/0.1275^0.5=	-0.73	-0.007/0.003^0.5=	-0.13	(-0.007+0.262)/0.003^0.5=	**4.66**
cd	-0.142/0.0256^0.5=	-0.89	0.042/0.0101^0.5=	0.42	(0.042+0.142)/0.0101^0.5=	1.83
ce	-0.217/0.0309^0.5=	-1.23	-0.193/0.0087^0.5=	-2.07	(-0.193+0.217)/0.0087^0.5=	0.26
cf	-0.342/1.0295^0.5=	-0.34	-0.148/0.0057^0.5=	-1.96	(-0.148+0.342)/0.0057^0.5=	**2.57**
de	-0.009/0.0116^0.5=	-0.08	0.053/0/0076^0.5=	0.61	(0.053+0.009)/0/0076^0.5=	0.71
df	-0.3/0.0693^0.5=	-1.14	-0.109/0.0158^0.5=	-0.87	(-0.109+0.3)/0.0158^0.5=	1.52
ef	0.229/0.1479^0.5=	0.60	-0.065/0.0077^0.5=	-0.74	(-0.065-0.229)/0.0077^0.5=	**-3.35**
abc	0.014/0.0028^0.5=	0.26	-0.038/0.0023^0.5=	-0.79	(-0.038-0.014)/0.0023^0.5=	-1.08
abd	0.002/0.0032^0.5=	0.04	0.03/0.0026^0.5=	0.59	(0.03-0.002)/0.0026^0.5=	0.55
abe	-0.0035/0.0027^0.5=	-0.06	-0.017/0.0024^0.5=	-0.35	(-0.017+0.003)/0.0024^0.5=	-0.29
abf	0.013/0.0028^0.5=	0.25	-0.026/0.0017^0.5=	-0.63	(-0.026-0.013)/0.0017^0.5=	-0.95
acd	0.021/0.003^0.5=	0.38	0.038/0.0023^0.5=	0.79	(0.038-0.021)/0.0023^0.5=	0.35
ace	0/0.0029^0.5=	0.00	0.007/0.0023^0.5=	0.15	(0.007-0)/0.0023^0.5=	0.15
acf	-0.009/0.0031^0.5=	-0.16	-0.01/0.002^0.5=	-0.22	(-0.01+0.009)/0.002^0.5=	-0.02
ade	-0.017/0.0029^0.5=	-0.32	-0.046/0.0025^0.5=	-0.92	(-0.046+0.017)/0.0025^0.5=	-0.58
adf	0.001/0.0032^0.5=	0.02	-0.022/0.0027^0.5=	-0.42	(-0.022-0.001)/0.0027^0.5=	-0.44
aef	0.003/0.0032^0.5=	0.05	-0.022/0.0023^0.5=	-0.46	(-0.022-0.003)/0.0023^0.5=	-0.52
bcd	0.017/0.0027^0.5=	0.33	-0.017/0.0024^0.5=	-0.35	(-0.017-0.017)/0.0024^0.5=	-0.69
bce	0.033/0.0026^0.5=	0.65	0/0.0022^0.5=	0.00	(0-0.033)/0.0022^0.5=	-0.70
bcf	0.01/0.0031^0.5=	0.18	0.01/0.0028^0.5=	0.19	(0.01-0.01)/0.0028^0.5=	0.00
bde	-0.012/0.0027^0.5=	-0.23	-0.025/0.0024^0.5=	-0.51	(-0.025+0.012)/0.0024^0.5=	-0.27
bdf	0.009/0.0028^0.5=	0.17	0.009/0.0024^0.5=	0.18	(0.009-0.009)/0.0024^0.5=	0.00
bef	-0.003/0.0032^0.5=	-0.05	-0.018/0.0025^0.5=	-0.36	(-0.018+0.003)/0.0025^0.5=	-0.30
cde	-0.015/0.0029^0.5=	-0.28	-0.03/0.0025^0.5=	-0.60	(-0.03+0.015)/0.0025^0.5=	-0.30
cdf	0.011/0.0031^0.5=	0.20	0.079/0.0026^0.5=	1.55	(0.079-0.011)/0.0026^0.5=	1.33
cef	0.015/0.0031^0.5=	0.27	0.087/0.0023^0.5=	1.81	(0.087-0.015)/0.0023^0.5=	1.50
def	-0.004/0.0032^0.5=	-0.07	-0.064/0.0028^0.5=	-1.21	(-0.064+0.004)/0.0028^0.5=	-1.13
a^2	-0.199/0.0341^0.5=	-1.08	-0.288/0.0028^0.5=	-5.44	(-0.288+0.199)/0.0028^0.5=	-1.68
c^2	0.199/0.0341^0.5=	1.08	0.034/0.0028^0.5=	0.64	(0.034-0.199)/0.0028^0.5=	**-3.12**
e^2	-0.199/0.0341^0.5=	-1.08	-0.047/0.0028^0.5=	-0.89	(-0.047+0.199)/0.0028^0.5=	**2.87**

Note: Numbers above 1.96 correspond to 5% significance level
Numbers above 1.65 correspond to 10% significance level
Numbers above 1.04 correspond to 30% significance level

Prior Covariance Matrix = 3 x Posterior Covariance Matrix of Quadratic Synthetic Water Model

test #1: (prior mean)/(prior standard deviation)

test #2: (posterior mean)/(posterior standard deviation)

test #3: (posterior mean - prior mean)/(posterior standard deviation)

	test #1		test #2		test #3	
	formula	ratio	formula	ratio	formula	ratio
1	5.323/0.1791^0.5=	**12.58**	4.44/0.0134^0.5=	**38.36**	(4.44-5.323)/0.0134^0.5=	**-7.63**
a	0.364/0.0379^0.5=	1.87	0.318/0.0127^0.5=	**2.82**	(0.318-0.364)/0.0127^0.5=	-0.41
b	-0.229/0.0886^0.5=	-0.77	0.102/0.0043^0.5=	1.56	(0.102+0.229)/0.0043^0.5=	**5.05**
c	-1.595/0.0327^0.5=	**-8.82**	-1.381/0.0112^0.5=	**-13.05**	(-1.381+1.595)/0.0112^0.5=	**2.02**
d	-0.177/0.1675^0.5=	-0.43	-0.074/0.0234^0.5=	-0.48	(-0.074+0.177)/0.0234^0.5=	0.67
e	0.515/0.2431^0.5=	1.04	0.228/0.0136^0.5=	**1.96**	(0.228-0.515)/0.0136^0.5=	**-2.46**
f	0.23/0.1802^0.5=	0.54	0.181/0.005^0.5=	**2.56**	(0.181-0.23)/0.005^0.5=	-0.69
ab	-0.006/0.0043^0.5=	-0.09	0.003/0.0039^0.5=	0.05	(0.003+0.006)/0.0039^0.5=	0.14
ac	-0.233/0.0264^0.5=	-1.43	-0.107/0.0079^0.5=	-1.20	(-0.107+0.233)/0.0079^0.5=	1.42
ad	-0.079/0.1214^0.5=	-0.23	-0.234/0.0153^0.5=	-1.89	(-0.234+0.079)/0.0153^0.5=	-1.25
ae	0.211/0.017^0.5=	1.62	0.226/0.0133^0.5=	**1.96**	(0.226-0.211)/0.0133^0.5=	0.13
af	0.003/0.0048^0.5=	0.04	0.021/0.003^0.5=	0.38	(0.021-0.003)/0.003^0.5=	0.33
bc	0.017/0.0182^0.5=	0.13	0.011/0.0094^0.5=	0.11	(0.011-0.017)/0.0094^0.5=	-0.06
bd	-0.016/0.0042^0.5=	-0.25	-0.019/0.0039^0.5=	-0.30	(-0.019+0.016)/0.0039^0.5=	-0.05
be	-0.107/0.0502^0.5=	-0.48	0/0.0131^0.5=	0.00	(0+0.107)/0.0131^0.5=	0.93
bf	-0.262/0.1913^0.5=	-0.60	-0.005/0.0038^0.5=	-0.08	(-0.005+0.262)/0.0038^0.5=	**4.17**
cd	-0.142/0.0384^0.5=	-0.72	0.054/0.0162^0.5=	0.42	(0.054+0.142)/0.0162^0.5=	1.54
ce	-0.217/0.0463^0.5=	-1.01	-0.186/0.0135^0.5=	-1.60	(-0.186+0.217)/0.0135^0.5=	0.27
cf	-0.342/1.0442^0.5=	-0.33	-0.15/0.0081^0.5=	-1.67	(-0.15+0.342)/0.0081^0.5=	**2.13**
de	-0.009/0.0174^0.5=	-0.07	0.06/0115^0.5=	0.56	(0.06+0.009)/0115^0.5=	0.64
df	-0.3/0.104^0.5=	-0.93	-0.114/0.0222^0.5=	-0.77	(-0.114+0.3)/0.0222^0.5=	1.25
ef	0.229/0.2218^0.5=	0.49	-0.054/0.011^0.5=	-0.51	(-0.054-0.229)/0.011^0.5=	**-2.70**
abc	0.014/0.0041^0.5=	0.22	-0.044/0.0034^0.5=	-0.75	(-0.044-0.014)/0.0034^0.5=	-0.99
abd	0.002/0.0048^0.5=	0.03	0.035/0.0044^0.5=	0.53	(0.035-0.002)/0.0044^0.5=	0.50
abe	-0.0035/0.0041^0.5=	-0.05	-0.02/0.0037^0.5=	-0.33	(-0.02+0.0035)/0.0037^0.5=	-0.28
abf	0.013/0.0042^0.5=	0.20	-0.03/0.0026^0.5=	-0.59	(-0.03-0.013)/0.0026^0.5=	-0.84
acd	0.021/0.0044^0.5=	0.32	0.035/0.0036^0.5=	0.58	(0.035-0.021)/0.0036^0.5=	0.23
ace	0/0.0044^0.5=	0.00	0.01/0.0035^0.5=	0.17	(0.01-0)/0.0035^0.5=	0.17
acf	-0.009/0.0047^0.5=	-0.13	-0.025/0.0028^0.5=	-0.47	(-0.025+0.009)/0.0028^0.5=	-0.30
ade	-0.017/0.0043^0.5=	-0.26	-0.049/0.0039^0.5=	-0.78	(-0.049+0.017)/0.0039^0.5=	-0.51
adf	0.001/0.0048^0.5=	0.01	-0.021/0.004^0.5=	-0.33	(-0.021-0.001)/0.004^0.5=	-0.35
aef	0.003/0.0047^0.5=	0.04	-0.023/0.0033^0.5=	-0.40	(-0.023-0.003)/0.0033^0.5=	-0.45
bcd	0.017/0.0041^0.5=	0.27	-0.02/0.0037^0.5=	-0.33	(-0.02-0.017)/0.0037^0.5=	-0.61
bce	0.033/0.0039^0.5=	0.53	0/0.0034^0.5=	0.00	(0-0.033)/0.0034^0.5=	-0.57
bcf	0.01/0.0047^0.5=	0.15	0.009/0.0042^0.5=	0.14	(0.009-0.01)/0.0042^0.5=	-0.02
bde	-0.012/0.0041^0.5=	-0.19	-0.029/0.0038^0.5=	-0.47	(-0.029+0.012)/0.0038^0.5=	-0.28
bdf	0.009/0.0042^0.5=	0.14	0.012/0.0036^0.5=	0.20	(0.012-0.009)/0.0036^0.5=	0.05
bef	-0.003/0.0047^0.5=	-0.04	-0.025/0.0035^0.5=	-0.42	(-0.025+0.003)/0.0035^0.5=	-0.37
cde	-0.015/0.0043^0.5=	-0.23	-0.029/0.004^0.5=	-0.46	(-0.029+0.015)/0.004^0.5=	-0.22
cdf	0.011/0.0047^0.5=	0.16	0.083/0.0039^0.5=	1.33	(0.083-0.011)/0.0039^0.5=	1.15
cef	0.015/0.0046^0.5=	0.22	0.089/0.0034^0.5=	1.53	(0.089-0.015)/0.0034^0.5=	1.27
def	-0.004/0.0047^0.5=	-0.06	-0.03/0.0041^0.5=	0.47	(-0.03+0.004)/0.0041^0.5=	0.53
a^2	-0.199/0.0511^0.5=	-0.88	-0.278/0.0131^0.5=	**-2.43**	(-0.278+0.199)/0.0131^0.5=	-0.69
c^2	0.199/0.0511^0.5=	0.88	0.037/0.0387^0.5=	0.19	(0.037-0.199)/0.0387^0.5=	-0.82
e^2	-0.199/0.0511^0.5=	-0.88	-0.05/0.029^0.5=	-0.29	(-0.05+0.199)/0.029^0.5=	0.87

Note: Numbers above 1.96 correspond to 5% significance level
Numbers above 1.65 correspond to 10% significance level
Numbers above 1.04 correspond to 30% significance level

Appendix E/3: Residual Plots - Real Water Models

Fit of Linear Real Water Models to Real Water Data

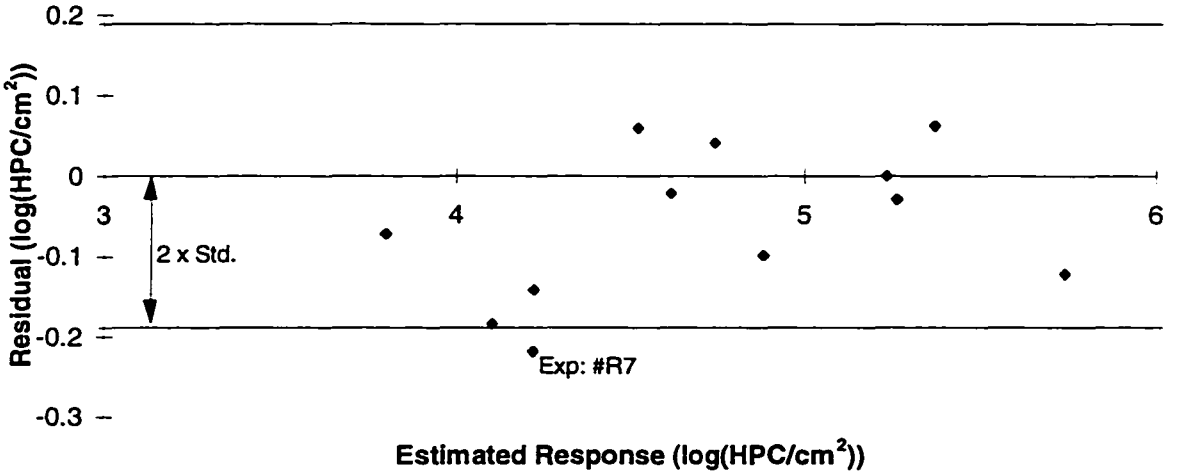


Figure E/3.1: Residual plot - linear real water model fitted to real water data
prior covariance matrix = 2 x posterior covariance matrix of linear synthetic water model
5% confidence, Var: 0.009, Std.: 0.094
9 reestimated parameters: l=4.268; a=0.141; c=-1.321; e=0.117; f=0.173; ad=-0.2; ae=0.159; ce=-0.173; cf=-0.116

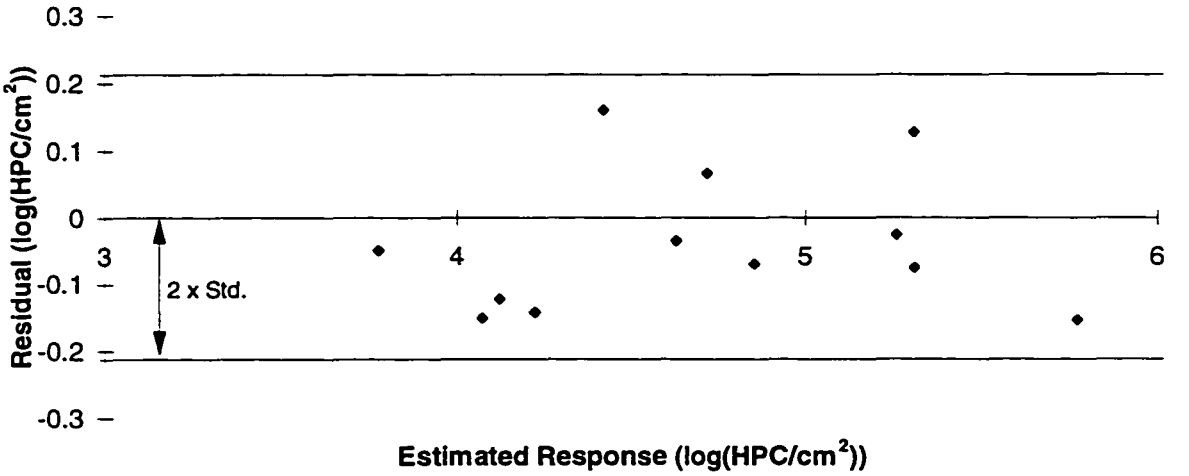


Figure E/3.2: Residual plot - linear real water model fitted to real water data
prior covariance matrix = 2 x posterior covariance matrix of linear synthetic water model
30% confidence, Var: 0.011, Std.: 0.106
20 reestimated parameters: l=4.324; a=0.176; b=0.058; c=-1.329; d=-0.059; e=0.152; f=0.170; ab=0.064; ac=-0.081; ad=-0.292; ae=0.198; be=0.129; ce=-0.266; cf=-0.155; df=-0.055; abd=0.055; ade=-0.067; cde=-0.061; cdf=0.064; cef=0.082

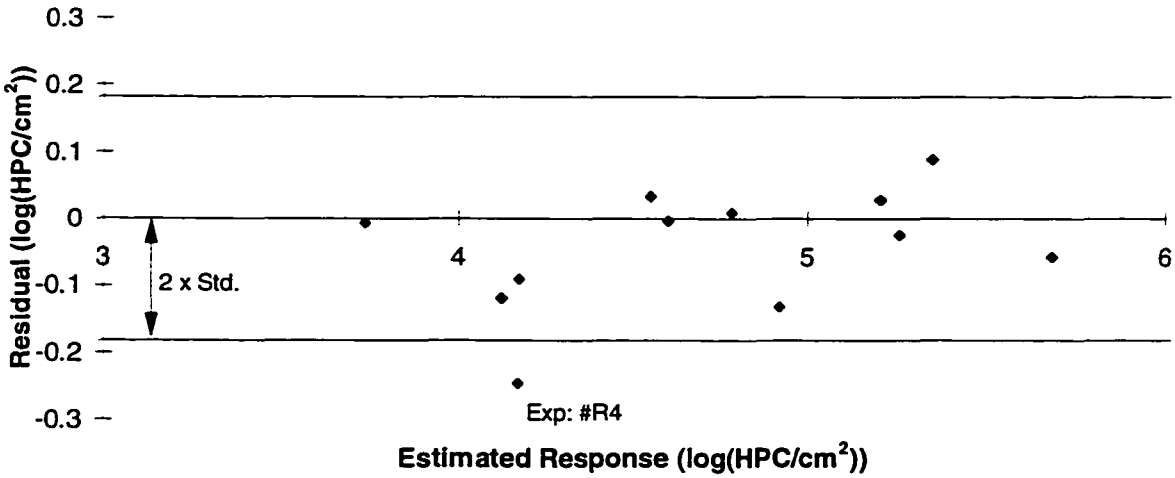


Figure E/3.3: Residual plot - linear real water model fitted to real water data
prior covariance matrix = 3 x posterior covariance matrix of linear synthetic water model
5% confidence, Var: 0.008, Std.: 0.091
7 reestimated parameters: l=4.292; a=0.208; c=-1.331; e=0.060; f=0.213;ad=-0.186; ce=-0.161

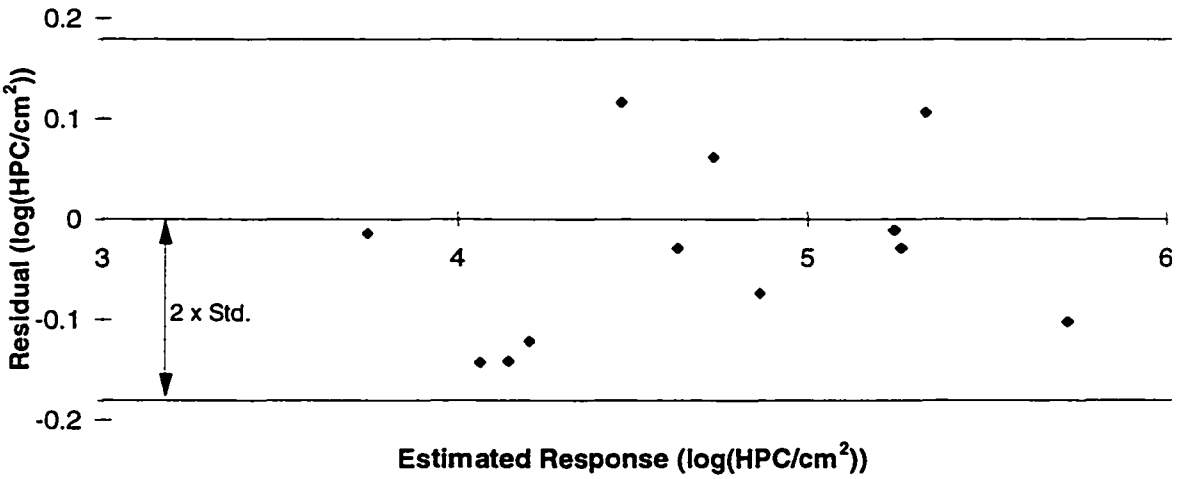


Figure E/3.4: Residual plot - linear real water model fitted to real water data
prior covariance matrix = 3 x posterior covariance matrix of linear synthetic water model
30% confidence, Var: 0.008, Std.: 0.090
19 reestimated parameters: l=4.321; a=0.209; b=0.067; c=-1.340; d=-0.051; e=0.153; f=0.181; ab=0.073; ad=-0.253; ae=0.174; be=0.119; ce=-0.248; cf=-0.138; df=-0.070; abd=0.057; ade=-0.080; cde=-0.059; cdf=0.051; cef=0.065

Fit of Quadratic Real Water Models to Real Water Data

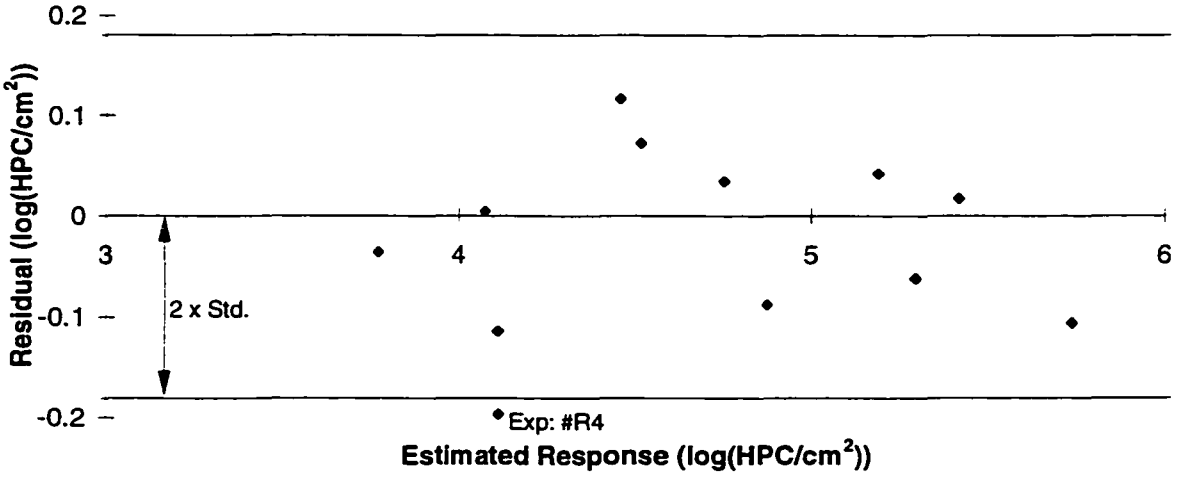


Figure E/3.5: Residual plot - quadratic real water model fitted to real water data
prior covariance matrix = 2 x posterior covariance matrix of quadratic synthetic water model
5% confidence, Var: 0.008, Std.: 0.090
10 reestimated parameters: l=4.348; a=0.253; c=-1.369; e=0.133; f=0.198; ad=-0.169; ae=0.246; ce=-0.170; cf=-0.121; a^2=-0.196

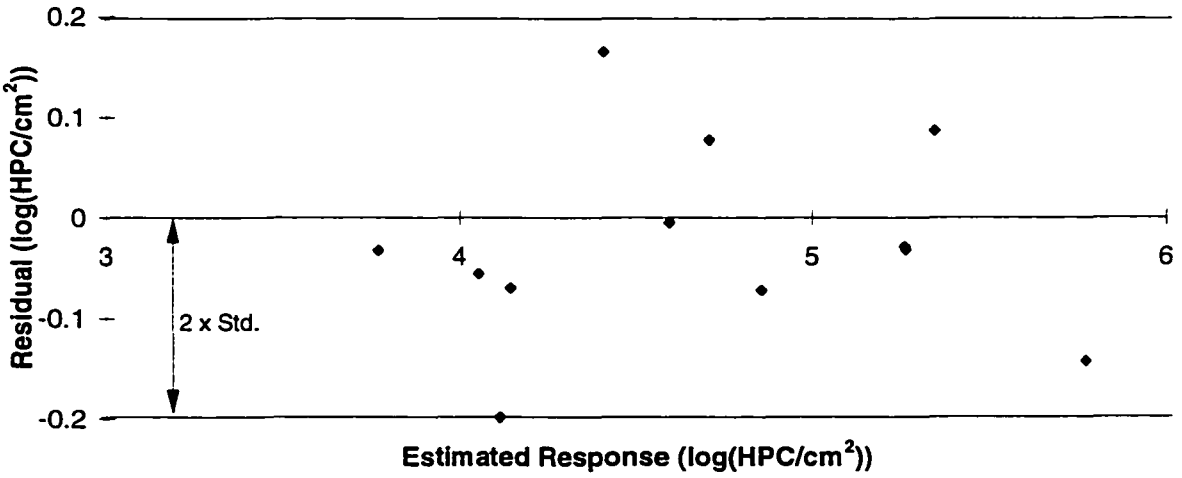


Figure E/3.6: Residual plot - quadratic real water model fitted to real water data
prior covariance matrix = 2 x posterior covariance matrix of quadratic synthetic water model
30% confidence, Var: 0.009, Std.: 0.099
15 reestimated parameters: l=4.409; a=0.275; b=0.057; c=-1.357; e=0.106; f=0.197; ac=-0.100; ad=-0.215; ae=0.211; ce=-0.181; cf=-0.127; cdf=-0.017; cef=0.062; def=0.068; a^2=-0.206

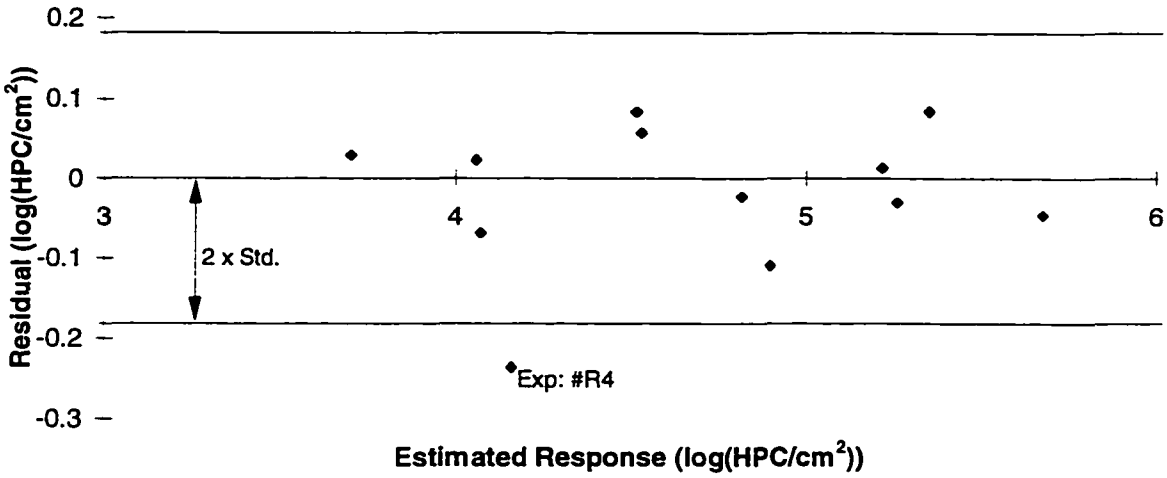


Figure E/3.7: Residual plot - quadratic real water model fitted to real water data
prior covariance matrix = 3 x posterior covariance matrix of quadratic synthetic water model
5% confidence, Var: 0.008, Std.: 0.091
7 reestimated parameters: l=4.311; a=0.238; c=-1.435; e=0.207; f=0.226; ae=0.275; a^2=0.198

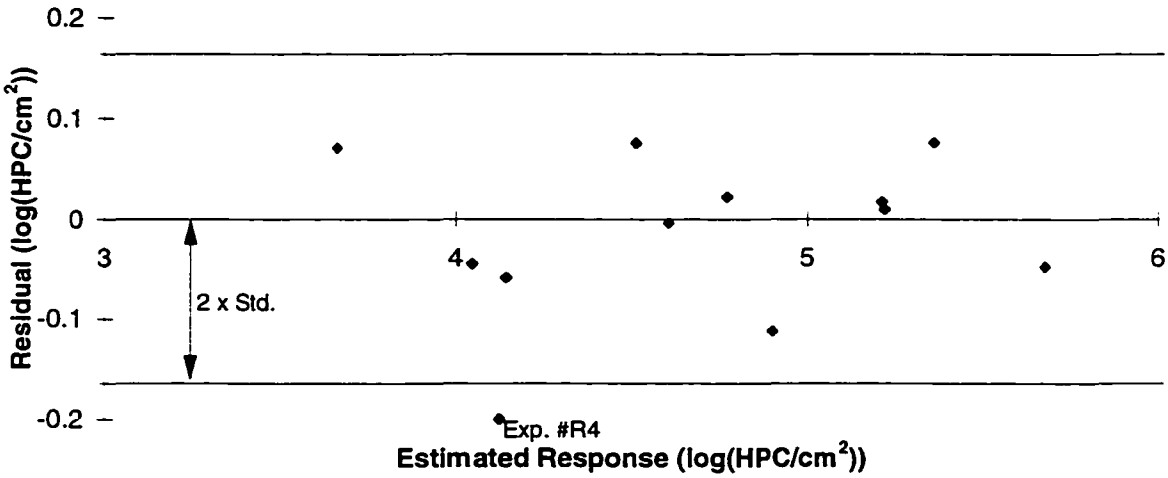


Figure E/3.8: Residual plot - quadratic real water model fitted to real water data
prior covariance matrix = 3 x posterior covariance matrix of quadratic synthetic water model
5% confidence + 'd' (type), Var: 0.006765, Std.: 0.082252
8 reestimated parameters: l=4.490725; a=0.415003; b=0.138714; c=-1.460490; e=0.236191; f=0.228892; ae=0.212368; a^2=-0.337130

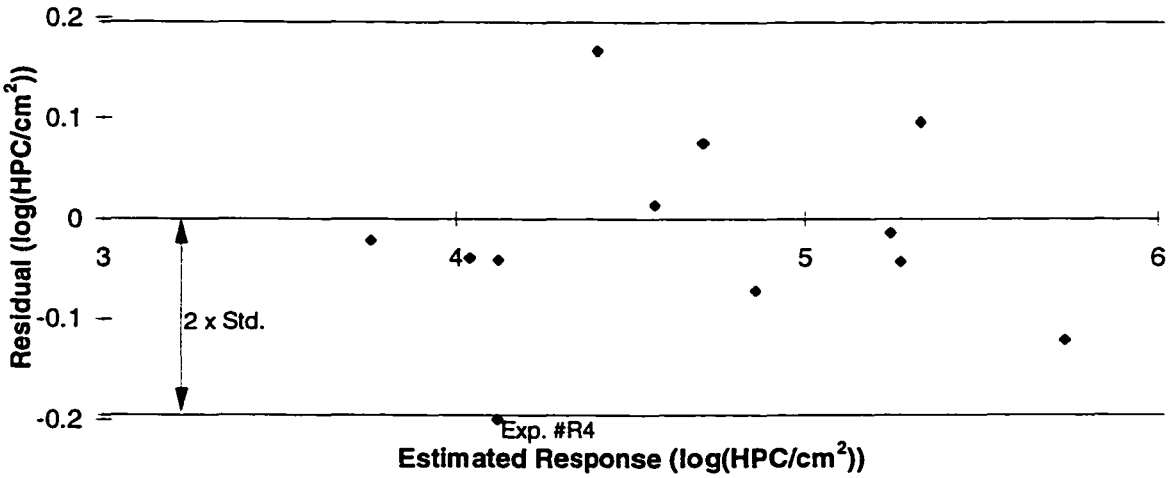


Figure E/3.9: Residual plot - quadratic real water model fitted to real water data
prior covariance matrix = 3 x posterior covariance matrix of quadratic synthetic water model
30% confidence, Var: 0.010, Std.: 0.098
14 reestimated parameters: l=4.473; a=0.351; b=0.080; c=-1.367; e=0.107; f=0.202; ac=-0.093; ad=-0.183; ae=0.217; ce=-0.162; cf=-0.124; cdf=0.069; cef=0.080; a^2=-0.290

Appendix F: Most Informative Model - Prior data, Parameter Reestimates, Covariance and Correlation Matrices, Residuals

Appendix F/1: Most Informative Model - Parameter Reestimates, Covariance and Correlation Matrices, Residuals

```
format short e
a = [5.323;0.364;-0.229;-1.595;0.515;0.230;0.211;-0.199]
y = [4.785;5.23;3.732;3.919;5.23;5.623;4;4.578;4.785;5.431;4.079;4.591]

u =   [0.1791  0.0311  0.0693  0.0249  -0.1360  0.1258  0.0035  0;
       0.0311  0.0379  0.0070  -0.0025 -0.0325  0.0428  -0.0061 0;
       0.0693  0.0070  0.0886  0.3080  -0.1208  0.0727  0.0006  0;
       0.0249  -0.0025 0.0380  0.0327  -0.0588  0.0319  0.0043  0;
       -0.1360 -0.0325 -0.1208 -0.0588 0.2431   -0.1754 -0.0105 0;
       0.1258  0.0428  0.0727  0.0319  -0.1754  0.1802  0.0086  0;
       0.0035  -0.0061 0.0006  0.0043  -0.0105  0.0086  0.0170  0;
       -0.0027 0.0005  0.0016  0.0008  0.0015   -0.0023 0.0002  0.0511]

s = 0.0189

x =   [1   -0.4   -1   -0.6   -0.11   -1   0.04   0.16;
       1   -0.4   -1   -0.6   -0.11   1    0.04   0.16;
       1   -0.4   -1   0.2    0.33    -1   -0.13  0.16;
       1   -0.4   -1   0.2    0.33    1    -0.13  0.16;
       1   1.4    -1   -0.6   0.55    -1   0.77   1.96;
       1   1.4    -1   -0.6   0.55    1    0.77   1.96;
       1   1.4    -1   0.2    0.55    -1   0.77   1.96;
       1   1.4    -1   0.2    0.55    1    0.77   1.96;
       1   -0.4   -1   -0.6   0.78    -1   -0.31  0.16;
       1   -0.4   -1   -0.6   0.78    1    -0.31  0.16;
       1   -0.8   1    -0.2   -0.11   -1   0.09   0.64;
       1   -0.8   1    -0.2   -0.11   1    0.09   0.64]


covmat = inv(inv(u)+(1/s)*x'*x)
parest = inv(inv(u)+(1/s)*x'*x)*(inv(u)*a+(1/s)*x'*y)

wk1write('a',a)
wk1write('y',y)
wk1write('u',u)
wk1write('x',x)
wk1write('covmat',covmat)
wk1write('parest',parest)
```

Reestimated Parameters:

```
  1     4.490725198
  a     0.415002937
  b      0.13871358
  c     -1.46049132
  e     0.236190789
  f      0.22889209
 ae      0.21236774
 a2     -0.33712887
```

Covariance Matrix:

0.0128225933	0.0122680600	0.0058940783	-0.0084195146	-0.0063715562	0.0003904101	-0.0034494940	-0.0102318618
0.0094538129	*0.0149516507*	0.0058060708	-0.0129190207	-0.0060004438	0.0003668184	-0.0065607187	-0.0093313188
-0.0005544676	0.0033359720	*0.0050719283*	-0.0190027480	-0.0022483340	-0.0000488068	-0.0007118567	-0.0008704614
0.0053559603	0.0003328439	-0.0008052273	*0.0191373767*	0.0013477700	0.0000583247	-0.0007686800	-0.0014136089
-0.0152969251	-0.0080998003	0.0007743049	-0.0302193025	*0.0082185593*	-0.0006849781	0.0063981247	0.0058214506
0.0003007214	0.0003794882	0.0000689747	-0.0004245137	-0.0005537195	*0.0015288910*	-0.0000392060	-0.0002591792
-0.0081226386	-0.0093136419	-0.0029636375	-0.0092565126	0.0010443244	-0.0001543562	*0.0165504348*	0.0014642663
-0.0056231642	-0.0088538421	-0.0038280793	0.0179262460	0.0038816499	-0.0002201308	-0.0022692983	*0.0099454266*

Square root of diagonal elements:

0.1132368902	0.1222769427	0.0712174722	0.1383379077	0.0906562700	0.0391010361	0.1286484930	0.0997267595

Correlation matrix:

1.0000000029	0.8860197162	0.7308721469	-0.5374745680	-0.6206685513	0.0881748736	-0.2367896706	-0.9060559672
0.6827530376	*0.9999749948*	0.6667151803	-0.7637178813	-0.5412901108	0.0767197910	-0.4170530347	-0.7652016309
-0.0687521528	0.3830679835	*0.9999645087*	-1.9287379970	-0.3482258545	-0.0175262978	-0.0776937193	-0.1225563564
0.3419020002	0.0196765173	-0.0817305016	*0.9999848736*	0.1074657747	0.0107824238	-0.0431909862	-0.1024636395
-1.4900487988	-0.7306578030	0.1199249967	-2.4095032560	*0.9999588580*	-0.1932291754	0.5485702925	0.6438783091
0.0679203006	0.0793737690	0.0247700122	-0.0784826684	-0.1562123069	*1.0000265036*	-0.0077941871	-0.0664678476
-0.5575700106	-0.5920592555	-0.3234660766	-0.5201114360	0.0895422017	-0.0306849890	*0.9999882865*	0.1141296694
-0.4979285357	-0.7260413945	-0.5389749150	1.2993385695	0.4293314388	-0.0564503689	-0.1768728031	*0.9999675023*

Model Parameters:

t0=4.490725	t13=0	t34=0
t1=0.415003	t14=0	t35=0
t2=0.138714	t15=0.212368	t36=0
t3=-1.46049	t16=0	t45=0
t4=0	t23=0	t46=0
t5=0.236191	t24=0	t56=0
t6=0.228892	t25=0	t1s=-0.33713
t12=0	t26=0	t3s=0
		t5s=0

% Actual setting according to design matrix (real water):
x1=-0.4 / 1.4 /-0.8
x2=-1 / +1
x3=-0.6 / 0.2
x4=0
x5=-0.11 /0.33 / 0.55 / 0.78
x6=-1 / +1

% Regression Model (Quadratic):
f=t0+t1*x1+t1s*x1^2+t2*x2+t3*x3+t3s*x3^2+t4*x4+t5*x5+t5s*x5^2+t6*x6+t12*x1*x2+t13*x1*x3+
+t14*x1*x4+t15*x1*x5+t16*x1*x6+t23*x2*x3+t24*x2*x4+t25*x2*x5+t26*x2*x6+t34*x3*x4+
+t35*x3*x5+t36*x3*x6+t45*x4*x5+t46*x4*x6+t56*x5*x6

Design Matrix and Residuals:

Exp #	Water Source	BOM Supplement (mg/L)	Dis Type - chlorine + chloramine	Disin. Conc. (mg/L)	Shear (N/m2)	Temp (C)	Substr - PC + DI	Actual	Estimated	Residual
1	filter effluent	150	-	0.1	1.2	16	-	4.7850	4.7628	0.0222
2	filter effluent	150	-	0.1	1.2	16	+	5.2300	5.2206	0.0094
3	filter effluent	150	-	0.3	1.2	20	-	3.7320	3.6610	0.071
4	filter effluent	150	-	0.3	1.2	20	+	3.9190	4.1188	-0.1998
5	filter influent	600	-	0.1	1.2	22	-	5.2300	5.2131	0.0169
6	filter influent	600	-	0.1	1.2	22	+	5.6230	5.6709	-0.0479
7	filter influent	600	-	0.3	1.2	22	-	4.0000	4.0447	-0.0447
8	filter influent	600	-	0.3	1.2	22	+	4.5780	4.5025	0.0755
9	filter effluent	150	-	0.1	1.2	24	-	4.7850	4.8974	-0.1124
10	filter effluent	150	-	0.1	1.2	24	+	5.4310	5.3552	0.0758
11	raw ground water	50	+	0.8	1.2	16	-	4.0790	4.1376	-0.0586
12	raw ground water	50	+	0.8	1.2	16	+	4.5910	4.5954	-0.0044
								Variance:		0.00677
								Standard deviation:		0.08225
			Confidence regions:				1	0.1645	-0.1645	
			(2 x Std.)				8	0.1645	-0.1645	

Appendix G: User-Friendly Model Interface

G/1 Interface Code

G/2 Executable File of Interface

Appendix G/1: Interface Code (Visual Basic© version 4)

1. Globals

```
'Global dimensions...
   Global constindex
   Global arrIcol(45)
   Global arrDisin(45)
   Global arrSubst(45)
   Global arrConst(45)
   Global arrVariable(45)
   Global calCounter As Integer
   Global sending As Integer
   Global exceeded As Integer
```

2.. Model

```
Dim x1#, x2#, x3#, x5#, x6#
Dim c1, c2, c3a, c3b, c5, c6 As Single
Dim linecolor As Integer
Dim a, b, c As Single

Sub AssignColor()
calCounter = calCounter + 1
linecolor = linecolor + 1
If linecolor = 7 Then linecolor = linecolor + 1
If linecolor >= 15 Then 'Reset
   linecolor = 0
End If

If calCounter >= 24 Then
   MsgBox "Warning! This is exceeding the number of allowable calculations", 64
   exceeded = 1
End If

arrIcol(calCounter) = linecolor

End Sub

Sub graphic(XVar(), YVar())
'Scale the picture box:
'vertical axis...
MinH = Val(txtMin(1))
MaxH = Val(txtMax(1))
'horizontal axis...
MinK = Val(txtMin(2))
MaxK = Val(txtMax(2))
Picture1.Scale (MinK, MaxH)-(MaxK, MinH)

If calCounter < 15 Then Picture1.DrawStyle = 0 Else Picture1.DrawStyle = 2
Picture1.Line (XVar(1), YVar(1))-(XVar(1), YVar(1)), QBColor(linecolor)
```

```
For cntr = 0 To UBound(YVar())
   Picture1.Line -(XVar(cntr), YVar(cntr)), QBColor(linecolor)
Next cntr

End Sub

Sub VerifyData(Cancel)
'Check for validity of data

'Case 1
If IsNumeric(txtConstant) Then
   Else: MsgBox "Constant must be number. Select a number", 0 + 16, "Steady-state Biofilm Accumulation
Model"
   Cancel = 1
   Exit Sub
End If

'Case 2
If IsNumeric(txtMin(1)) Then
   Else: MsgBox "Minimum value of ordinate must be a number. Select a number", 0 + 16, "Steady-state Biofilm
Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 3
If IsNumeric(txtMin(2)) Then
   Else: MsgBox "Minimum value of abscissa must be a number. Select a number", 0 + 16, "Steady-state Biofilm
Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 4
If IsNumeric(txtMin(3)) Then
   Else: MsgBox "Minimum value of third variable must be a number. Select a number", 0 + 16, "Steady-state
Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 5
If IsNumeric(txtMax(1)) Then
   Else: MsgBox "Maximum value of ordinate must be a number. Select a number", 0 + 16, "Steady-state
Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 6
If IsNumeric(txtMax(2)) Then
   Else: MsgBox "Maximum value of abscissa must be a number. Select a number", 0 + 16, "Steady-state
Biofilm Accumulation Model"
   Cancel = 1
```

```
    Exit Sub
End If

'Case 7
If IsNumeric(txtMax(3)) Then
    Else: MsgBox "Maximum value of third variable must be a number. Select a number", 0 + 16, "Steady-state
Biofilm Accumulation Model"
    Cancel = 1
    Exit Sub
End If

'Case 8
If IsNumeric(txtInc) Then
    Else: MsgBox "Increment of third variable must be a number. Select a number", 0 + 16, "Steady-state Biofilm
Accumulation Model"
    Cancel = 1
    Exit Sub
End If

'Case 9: -ve HPC at HPC const.
If (Val(txtConstant) < 0 And optConstant(0)) Then
    MsgBox "Steady-state HPC number cannot be negative value. Select another value.", 0 + 16, "Steady-state
Biofilm Accumulation Model"
    Cancel = 1
    Exit Sub
End If

'Case 10: -ve chlorine residual at residual const.
If (Val(txtConstant) < 0 And optConstant(1) And optDisinfectant(0)) Then
    MsgBox "Free chlorine residual concentration cannot be negative value. Select another value.", 0 + 16,
"Steady-state Biofilm Accumulation Model"
    Cancel = 1
    Exit Sub
End If

'Case 11: -ve chloramine residual at residual const.
If (Val(txtConstant) < 0 And optConstant(1) And optDisinfectant(1)) Then
    MsgBox "Combined chlorine residual concentration cannot be negative value. Select another value.", 0 + 16,
"Steady-state Biofilm Accumulation Model"
    Cancel = 1
    Exit Sub
End If

'Case 12: -ve temperature at temp constant.
If (Val(txtConstant) < 0 And optConstant(2)) Then
    MsgBox "Temperature cannot be negative value. Select another value.", 0 + 16, "Steady-state Biofilm
Accumulation Model"
    Cancel = 1
    Exit Sub
End If

'Case 13: -ve BOM at BOM constant.
If (Val(txtConstant) < 0 And optConstant(3)) Then
```

```
   MsgBox "BOM concentration cannot be negative value. Select another value.", 0 + 16, "Steady-state Biofilm
Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 14: -ve BOM at HPC constant.
If Val(txtMin(1)) < 0 And optConstant(0) Then
   MsgBox "BOM concentration cannot be negative value. Select another value.", 0 + 16, "Steady-state Biofilm
Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 15: -ve Temp at HPC constant.
If Val(txtMin(2)) < 0 And optConstant(0) Then
   MsgBox "Temperature cannot be negative value. Select another value.", 0 + 16, "Steady-state Biofilm
Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 16: -ve chlorine at HPC constant.
If Val(txtMin(3)) < 0 And optConstant(0) And optDisinfectant(0) Then
   MsgBox "Free chlorine residual concentration cannot be negative value. Select another value.", 0 + 16,
"Steady-state Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 17: -ve chlorine at HPC constant.
If Val(txtMin(3)) < 0 And optConstant(0) And optDisinfectant(1) Then
   MsgBox "Combined chlorine residual concentration cannot be negative value. Select another value.", 0 + 16,
"Steady-state Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 18: -ve BOM at residual constant.
If Val(txtMin(1)) < 0 And optConstant(1) Then
   MsgBox "BOM cannot be negative value. Select another value.", 0 + 16, "Steady-state Biofilm Accumulation
Model"
   Cancel = 1
   Exit Sub
End If

'Case 19: -ve Temp at residual constant.
If Val(txtMin(2)) < 0 And optConstant(1) Then
   MsgBox "Temperature cannot be negative value. Select another value.", 0 + 16, "Steady-state Biofilm
Accumulation Model"
   Cancel = 1
   Exit Sub
End If
```

```
'Case 20: -ve HPC at residual constant.
If Val(txtMin(3)) < 0 And optConstant(1) Then
   MsgBox "Steady-state HPC number cannot be negative value. Select another value.", 0 + 16, "Steady-state
Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 21: -ve HPC at temp constant.
If Val(txtMin(1)) < 0 And optConstant(2) Then
   MsgBox "Steady-state HPC number cannot be negative value. Select another value.", 0 + 16, "Steady-state
Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 22: -ve BOM at temp constant.
If Val(txtMin(2)) < 0 And optConstant(2) Then
   MsgBox "BOM concentration cannot be negative value. Select another value.", 0 + 16, "Steady-state Biofilm
Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 23: -ve chlorine at temp constant.
If Val(txtMin(3)) < 0 And optConstant(2) And optDisinfectant(0) Then
   MsgBox "Free chlorine residual concentration cannot be negative value. Select another value.", 0 + 16,
"Steady-state Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 24: -ve chloramine at temp constant.
If Val(txtMin(3)) < 0 And optConstant(2) And optDisinfectant(1) Then
   MsgBox "Combined chlorine residual concentration cannot be negative value. Select another value.", 0 + 16,
"Steady-state Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 25: -ve HPC at BOM constant.
If Val(txtMin(1)) < 0 And optConstant(3) Then
   MsgBox "Steady-state HPC number cannot be negative value. Select another value.", 0 + 16, "Steady-state
Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 26: -ve chlorine at BOM constant.
If Val(txtMin(2)) < 0 And optConstant(3) And optDisinfectant(0) Then
   MsgBox "Free chlorine residual concentration cannot be negative value. Select another value.", 0 + 16,
"Steady-state Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
```

```
End If

'Case 27: -ve chloramine at BOM constant.
If Val(txtMin(2)) < 0 And optConstant(3) And optDisinfectant(1) Then
   MsgBox "Combined chlorine residual concentration cannot be negative value. Select another value.", 0 + 16,
"Steady-state Biofilm Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 28: -ve temperature at BOM constant.
If Val(txtMin(3)) < 0 And optConstant(3) Then
   MsgBox "Temperature cannot be negative value. Select another value.", 0 + 16, "Steady-state Biofilm
Accumulation Model"
   Cancel = 1
   Exit Sub
End If

'Case 29: warning for all three ranges.
For icount = 1 To 3
   If Val(txtMin(icount)) >= Val(txtMax(icount)) Then
      MsgBox "Maximum value is not greater then selected minimum", 16, "Steady-state Biofilm Accumulation
Model"
      Cancel = 1
      Exit Sub
   End If
Next icount

'Case 30: increment is too large.
If Val(txtInc) > (Val(txtMax(3)) - Val(txtMin(3))) Then
   MsgBox "Increment is larger then selected range", 0 + 48, "Steady-state Biofilm Accumulation Model"
   Cancel = 0
   Exit Sub
End If

'Case 31: HPC constant - temperature too low.
If (Val(txtMin(2)) < 8 And optConstant(0)) Then
   MsgBox "Selected temperature is below tested range. Proceed with caution", 0 + 48, "Steady-state Biofilm
Accumulation Model"
   Cancel = 0
   Exit Sub
End If

'Case 32: Disinfectant constant - temperature too low.
If (Val(txtMin(2)) < 8 And optConstant(1)) Then
   MsgBox "Selected temperature is below tested range. Proceed with caution", 0 + 48, "Steady-state Biofilm
Accumulation Model"
   Cancel = 0
   Exit Sub
End If

'Case 33: HPC - temperature too high.
If (Val(txtMax(2)) > 26 And optConstant(0)) Then
```

```
    MsgBox "Selected temperature is above tested range. Proceed with caution", 0 + 48, "Steady-state Biofilm
Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 34: Disinfectant constant - temperature too high.
If (Val(txtMax(2)) > 26 And optConstant(1)) Then
    MsgBox "Selected temperature is above tested range. Proceed with caution", 0 + 48, "Steady-state Biofilm
Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 35: Temperature constant; chlorine on both substrata - BOM too low.
If (Val(txtMax(2)) < 50 And optConstant(2)) Then
    MsgBox "Selected BOM concentration is below tested range. Proceed with caution", 0 + 48, "Steady-state
Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 36: Temperature constant; - BOM too high.
If (Val(txtMax(2))) > 400 And optConstant(2) Then
    MsgBox "Selected BOM concentration is above tested range. Proceed with caution", 0 + 48, "Steady-state
Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 37: Temperature constant; - BOM too low.
If (Val(txtMin(2))) < 50 And optConstant(2) Then
    MsgBox "Selected BOM concentration is below tested range. Proceed with caution", 0 + 48, "Steady-state
Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 38: Disinfectant constant; chlorine (too high) on both substrata.
If (Val(txtMax(2)) > 0.5 And optConstant(3) And optDisinfectant(0)) Then
    MsgBox "Selected free chlorine residual concentration is above tested range. Proceed with caution", 0 + 48,
"Steady-state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 39: Disinfectant constant; chloramine (too high) on polycarbonate.
If (Val(txtMax(2)) > 0.5 And optConstant(3) And optDisinfectant(1) And optSubstratum(0)) Then
    MsgBox "Selected combined chlorine residual concentration is above tested range. Proceed with caution", 0 +
48, "Steady-state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If
```

```
'Case 40: Disinfectant constant; chloramine (too high) on ductile iron.
If (Val(txtMax(2)) > 2.5 And optConstant(3) And optDisinfectant(1) And optSubstratum(1)) Then
    MsgBox "Selected combined chlorine residual concentration is above tested range. Proceed with caution", 0 +
48, "Steady-state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 41: HPC constant; chlorine on both substrata (too high).
If (Val(txtMax(3)) > 0.7 And optConstant(0) And optDisinfectant(0)) Then
    MsgBox "Selected free chlorine residual concentration is above tested range. Proceed with caution", 0 + 48,
"Steady-state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 42: HPC constant; chloramine (too high) on polycarbonate.
If (Val(txtMax(3)) > 0.7 And optConstant(0) And optDisinfectant(1) And optSubstratum(0)) Then
    MsgBox "Selected combined chlorine residual concentration is above tested range. Proceed with caution", 0 +
48, "Steady-state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 43: HPC constant; chloramine on ductile iron (too high).
If (Val(txtMax(3)) > 3# And optConstant(0) And optDisinfectant(1) And optSubstratum(1)) Then
    MsgBox "Selected combined chlorine residual concentration is above tested range. Proceed with caution", 0 +
48, "Steady-state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 44: disinfectant constant on both substrata; HPC too high.
If (Val(txtMax(3)) > 7.5 And optConstant(1)) Then
    MsgBox "Selected steady-state HPC number is above tested range. Proceed with caution", 0 + 48, "Steady-
state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 45: disinfectant constant on both substrata; HPC too low.
If (Val(txtMin(3)) < 4 And optConstant(1)) Then
    MsgBox "Selected steady-state HPC number is below tested range. Proceed with caution", 0 + 48, "Steady-
state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 46: Temperature constant; chlorine on both substrata (too high).
If (Val(txtMax(3)) > 0.5 And optConstant(2) And optDisinfectant(0)) Then
    MsgBox "Selected free chlorine residual concentration is above tested range. Proceed with caution", 0 + 48,
"Steady-state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
```

```
End If

'Case 47: Temperature constant; chloramine on polycarbonate (too high).
If (Val(txtMax(3)) > 0.5 And optConstant(2) And optDisinfectant(1) And optSubstratum(0)) Then
   MsgBox "Selected combined chlorine residual concentration is above tested range. Proceed with caution", 0 +
48, "Steady-state Biofilm Accumulation Model"
   Cancel = 0
   Exit Sub
End If

'Case 48: Temperature constant; chloramine on ductile iron (too high).
If (Val(txtMax(3)) > 2.5 And optConstant(2) And optDisinfectant(1) And optSubstratum(1)) Then
   MsgBox "Selected combined chlorine residual concentration is above tested range. Proceed with caution", 0 +
48, "Steady-state Biofilm Accumulation Model"
   Cancel = 0
   Exit Sub
End If

'Case 49: BOM constant on both substrata; temperature too high).
If (Val(txtMax(3)) > 26 And optConstant(3)) Then
   MsgBox "Selected temperature is above tested range. Proceed with caution", 0 + 48, "Steady-state Biofilm
Accumulation Model"
   Cancel = 0
   Exit Sub
End If

'Case 50: BOM constant on both substrata - temperature too low.
If (Val(txtMin(3)) < 8 And optConstant(3)) Then
   MsgBox "Selected temperature is below tested range. Proceed with caution", 0 + 48, "Steady-state Biofilm
Accumulation Model"
   Cancel = 0
   Exit Sub
End If

'Case 51: HPC constant is too low.
If (Val(txtConstant) < 3 And optConstant(0)) Then
   MsgBox "Selected steady-state HPC number is below tested range. Proceed with caution", 0 + 48, "Steady-
state Biofilm Accumulation Model"
   Cancel = 0
   Exit Sub
End If

'Case 52: HPC constant is too large.
If (Val(txtConstant) > 7 And optConstant(0)) Then
   MsgBox "Selected steady-state HPC number is above tested range. Proceed with caution", 0 + 48, "Steady-
state Biofilm Accumulation Model"
   Cancel = 0
   Exit Sub
End If

'Case 53: Chlorine residual on both substrata is constant (too large).
If (Val(txtConstant) > 0.5 And optConstant(1) And optDisinfectant(0)) Then
   MsgBox "Selected free chlorine residual concentration is above tested range. Proceed with caution", 0 + 48,
"Steady-state Biofilm Accumulation Model"
```

```
    Cancel = 0
    Exit Sub
End If

'Case 54: Chloramine residual on polycarbonate is constant (too large).
If (Val(txtConstant) > 0.5 And optConstant(1) And optDisinfectant(1) And optSubstratum(0)) Then
    MsgBox "Selected combined chlorine residual concentration is above tested range. Proceed with caution", 0 +
48, "Steady-state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 55: Chloramine residual on ductile iron is constant (too large).
If (Val(txtConstant) > 2 And optConstant(1) And optDisinfectant(1) And optSubstratum(1)) Then
    MsgBox "Selected combined chlorine residual concentration is above tested range. Proceed with caution", 0 +
48, "Steady-state Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 56: Temperature is constant (too low).
If (Val(txtConstant) < 8 And optConstant(2)) Then
    MsgBox "Selected temperature is below tested range. Proceed with caution", 0 + 48, "Steady-state Biofilm
Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 57: Temperature is constant (too high).
If (Val(txtConstant) > 26 And optConstant(2)) Then
    MsgBox "Selected temperature is above tested range. Proceed with caution", 0 + 48, "Steady-state Biofilm
Accumulation Model"
    Cancel = 0
    Exit Sub
End If

'Case 58: BOM is constant (too high).
If (Val(txtConstant) > 600 And optConstant(3)) Then
    MsgBox "Selected BOM concentration is above tested range. Proceed with caution", 0 + 48, "Steady-state
Biofilm Accumulation Model"
    Cancel = 0
    Exit Sub
End If

End Sub

Private Sub cmdCalculate_Click()
Unload Legend
'Calculate the equation according to selected case...
Dim XVar(), YVar() As Variant
Dim icount As Integer
Dim Intervals As Integer

Cancel = 0
```

```
Call VerifyData(Cancel)
If Cancel = 1 Then Exit Sub

If exceeded = 1 Then
   MsgBox "Sorry! No more runs allowed before you press the Clear button", 64
   Exit Sub
End If

Intervals = 30 'adjust speed of calc
ReDim XVar(Intervals), YVar(Intervals)

Screen.MousePointer = 11

'Case HPC constant...
If optConstant(0).Value = True Then
   Increment = (Val(txtMax(2)) - Val(txtMin(2))) / Intervals
   For c3 = Val(txtMin(3)) To Val(txtMax(3) + Val(txtInc)) Step Val(txtInc) 'conc
   If c3 > Val(txtMax(3)) Then GoTo 10
      If optDisinfectant(0).Value Then arrDisin(calCounter) = "Cl2" Else arrDisin(calCounter) = "NH2Cl"
      If optSubstratum(0).Value Then arrSubst(calCounter) = "PC" Else arrSubst(calCounter) = "DI"
      arrConst(calCounter) = txtConstant
      arrVariable(calCounter) = c3

      If (optDisinfectant(1) And optSubstratum(1)) Then x3 = -1 + c3 Else x3 = -1 + (c3 / 0.25)
      c5 = Val(txtMin(2))
      For cnter = 0 To Intervals
         x5 = -1.89 + (c5 / 9)
         H = Val(txtConstant) 'HPC
         '0.33713 * x1 ^2 - (0.415003 + 0.212368 * x5) * x1 + H - 4.490725 - 0.138714 * x2 + 1.46049 * x3 -
0.236191 * x5 - 0.228892 * x6 = 0
         a = 0.33713
         b = -(0.415003 + 0.212368 * x5)
         c = H - 4.490725 - 0.138714 * x2 + 1.46049 * x3 - 0.236191 * x5 - 0.228892 * x6
         x1 = (-b - (b ^ 2 - 4 * a * c) * 0.5) / (2 * a)
         XVar(cnter) = c5
         YVar(cnter) = (x1 + 1) * 250
         c5 = c5 + Increment
      Next cnter
      AssignColor
      graphic XVar(), YVar()
   Next c3
10
End If

'Case Residual constant
If optConstant(1).Value = True Then
   Increment = (Val(txtMax(2)) - Val(txtMin(2))) / Intervals
   For H = Val(txtMin(3)) To Val(txtMax(3)) Step Val(txtInc) 'HPC
      If optDisinfectant(0).Value Then arrDisin(calCounter) = "Cl2" Else arrDisin(calCounter) = "NH2Cl"
      If optSubstratum(0).Value Then arrSubst(calCounter) = "PC" Else arrSubst(calCounter) = "DI"
      arrConst(calCounter) = txtConstant
      arrVariable(calCounter) = H

      c5 = Val(txtMin(2))
```

```
      For cnter = 0 To Intervals
         x5 = -1.89 + (c5 / 9)
         c3 = Val(txtConstant) 'conc
         If (optDisinfectant(1) And optSubstratum(1)) Then x3 = -1 + c3 Else x3 = -1 + (c3 / 0.25)
        '0.33713 * x1 ^2 - (0.415003 + 0.212368 * x5) * x1 + H - 4.490725 - 0.138714 * x2 + 1.46049 * x3 -
0.236191 * x5 - 0.228892 * x6 = 0
         a = 0.33713
         b = -(0.415003 + 0.212368 * x5)
         c = H - 4.490725 - 0.138714 * x2 + 1.46049 * x3 - 0.236191 * x5 - 0.228892 * x6
         x1 = (-b - (b ^ 2 - 4 * a * c) * 0.5) / (2 * a)
         XVar(cnter) = c5
         YVar(cnter) = (x1 + 1) * 250
         c5 = c5 + Increment
      Next cnter
      AssignColor
      graphic XVar(), YVar()
   Next H
End If

'Case Temperature constant...
If optConstant(2).Value = True Then
   Increment = (Val(txtMax(2)) - Val(txtMin(2))) / Intervals
   For c3 = Val(txtMin(3)) To Val(txtMax(3) + Val(txtInc)) Step Val(txtInc) 'conc
   If c3 > Val(txtMax(3)) Then GoTo 20
      If optDisinfectant(0).Value Then arrDisin(calCounter) = "Cl2" Else arrDisin(calCounter) = "NH2Cl"
      If optSubstratum(0).Value Then arrSubst(calCounter) = "PC" Else arrSubst(calCounter) = "DI"
      arrConst(calCounter) = txtConstant
      arrVariable(calCounter) = c3

      If (optDisinfectant(1) And optSubstratum(1)) Then x3 = -1 + c3 Else x3 = -1 + (c3 / 0.25)
      c1 = Val(txtMin(2))
      For cnter = 0 To Intervals
         x1 = -1 + (c1 / 250)
         c5 = Val(txtConstant) 'temp
         x5 = -1.89 + (c5 / 9)
         H = 4.490725 + 0.415003 * x1 - 0.33713 * x1 ^ 2 + 0.138714 * x2 - 1.46049 * x3 + 0.236191 * x5 +
0.228892 * x6 + 0.212368 * x1 * x5
         XVar(cnter) = c1
         YVar(cnter) = H
         c1 = c1 + Increment
      Next cnter
      AssignColor
      graphic XVar(), YVar()
   Next c3
20
End If

'Case BOM constant
If optConstant(3).Value = True Then
   Increment = (Val(txtMax(2)) - Val(txtMin(2))) / Intervals
   For c5 = Val(txtMin(3)) To Val(txtMax(3)) Step Val(txtInc) 'temp
      If optDisinfectant(0).Value Then arrDisin(calCounter) = "Cl2" Else arrDisin(calCounter) = "NH2Cl"
      If optSubstratum(0).Value Then arrSubst(calCounter) = "PC" Else arrSubst(calCounter) = "DI"
      arrConst(calCounter) = txtConstant
```

```
      arrVariable(calCounter) = c5

      x5 = -1.89 + (c5 / 9)
      c3 = Val(txtMin(2))
      For cnter = 0 To Intervals
        If (optDisinfectant(1) And optSubstratum(1)) Then x3 = -1 + c3 Else x3 = -1 + (c3 / 0.25)
        c1 = Val(txtConstant) 'BOM
        x1 = -1 + (c1 / 250)
        H = 4.490725 + 0.415003 * x1 - 0.33713 * x1 ^ 2 + 0.138714 * x2 - 1.46049 * x3 + 0.236191 * x5 +
0.228892 * x6 + 0.212368 * x1 * x5
        XVar(cnter) = c3
        YVar(cnter) = H
        c3 = c3 + Increment
      Next cnter
      AssignColor
      graphic XVar(), YVar()
    Next c5
End If

Screen.MousePointer = 0

End Sub

Private Sub cmdCalculate_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = "Calculate = Draw graphs of selected data"
End Sub

Private Sub cmdClear_Click()
  Picture1.Cls
  linecolor = 0
  calCounter = 0
  exceeded = 0
  Unload Legend
End Sub

Private Sub cmdClear_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = "Clear = Delete all graphs and remove legend from screen"
End Sub

Private Sub cmdExit_Click()
  Unload Me
  Unload Legend
End Sub

Private Sub cmdExit_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = "Exit = Finish running program (program is at design status)"
End Sub

Private Sub cmdLegend_Click()
  Legend.Show
End Sub

Private Sub cmdLegend_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = "Display Legend = Describe parameters of graphed models"
```

```
End Sub

Private Sub Form_Load()
constindex = 0
lblMinX = txtMin(2)
lblMinX = Format(lblMinX, "#0.0")
lblMinH = txtMin(1)
lblMinH = Format(lblMinH, "#0.0")
lblMaxX = txtMax(2)
lblMaxX = Format(lblMaxX, "#0.0")
lblMaxH = txtMax(1)
lblMaxH = Format(lblMaxH, "#0.0")
lblXPic.Caption = "Temperature (°C)"
lblYPic.Caption = "BOM (µg/L)"
End Sub

Private Sub Form_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = ""
End Sub

Private Sub fraData_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = ""
End Sub

Private Sub Frame1_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = ""
End Sub

Private Sub Frame3_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = ""
End Sub

Private Sub fraOptions_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = ""
End Sub

Private Sub lblConstant_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = "Constant and unit"
End Sub

Private Sub lblInc_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = "increment = selected incremental value of the third variable"
End Sub

Private Sub lblMax_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = "maximum = selected maximum values of the variables"
End Sub

Private Sub lblMin_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = "minimum = selected minimum values of the variables"
End Sub

Private Sub lblVariables_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As
Single)
```

```
   Describe = "Variables"
End Sub

Private Sub lblXPic_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
If optConstant(0) Then
   Describe = "Temperature (°C)"
ElseIf optConstant(1) Then
   Describe = "Temperature (°C)"
ElseIf optConstant(2) Then
   Describe = "BOM = Biodegradable Organic Matter (µg/L)"
ElseIf optConstant(3) Then
      If optDisinfectant(0) Then
         Describe = "Free chlorine residual (mg/L)"
         Else
         Describe = "Combined chlorine (predominantly monochloramine) residual (mg/L)"
      End If
End If

End Sub

Private Sub lblYPic_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
If optConstant(0) Then
   Describe = "BOM = Biodegradable Organic Matter (µg/L)"
ElseIf optConstant(1) Then
   Describe = "BOM = Biodegradable Organic Matter (µg/L)"
ElseIf optConstant(2) Then
   Describe = "HPC = 10 based Logarithm of Heterotrophic Plate Count (CFU/cm2)"
ElseIf optConstant(3) Then
   Describe = "HPC = Heterotrophic Plate Count (CFU/cm2)"
End If

End Sub

Private Sub optConstant_Click(Index As Integer)
If sending = 0 Then cmdClear_Click
sending = 0

constindex = Index
Dim count&
For count = 0 To 3: lblVariables(count).Visible = False: Next count
txtInc = "": txtConstant = ""

Select Case Index
Case 0   'HPC constant
   lblConstant.Caption = "log(" & optConstant(Index).Caption & " )[CFU/cm2]"
   lblVariables(Index).Visible = True
      txtMin(1) = 0: txtMax(1) = 1000  'BOM
      txtMin(2) = 8: txtMax(2) = 26   'Temp
      'Residual:
      If (optDisinfectant(1) And optSubstratum(1)) Then txtMin(3) = 0.5 Else txtMin(3) = 0.1
      If (optDisinfectant(1) And optSubstratum(1)) Then txtMax(3) = 3# Else txtMax(3) = 0.7
      If (optDisinfectant(1) And optSubstratum(1)) Then txtInc = 0.5 Else txtInc = 0.1
      txtConstant = 4
      lblYPic.Caption = "BOM (µg/L)"
```

```
    lblXPic.Caption = "Temperature (°C)"

Case 1   'Residual constant
  lblConstant.Caption = optConstant(Index).Caption & " [mg/L]"
  lblVariables(Index).Visible = True
    txtMin(1) = 0: txtMax(1) = 1000  'BOM
    txtMin(2) = 8: txtMax(2) = 26   'Temp
    'HPC:
    txtMin(3) = 4#: txtMax(3) = 7.5
    txtInc = 0.5
    If (optDisinfectant(1) And optSubstratum(1)) Then txtConstant = 0.4 Else txtConstant = 0.1
    lblYPic.Caption = "BOM (µg/L)"
    lblXPic.Caption = "Temperature (°C)"

Case 2   'Temperature constant
  lblConstant.Caption = optConstant(Index).Caption & " [°C]"
  lblVariables(Index).Visible = True
    txtMin(1) = 2.5: txtMax(1) = 6 'HPC
    txtMin(2) = 50
    txtMax(2) = 400
    'Residual:
    txtMin(3) = 0: If (optDisinfectant(1) And optSubstratum(1)) Then txtMax(3) = 2.5 Else txtMax(3) = 0.5
    If (optDisinfectant(1) And optSubstratum(1)) Then txtInc = 0.5 Else txtInc = 0.1
    txtConstant = 15
    lblYPic.Caption = "HPC (CFU/cm2)"
    lblXPic.Caption = "BOM (µg/L)"

Case 3   'BOM constant
  lblConstant.Caption = optConstant(Index).Caption & " [µg/L]"
  lblVariables(Index).Visible = True
    txtMin(1) = 3: txtMax(1) = 7   'HPC
    'Residual:
    txtMin(2) = 0: If (optDisinfectant(1) And optSubstratum(1)) Then txtMax(2) = 2.5 Else txtMax(2) = 0.5
    txtMin(3) = 8: txtMax(3) = 26   'Temp
    txtInc = 9
    txtConstant = 150
    lblYPic.Caption = "HPC (CFU/cm2)"
    If optDisinfectant(0) Then
      lblXPic.Caption = "Free chlorine residual (mg/L)"
    Else
      lblXPic.Caption = "Combined chlorine residual (mg/L)"
    End If

End Select

End Sub

Private Sub optConstant_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As
Single)
Select Case Index
Case 0
  Describe = "HPC - Heterotrophic Plate Count (CFU/cm2)"
Case 1
  Describe = "Disinfectant: " & optConstant(1).Caption & " (mg/L)"
```

```
Case 2
   Describe = "Temperature (°C)"
Case 3
   Describe = "BOM - Biodegradable Organic Matter (µg/L)"
End Select

End Sub

Private Sub optDisinfectant_Click(Index As Integer)
If optDisinfectant(0) Then
   optConstant(1).Caption = optDisinfectant(0).Caption & " residual"
   x2 = -1
Else
   optConstant(1).Caption = optDisinfectant(1).Caption & " residual"
   x2 = 1
End If
If optConstant(1) Then
   sending = 1
   optConstant_Click (1)
End If
sending = 1
optConstant_Click (constindex)
End Sub

Private Sub optDisinfectant_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y
As Single)
Select Case Index
Case 0
   Describe = "Disinfectant: Chlorine = Cl2"
Case 1
   Describe = "Disinfectant: Chloramine = NH2Cl"
End Select
End Sub

Private Sub optSubstratum_Click(Index As Integer)
If optSubstratum(0) Then
   x6 = -1
Else
   x6 = 1
End If
sending = 1
optConstant_Click (constindex)
End Sub

Private Sub optSubstratum_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y
As Single)
Select Case Index
Case 0
   Describe = "Substratum: Polycarbonate = PC"
Case 1
   Describe = "Substratum: Ductile Iron = DI"
End Select
End Sub
```

```
Private Sub Picture1_MouseMove(Button As Integer, Shift As Integer, X As Single, Y As Single)
  Describe = ""
End Sub

Private Sub Reset_Click()
Describe = "Mark on = graph is reset;  Mark off = graph is not reset"
Cancel = 1
  If Val(Reset) = 0 Then
    MsgBox "Are you sure you do not wish to reset graph?", 16, "Steady-state Biofilm Accumulation Model"
    Exit Sub
    If (vbOK) Then
      Cancel = 0
    End If
  End If

  If Val(Reset) = 1 Then
    MsgBox "Are you sure you wish to reset graph?", 16, "Steady-state Biofilm Accumulation Model"
    Exit Sub
    If (vbOK) Then
      Cancel = 0
    End If
  End If
End Sub

Private Sub txtMax_Change(Index As Integer)
If Reset.Value Then Picture1.Cls: calCounter = 0
Unload Legend
linecolor = 0
lblMaxX = txtMax(2)
lblMaxX = Format(lblMaxX, "#0.0")
lblMaxH = txtMax(1)
lblMaxH = Format(lblMaxH, "#0.0")
End Sub

Private Sub txtMin_Change(Index As Integer)
If Reset.Value Then Picture1.Cls: calCounter = 0
Unload Legend
linecolor = 0
lblMinX = txtMin(2)
lblMinX = Format(lblMinX, "#0.0")
lblMinH = txtMin(1)
lblMinH = Format(lblMinH, "#0.0")
End Sub

Sub Form_Load()

Height = calCounter * 255 + 850

LegendLine(1).BorderColor = QBColor(arrlcol(1))
lblDisin(1).Caption = arrDisin(0)
lblSubst(1).Caption = arrSubst(0)
lblConst(1).Caption = arrConst(0)
lblVariable(1).Caption = arrVariable(0)
```

```
For X = 2 To calCounter

   Load LegendLine(X)
   If X < 15 Then LegendLine(X).BorderStyle = 1 Else LegendLine(X).BorderStyle = 3
   LegendLine(X).Y1 = LegendLine(X - 1).Y1 + 255
   LegendLine(X).Y2 = LegendLine(X - 1).Y2 + 255
   LegendLine(X).BorderColor = QBColor(arrIcol(X))
   LegendLine(X).Visible = True

   Load lblDisin(X)
   lblDisin(X).Top = lblDisin(X - 1).Top + 255
   lblDisin(X).Caption = arrDisin(X - 1)
   lblDisin(X).Visible = True

   Load lblSubst(X)
   lblSubst(X).Top = lblSubst(X - 1).Top + 255
   lblSubst(X).Caption = arrSubst(X - 1)
   lblSubst(X).Visible = True

   Load lblConst(X)
   lblConst(X).Top = lblConst(X - 1).Top + 255
   lblConst(X).Caption = arrConst(X - 1)
   lblConst(X).Visible = True

   Load lblVariable(X)
   lblVariable(X).Top = lblVariable(X - 1).Top + 255
   lblVariable(X).Caption = arrVariable(X - 1)
   lblVariable(X).Visible = True

Next X

If RegModel!optConstant(0).Value = True Then
      lblC(0).Caption = "HPC": lblVar(0).Caption = "Residual"
   ElseIf RegModel!optConstant(1).Value = True Then
      lblC(0).Caption = "Residual": lblVar(0).Caption = "HPC"
   ElseIf RegModel!optConstant(2).Value = True Then
      lblC(0).Caption = "Temp": lblVar(0).Caption = "Residual"
   ElseIf RegModel!optConstant(3).Value = True Then
      lblC(0).Caption = "BOM": lblVar(0).Caption = "Temp"
End If

End Sub

Private Sub lblC_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As Single)

If RegModel!optConstant(0).Value = True Then
      RegModel!Describe = "Constant: HPC = Heterotrophic Plate Count (CFU/cm2)"
   ElseIf RegModel!optConstant(1).Value = True Then
      RegModel!Describe = "Constant: " & RegModel!optConstant(1).Caption & " (mg/L)"
   ElseIf RegModel!optConstant(2).Value = True Then
      RegModel!Describe = "Constant: Temperature (°C)"
   ElseIf RegModel!optConstant(3).Value = True Then
      RegModel!Describe = "Constant: BOM = Biodegradable Organic Matter (µg/L)"
End If
```

```
End Sub

Private Sub lblConst_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As
Single)

If RegModel!optConstant(0).Value = True Then
    RegModel!Describe = "CFU/cm2"
  ElseIf RegModel!optConstant(1).Value = True Then
    RegModel!Describe = "mg/L"
  ElseIf RegModel!optConstant(2).Value = True Then
    RegModel!Describe = "°C"
  ElseIf RegModel!optConstant(3).Value = True Then
    RegModel!Describe = "µg/L"
End If

End Sub

Private Sub lbld_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As Single)
RegModel!Describe = "D = Disinfectant"
End Sub

Private Sub lblDisin_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As
Single)
If lblDisin(Index) = "Cl2" Then RegModel!Describe = "Disinfectant: Cl2 = chlorine"
If lblDisin(Index) = "NH2Cl" Then RegModel!Describe = "Disinfectant: NH2Cl = monochloramine"

End Sub

Private Sub lblS_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As Single)
RegModel!Describe = "S = Substratum"
End Sub

Private Sub lblSubst_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As
Single)
If lblSubst(Index) = "PC" Then RegModel!Describe = "Substratum: PC = Polycarbonate"
If lblSubst(Index) = "DI" Then RegModel!Describe = "Substratum: DI = Ductile Iron"
End Sub

Private Sub lblVar_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As
Single)

If RegModel!optConstant(0).Value = True Then
    RegModel!Describe = "Variable:Disinfectant Residual (mg/L)"
  ElseIf RegModel!optConstant(1).Value = True Then
    RegModel!Describe = "Variable: HPC = 10 base logarithm of Heterotrophic Plate Counts"
  ElseIf RegModel!optConstant(2).Value = True Then
    RegModel!Describe = "Variable: " & RegModel!optConstant(1).Caption & " (mg/L)"
  ElseIf RegModel!optConstant(3).Value = True Then
    RegModel!Describe = "Variable: Temperature (°C)"
End If

End Sub
```

```
Private Sub lblVariable_MouseMove(Index As Integer, Button As Integer, Shift As Integer, X As Single, Y As
Single)

If RegModel!optConstant(0).Value = True Then
    RegModel!Describe = "(mg/L)"
  ElseIf RegModel!optConstant(1).Value = True Then
    RegModel!Describe = "Heterotrophic Plate Count (CFU/cm2)"
  ElseIf RegModel!optConstant(2).Value = True Then
    RegModel!Describe = "(mg/L)"
  ElseIf RegModel!optConstant(3).Value = True Then
    RegModel!Describe = "Temperature (°C)"

End If

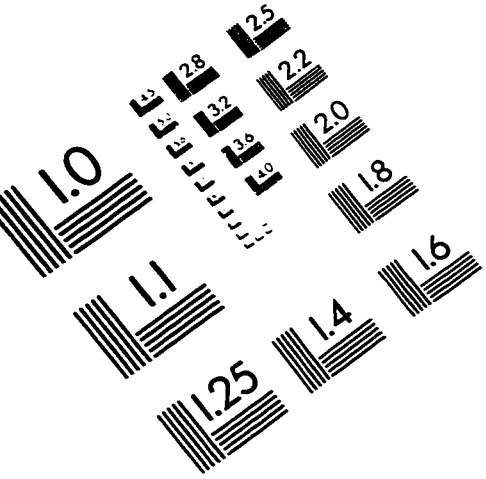
End Sub
```

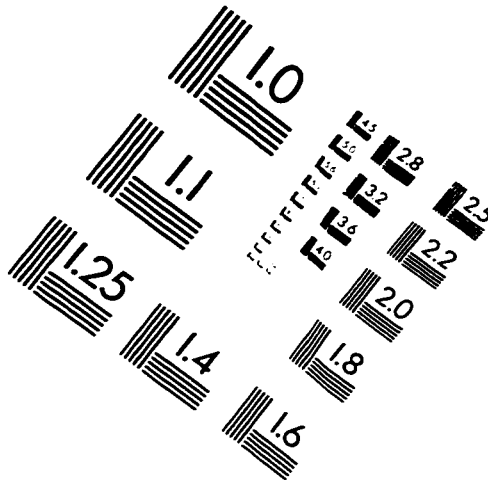
Appendix G/2: Executable File of Interface

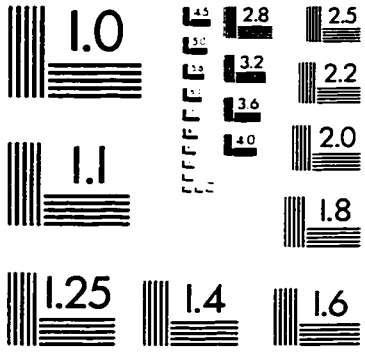
Disks are in holders on the inside of the back cover page

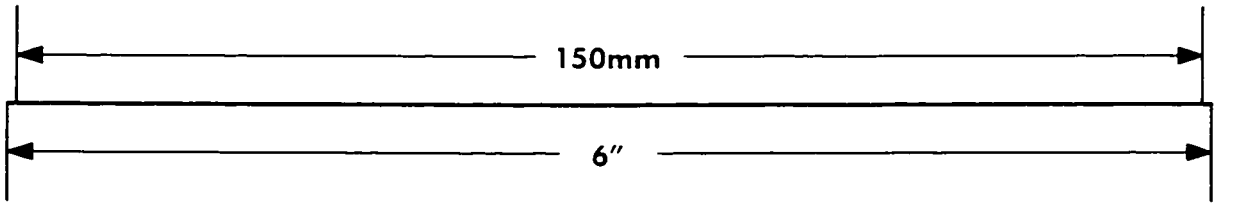
(ENJOY IT!)

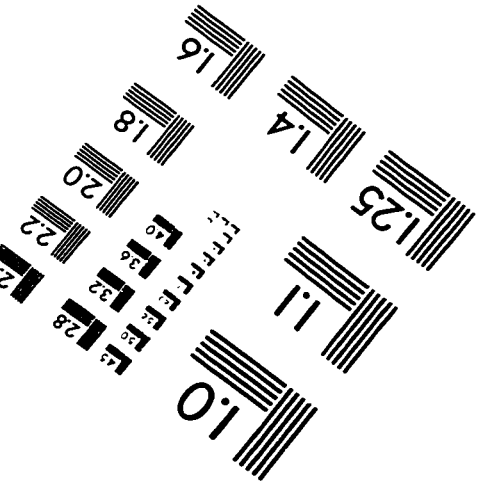
IMAGE EVALUATION
TEST TARGET (QA-3)

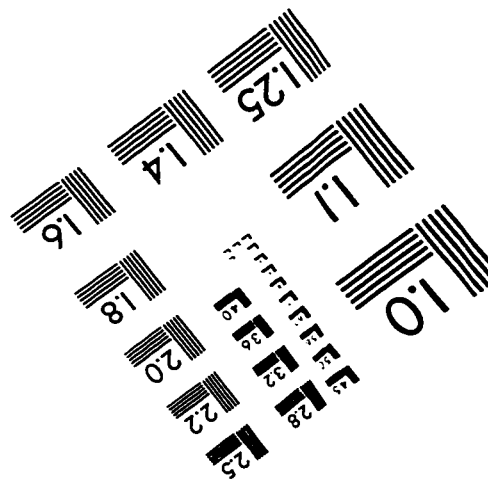












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