



Measuring antimicrobial efficacy against sulfate-reducing bacterial biofilms
by Lawrence Robert Gardner

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Chemical Engineering
Montana State University
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Abstract:

A laboratory reactor system was used to measure the efficacy of glutaraldehyde and nitrite treatments in controlling sulfide generation by sulfate-reducing bacterial biofilms. The experimental system consisted of an annular reactor inoculated with an undefined mixed population from oilfield produced water and fed continuously with Postgate C medium. An anoxic environment and a temperature of 36°C were maintained in the reactor. Glutaraldehyde (500 mg/L, slug dose) and nitrite (100 mg-N/L, 24 h continuous feed dose) were administered to established sulfide-producing biofilms. Both treatments inhibited sulfide production. The glutaraldehyde treatment delayed recovery of sulfide generation (defined as the time required to attain 90 percent of pretreatment sulfide concentration) for an average of 73 h. Sulfide production began to recover within 2 to 4 h after the nitrite dosing was terminated and sulfide levels reached 90 percent of their steady-state values within approximately 24 to 32 h after the end of the dose. Both glutaraldehyde and nitrite penetrated the relatively thin biofilms (5 to 10 microns thick) readily. Biofilms recovered sulfide-generating capacity faster than planktonic cultures when treated with equivalent doses of glutaraldehyde. Corrosion of stainless steel reactor components was noted. Repeated doses of glutaraldehyde, on the same biofilm yielded similar results suggesting that adaptation of the biofilm in response to biocide treatment was not an important process in this case. While this laboratory system offers a reproducible approach to measuring antimicrobial efficacy against sulfate-reducing biofilms, an important outstanding question is how well it captures the performance of these treatments in the field.

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BACTERIAL BIOFILMS

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

A laboratory reactor system was used to measure the efficacy of glutaraldehyde and nitrite treatments in controlling sulfide generation by sulfate-reducing bacterial biofilms. The experimental system consisted of an annular reactor inoculated with an undefined mixed population from oilfield produced water and fed continuously with Postgate C medium. An anoxic environment and a temperature of 36°C were maintained in the reactor. Glutaraldehyde (500 mg/L, slug dose) and nitrite (100 mg-N/L, 24 h continuous feed dose) were administered to established sulfide-producing biofilms. Both treatments inhibited sulfide production. The glutaraldehyde treatment delayed recovery of sulfide generation (defined as the time required to attain 90 percent of pretreatment sulfide concentration) for an average of 73 h. Sulfide production began to recover within 2 to 4 h after the nitrite dosing was terminated and sulfide levels reached 90 percent of their steady-state values within approximately 24 to 32 h after the end of the dose. Both glutaraldehyde and nitrite penetrated the relatively thin biofilms (5 to 10 microns thick) readily. Biofilms recovered sulfide-generating capacity faster than planktonic cultures when treated with equivalent doses of glutaraldehyde. Corrosion of stainless steel reactor components was noted. Repeated doses of glutaraldehyde, on the same biofilm yielded similar results suggesting that adaptation of the biofilm in response to biocide treatment was not an important process in this case. While this laboratory system offers a reproducible approach to measuring antimicrobial efficacy against sulfate-reducing biofilms, an important outstanding question is how well it captures the performance of these treatments in the field.

INTRODUCTION

Background

Biofilms consist of micro and macroorganisms that adhere to and populate a surface (Costerton *et al.*, 1995; Costerton, 1995; Fletcher, 1996). Microbial cells and abiotic substances are embedded in a protective extracellular polymeric matrix attached to a substratum (Characklis *et al.*, 1990) whose size, shape, and location are determined by species-specific factors (Costerton, 1995; Rice, 1999). These aquatic communities are continuously bathed with bulk fluid nutrients via interspersed water channels of the biofilm matrix. Biofilm formation confers protective and commensalistic effects for the participating organisms. Microorganisms in the biofilm mode of growth are less susceptible to deleterious chemical, physical and biological factors. Even when oligotrophic conditions persist, the biofilm matrix sequesters critical organic carbon and energy sources necessary for survival (Costerton and Lappin-Scott, 1995).

The ever-present microbial biofilm mode of growth was first observed by Geesey *et al.*, 1977. Biofilms are detrimental to many industrial and medical processes including: drinking water quality, souring of oil, corrosion of metals, reduced efficiency of heat-transfer equipment, increased drag of ships due to fouling of hulls, human infections, and medical implants (Costerton *et al.*, 1995).

Implanted devices are non-exempt from bacterial colonization with infection being the number one cause of failure. Some of the clinical related diseases and infections include cystic fibrosis (Doggett, 1969), coronary artery disease {Biomaterials Symposiums, 1998}, inner ear infections (Rayner *et al.*, 1998), prosthetic heart valves and joints {Biomaterials Symposium, 1998}, catheters, and pacemakers {Khardori, 1995; Biomaterials Symposium, 1998}

Biofilm Formation and Heterogeneity

The ubiquitous quality of bacterial biofilms is independent of the surface material, e.g. metals, inert materials, and human tissues. The topography of a given surface, however, dictates the pattern of the biofilm colonization (Camper *et al.*, 1994; Hamilton *et al.*, 1995; Scheuerman *et al.*, 1998). The first step in biofilm formation is the "conditioning" of the surface by the accretion of inorganic ions and organic molecules (Cheung and Beech, 1996; Costerton *et al.*, 1995; Marshall, 1996). This phenomenon results in the establishment of an organic film. Freely suspended organisms in the passing bulk fluid are then able to initiate colonization by adhering to these conditioned films. Depending on the environmental conditions, biofilm colonization may be driven by an affinity for specific adhesion receptors, e.g. substrates, proteins, or even the surface material.

Direct observation via various forms of microscopy has enabled researchers to capture the majority of what we know about microbial biofilms. Coupling of microprobes with confocal scanning laser microscopy (CSLM), has illuminated a

more definitive snapshot of the intrinsic complexity, sophistication, and heterogeneity of bacterial biofilms through three-dimensional images and time-lapse photography (Costerton, 1995; Dalton *et al.*, 1994; Lawrence *et al.*, 1991; Lewandowski *et al.*, 1993; de Beer *et al.*, 1994). The term "biofilm" implies an encapsulated matrix of microorganisms protected from environmental stresses with the constitutive ability to adapt to adverse changes in their habitat. Biofilm cells are protected by a microbially produced layer of extracellular polymeric substances (EPS). The layer is composed of polysaccharides, proteins and other organic matter. The EPS matrix may aid in the protection from biocidal activity in the form of diffusional resistance (Stoodley *et al.*, 1994).

The initial planktonic cells that colonize a surface undergo a phenotypic transformation to a sessile state and in turn function as the parent population of the resulting biofilm. There is an apparent lag time in the subsequent replication associated with the attachment event (Rice, 1999). It has been speculated that during this lag phase in growth, the cells are increasing EPS production and switching to a biofilm phenotype, by inducing new genes. The progeny or daughter cells of the parent organisms have the ability to redistribute along the surface, establish dense colonies near the parent cells, detach into the bulk liquid, or form long chains of cells (Lawrence and Caldwell, 1987; Power and Marshall, 1988; Rice, 1999). In the case of a *Pseudomonas aeruginosa* PA01 strain, Rice (1999) observed that some replicated cells moved away from their original locations and subsequently colonized new regions of the surface.

A major difference between planktonic and biofilm mode of growth is the critical role of nutrient transfer to the adherent populations. Biofilms are often limited by the mass transport of available nutrients, which results in a concentration gradient from the bulk liquid to the underlying substratum. Just as the EPS provided a barrier to unwanted biocides, this layer can also diminish the nutrients necessary to the microorganisms deep within the biofilm structure. There are three potential avenues of obstruction to nutrient delivery: external mass transport from the bulk liquid to biofilm interface (Characklis *et al.*, 1990), diffusion into the biofilm (Hoyle *et al.*, 1993), and potential reaction with biofilm constituents prior to reaching the substratum. With the upper layers of the biofilm being readily accessible to passing nutrients, it has been proposed that the cells closer to the surface are actively growing while the organisms deeper into the structure are less active (Huang *et al.*, 1998; Wentland *et al.*, 1996). Inherent heterogeneity of the biofilm was confirmed by decreasing concentration profiles of metabolic substrates, oxygen (Stoodley *et al.*, 1994), biocides (Sanderson and Stewart, 1997), and tracers (Reinsel, 1995). In some cases, the diffusion profiles illustrated regions of limited permeability, i.e. water channels, followed by areas of transport limitation (Lewandowski *et al.*, 1993). However, metabolic cooperation between different microbial species, where the by-product of one species is a nutrient source for another species, may be an important aspect of natural biofilm growth (Costerton, 1995; MacLeod *et al.*, 1990)

Heterogeneity within biofilms has been traditionally documented with regards to

the chemical and structural aspects of the biofilm (de Beer *et al.*, 1994; Stoodley *et al.*, 1994). Little *et al.* 1987 acknowledged the existence of an electrical gradient between adjacent cells that manifested into a measurable corrosion potential for a conductive surface. Costerton *et al.* (1994) found electrical potentials were present regardless of the colonized surface conductivity. The corresponding movement of ions and charged molecules is dictated by this gradient which may impede the ability of charged biocides and antibiotics to diffuse into the biofilm matrix. Modification to the biofilm electrical potential by an externally applied electric field, termed the bioelectric effect (Costerton *et al.*, 1994), was shown to enhance the efficacy of antibiotics when used against a biofilm.

Biofilm Resistance to Antimicrobial Agents

It has been widely reported that biofilms possess a higher degree of resistance to antimicrobial agents (Cochran *et al.*, 1998; Characklis *et al.*, 1990; Chen *et al.*, 1993; de Beer *et al.*, 1994; Huang *et al.*, 1995; Sanderson and Stewart, 1997; Stewart, 1996; Srinivasan *et al.*, 1995; Xu *et al.*, 1995) when compared to their planktonic counterparts. There are four known mechanisms (Stewart *et al.*, 1998). These include, but are not limited to: (1) external mass transport from the bulk liquid to biofilm interface (Bott, 1992; Characklis *et al.*, 1990), (2) diffusion into the biofilm (Stewart, 1996; Stoodley *et al.*, 1994), (3) potential reaction or neutralization with constituents within the biofilm (Huang *et al.*, 1995; Sanderson and Stewart, 1997), and (4) physiological adaptation by the microorganisms

(Sanderson, 1996; Vransky *et al.*, 1997). Many antimicrobial agents that are tested under planktonic laboratory conditions have reduced effectiveness in field applications, where biofilms are predominant. Biocide concentrations that are effective against planktonic microorganisms tend to be less efficacious with bacterial biofilms due to the protective nature of the biofilm, EPS matrix, and physiological changes initiated by adverse conditions.

Antimicrobial delivery is quite different for suspended cultures compared to sessile populations. Planktonic organisms cannot escape the 360 degrees of exposed surface area whereas biofilm vulnerability is limited to the bulk liquid-biofilm interface. Antimicrobial delivery into a biofilm is actually a complex process. The delivery of the biocide must first overcome external mass transfer resistance. This reduces the biocide concentration at the biofilm interface relative to the bulk fluid concentration. The antimicrobial agent must then diffuse into the biofilm where it reacts with and is neutralized by components of the biofilm, resulting in further reductions in concentration (Gilbert *et al.*, 1990). De Beer *et al.* (1994) used a chlorine microelectrode and found chlorine concentration profiles within a binary species biofilm to be a function of chlorine levels and the neutralizing capacity of the biofilm matrix. In a similar fashion, Huang *et al.* (1995) documented a reduction in respiratory activity near the bulk liquid interface in response to a monochloramine dosing regime. Viability, however, was maintained adjacent to the substratum. The antibacterial activity of the monochloramine was diminished by the sacrificial layer of cells that were in close proximity with the bulk

fluid. This in turn resulted in a partial diffusion of the biocide and overall survival of the biofilm. Srinivasan *et al.* (1995) concluded that the decreased efficacy of monochloramine was attributed to transport limitations for thick, robust biofilms but physiological changes were hypothesized as resistance mechanisms for thinner structures. The neutralization and physiological responses by bacterial biofilms in response to monochloramine was further substantiated by the work of Sanderson and Stewart (1997). A phenomenological model was applied to a repetitive dosing regime and predicted the overall trend in viable cells but overestimated the efficacy of the second dose. The researchers hypothesized a physiological adaptation by the *P. aeruginosa* biofilm to explain this discrepancy.

Biofilm Control Strategies

While biofilms possess appealing qualities in the areas of wastewater management and biodegradation of toxic chemicals, the majority of biofilm awareness or research has been concentrated toward eradication of these structures. The economic impact coupled with health related issues are staggering. Control and/or removal of bacterial biofilms is (are) an overwhelming assignment. Historical approaches to dealing with unwanted biofilms have utilized mechanical and/or chemical therapies. Mechanical approaches are often expensive and difficult to implement due to limited accessibility of the fouled surfaces, whereas chemical treatments are universal in their delivery. Chemical reagents are quite diverse in their modes of action ranging from broad-spectrum

disinfection with oxidizing biocides to tailored mechanisms of microbial disruption, such as compromised cell membrane integrity and interference with nucleic acid and protein synthesis. In the cost conscious markets of today's business world, the selection of a given product is often driven by economics.

System geometry factor plays a primary role in the effective administration of biocides. Accessibility of the biocide to all areas of a given environment is difficult to overcome. Biocides may not adequately penetrate crevices and populations within these crevices can therefore serve as an inoculum for biofilm re-growth (Bott, 1992).

Broad-spectrum agents are generally less expensive and non-specific towards individual organisms. Oxidizing compounds, such hypochlorous acid and monochloramine fall into this category (Boivin, 1995; Bott, 1992). Over the years, these antimicrobial agents have been a staple mode of treatment for the drinking water industry. While these agents were implemented to deter microbial activity, their interaction causes potential harmful effects to the delivery system via corrosion, toxic by-products, and associated odors. The chloramines, however, do not pose health risks and are less reactive when compared to free chlorine (Chen *et al.*, 1993; Chen *et al.*, 1994; LeChevallier *et al.*, 1988). Monochloramine's less reactive nature imparts longer lasting residuals and was more effective than free chlorine for inactivation of *P. aeruginosa* biofilms (Chen *et al.*, 1993; Griebbe *et al.*, 1994).

Antimicrobial agents are applied into the bulk fluid in one of two ways: pulse injection (slug treatment) or a continuous dose for a specific period of time. The manner in which a biocide is applied to a system greatly influences how well the biocide will perform in that system. Slug treatments have been documented to provide a more cost effective and efficacious treatment as opposed to a continuous feed of low-levels of biocide (Dewar, 1986; McIlwaine, 1998). Antimicrobial agents control populations by killing or inhibiting bacterial metabolism but can also influence the dynamics of biofilm formation by reducing the initial adhesion of cells to surfaces. Biocide compounds change the surface free energy of a substratum by altering the conditioning layer. The modified surface may influence the number of microorganisms attaching to a surface. Since biocides alter cell wall integrity, adhesion of cells can either be promoted or inhibited due to changes of cell wall hydrophobicity (Cheung and Beech, 1996; Verran and Taylor, 1995).

Sulfate Reducing Bacteria in Oilfield Operations

Sulfate-reducing bacteria (SRB) are a group of phylogenetically diverse anaerobes which carry out dissimilatory reduction of sulfur containing compounds such as sulfate, sulfite, thiosulfate, and sulfur to sulfide (Bak and Cypionka, 1987; Lovley and Philips, 1994; Okabe *et al.*, 1994; Widdel, 1988). These organisms vary in their ability to influence metal deterioration (Cheung and Beech, 1996; Beech *et al.*, 1994). In the absence of sulfate or other inorganic electron

acceptors, some species of SRB can flourish by a fermentation pathway (Postgate, 1984). Sulfate-reducing bacteria metabolize a suite of organic compounds serving as electron donors. These compounds consist of hydrogen, lactate, pyruvate, the low molecular weight fatty acids of acetate, propionate, butyrate, etc. to the long chain fatty acids, keto acids, alcohols, and aromatic compounds (Postgate, 1984; Widdel, 1988). There are two primary nutritional classifications for sulfate-reducing bacteria. The first grouping conducts the incomplete oxidation of organic substrates to acetate while the second can completely oxidize the substrates to CO_2 (Okabe, 1992).

The activity of SRB in a number of natural and man-made systems is becoming more apparent and of economical concern (Bott, 1992; Grab and Theis, 1993). In particular, the oil and gas industries are seriously affected by the sulfides generated by SRB (Hamilton, 1994). Biogenic sulfide also poses health and safety problems, environmental hazards and severe economic losses due to corrosion of equipment (Odom and Singleton, 1992; Lee, 1990; Odom, 1990).

Biofouling in the Petroleum Industry

A subset of the detrimental effects caused by biofilm communities is the havoc rendered by sulfate-reducing bacterial biofilms in the petroleum industry. Biofilm accumulation in pipelines is of paramount importance to oilfield operations. Pipelines serve two primary objectives: transport of produced oil from the oilfield to refinery and secondary water injection into the oil-bearing formation to enhance oil

recovery (Bass *et al.*, 1998; Bass *et al.*, 1993; Herbert, 1994; Iverson and Olson, 1984; Lynch and Edyvean, 1988). The latter is the basis for this research.

Tardy-Jacquenod, *et al.* (1996) performed a study to determine the occurrence and metabolic capacity of sulfate-reducing bacteria from 14 different oilfield sites in France, the North Sea, and the Gulf of Guinea. This effort produced an isolation of thirty-seven sulfate-reducing bacteria strains: 16 from wellhead and 21 from pipeline operations. The results of their survey exemplify the ubiquitous nature of SRB populations in oil petroleum reservoirs and the problems faced with controlling their microbial activity.

Microbial activity by sulfate-reducing bacteria presents a foundation for multiple problems encountered in oilfield operations. These harmful effects include reservoir souring, decreased formation permeability, impeded secondary recovery operations, fouling of surfaces, and exacerbated corrosion. The technology for enhancing oil recovery has inadvertently increased down-hole microbial activity with a battery of field related problems. Sloughing of biomass contributes to the fouling of the reservoir by penetrating the porosity of the reservoir at a velocity front equivalent to the injected water (Rosnes *et al.*, 1991). SRB-mediated corrosion is a topic that has been extensively documented with links to localized corrosion, hydrogen induced cracking, stress corrosion cracking, corrosion fatigue (Hamilton, 1998; Lee *et al.*, 1995; Little and Wagner, 1994; Lynch and Edyvean, 1988). Corrosion in aqueous environments can be described as an electrochemical process that results in the destructive attack of metals. The significance and

extent of biocorrosion is readily apparent by the available literature devoted to corrosion by sulfate-reducing bacteria (Iverson and Olson, 1984). The existence of sulfodigens in biofilms attached to metal surfaces results in the initiation of corrosion due to the production of sulfide and organic acids (Bass *et al.*, 1998; Hamilton, 1985; Lee *et al.*, 1995).

Biofilm accumulation on the interior walls of the pipeline increases the fluid resistance with the potential for partially plugging the medium adjacent to the injection well. This results in restriction of flow (increased injection pressures or reduced injection rates) and as mentioned earlier, enhanced rates of corrosion. Previous work has shown a need for antimicrobial agents such as chlorine or glutaraldehyde in order to maintain biofilm activity and accumulation to a minimum (Cheung and Beech, 1996; Boivin, 1995; Vatsala and Mah, 1995; Grab and Theis, 1993; Whitham and Gilbert, 1993; Eagar *et al.*, 1988; Costerton and Lashen, 1983). The ramifications of sulfate-reducing bacteria respiration with respect to corroded surfaces are astronomical. While corrosion was not a monitored variable in this research, failure to mention this process would not present the complete picture of SRB activity in the field.

Microbiologically Influenced Corrosion

Corrosion is the naturally occurring electrochemical process by which materials fabricated of metal or their alloys undergo chemical oxidation from a ground state to an ionized species via two half-reactions: oxidation of the metal surface (the

anode), and the reduction of the chemical species (cathode) in contact with the metal. The oxidation products of a metal surface eventually serve as a diffusion barrier to further oxidation of the underlying metal. Changes in the environmental conditions can effect the stability of the protective metal oxide film and the susceptibility of the metal to corrosion (Scully, 1990). Microbiologically influenced corrosion (MIC) occurs when surface conditions are altered microbiologically via biochemical reactions. The resulting conditions prevail in the establishment and maintenance of the cathodic and anodic regions necessary for the promotion of corrosion (Stoecker II, 1995).

Bacterial influence on the corrosion of mild steel has been proposed by several models. Clearly, a number of factors are involved and not one predominant mechanism exists in the role of MIC by SRB's (Hamilton, 1998; Lee *et al.*, 1995). Cathodic depolarization refers to the anaerobic corrosion of ferrous metal by the removal of hydrogen, which is catalyzed by the presence of hydrogenase, and the production of iron sulfide (von Wolzogen Kuhr, 1934). The uptake of cathodic hydrogen comes at the expense of iron depletion at the anode. In a concerted fashion, the dissolution of iron reacts with bacterially produced sulfide generating a protective FeS film over the metal surface (Booth *et al.*, 1965). Ultimately the integrity of the film is compromised which exposes regions of unprotected and unreacted metal that can promote cathodic depolarization. Sulfide generation inhibits the formation of molecular hydrogen from atomic hydrogen thereby increasing the amount of atomic hydrogen at the metal surface and its deleterious

effects. Permeation of hydrogen into the metal structure causes embrittlement and stress cracking which are attributed to multiple types of failures in the petroleum industry. The ability to produce a broad spectrum of corrosive metabolic by-products over a wide range of environmental conditions makes bacteria a real threat to the stability of metals that have been engineered for corrosion resistance.

Biofilm Control in the Petroleum Industry

The choice and delivery of biocides to pipeline distribution systems in the petroleum industry are complex and often misunderstood. Early detection of microbiological activity is necessitated for a proactive approach in preventing the degradation of capital equipment and souring of oil. The physical characteristics of a pipeline vary in diameter, length, and material but possess one common characteristic, the lack of accessibility. This element impedes the field personnel's ability to assess and control microbial activity within the pipeline. Unfortunately, these inaccessible environments provide a "head start" for the proliferation of microbial communities on wall surfaces and in the oil-bearing formation. The presence and type of microbial activity is often difficult to detect, assess, and monitor. Another set of burdening factors are the time delay and available methodologies required in the assessment of biocide efficacy in controlling unwanted biofilms (Boivin, 1995; Eagar *et al.*, 1986; Herbert, 1994).

Ironically, the analysis of the inner surface of a pipeline yields approximately

1% of biotic material. The remaining material consists of inorganic scales, corrosion products, and entrained hydrocarbons. Accumulation of these bulk materials reduces the effective volume of the pipeline and increases the drag of the bulk liquid. Oil pipelines are one of the few aquatic environments with the affordable luxury to engage mechanical removal techniques with complimentary chemical strategies. In an attempt to remove the unwanted accumulation, the pipelines are routinely scraped by a process known as pigging. It is nearly impossible to entirely remove the deposits but the process disrupts the structures.

The incidence of reservoir souring has lead to the deployment of combined chemical and physical strategies to diminish sulfate-reducing bacterial biofilms (Bass *et al.*, 1993; Herbert, 1994). Utilization of physical and chemical strategies, however, provides a contradictory situation for pipeline operations even though cleanliness of a pipeline and antimicrobial efficacy are inter-related. Physical removal of pipewall accumulation aids in the delivery of the antimicrobial to the actively respiring bacteria, but the removed debris can re-attach, proliferate, plug reservoirs, and potentially be more detrimental than long-term accumulation. The amount of solids removed by the mechanical operations provides a qualitative indication of system cleanliness. Although it is desirable to minimize the scraping events, the use of chemicals alone to mitigate microbial activity is cost prohibitive. Chemical agents are developed to impede microbial activity using different mechanisms of attack. In an attempt to make microorganisms more vulnerable to chemical strategies, some treatment programs have been developed by pooling

individual capabilities into a more synergistic effect, i.e. an agent that might disperse the biofilm is followed by another chemical that proves to be lethal to the microorganisms. A common theme to biofilm control is that methodologies and successes from one situation may not be easily extrapolated to another (Dewar, 1986).

Chemical treatment programs for field applications are initially established by vendor recommendations that were validated in laboratory and/or field trials. Determination of lethal biocide concentrations in the laboratory may fail to adequately capture the environmental and bacterial demands on the biocide (Dewar, 1986). Application of biocides in the field is hindered by a complex environment with the presence of oil droplets, corrosion by-products, and metal scrapings. Individually or in concert, these variables restrict the interaction of the biocide with the microorganisms (Whitham and Gilbert, 1993). The concentration of the biocide is intuitively important, but the surface area of the system in question and the reactivity of the reagent with biomass, other constituents, surfaces, etc. must also be considered.

Common Antimicrobial Agents

Control of biofilms and biocorrosion are frequently addressed by the delivery of chemical reagents. Glutaraldehyde (Scott and Gorman, 1991), isothiazolone (Gilbert *et al.*, 1990), formaldehyde (Rossmore and Sondossi, 1988), and halogenated biocides (LeChevallier *et al.*, 1988) have traditionally been used to

control microbial activity (Cheung and Beech, 1996). As mentioned above, biocides are broadly classified according to their chemical character, i.e. either oxidizing or non-oxidizing. Chlorine is by far the most widely used oxidizing biocide particularly in cooling water and drinking water distribution systems (Bott, 1992). Oxidizing biocides encompass a wide range of chemicals and environments (Boivin, 1995).

Another widely used group of organic compounds is the quaternary ammonium salts. These compounds possess a cationic charge, which binds with the negatively charged cell wall of the microorganism. Cell lysis results from the stresses associated with this electrostatic charge. Cell wall permeability is another mechanism of attack due to denaturing of cell wall proteins. While these compounds are rapidly biodegradable in low concentrations and are good bacteriostats, they are slow bactericides and expensive (Bott, 1992; Boivin, 1995; Lynch and Edyvean, 1988).

Application of aldehydes, e.g. glutaraldehyde and formaldehyde, to oilfield settings has been the basis of widely used treatment procedures. Glutaraldehyde has been used by itself or in combination with other biocides to increase its overall performance. The broad spectrum activity, nonionic nature, and tolerance to sulfide make glutaraldehyde an indispensable biocide for the petroleum industry. Major disadvantages of formaldehyde are potential carcinogenic effects, high doses required for effectiveness, and adaptation by microorganisms that offset its economical feature (Bessemers, 1983; Boivin, 1995).

Glutaraldehyde, 1,5-pentaedial, has been employed for nearly 30 years in an effort to control unwanted bacterial colonization in industrial systems. Gorman and Scott (1991) have outlined some of the plausible mechanisms responsible for the efficacy of glutaraldehyde with microorganisms. It is believed that the biocide interacts with the proteins of the outer cell membrane thereby diminishing the permeability of the cell (Dewar, 1986). This reduction in permeability hinders the transfer of nutrients and harmful by-products resulting in cell death (Cheung and Beech, 1996). As with any other biocide, the efficacy of glutaraldehyde is governed by the pH and temperature of the environment, contact time, and concentration. Eagar, *et al.* (1988) also suggested that the apparent efficacy of glutaraldehyde is the combined result of the chemical activity and the hydrodynamics of the environmental system. First, the microbial mode of attachment is partially compromised when subjected to the glutaraldehyde. This altered state then subjects the microorganisms to accelerated detachment by the abrading bulk fluid.

Alternative Sulfide Inhibition Strategies

Reservoir souring, the result of hydrogen sulfide production, has an enormous economic impact for oil companies at a cost of billions of dollars per year (Lee, 1990). In 1949, Allen noted the addition of nitrate to waste water was successful in controlling noxious sulfide odors and Jennemen (1986) favorably reported that nitrate inhibited sulfide production in a variety of anaerobic systems. Investigation

by researchers at Montana State University further determined that nitrate was being microbiologically reduced to nitrite (Mueller, 1994; Reinsel, 1995) and concluded that nitrite inhibited microbial souring. Comparisons between microbially reduced nitrite and external additions of nitrite yielded similar results. In a comparative study with glutaraldehyde, nitrite was found to be more effective at inhibiting sulfide production in sandstone columns (Goeres, 1995; Reinsel, 1995). Deployment of this alternative nitrite strategy reduces the environmental and economic concerns that commonly plague biocide delivery programs.

Research Goal and Objectives

A general purpose of the research presented in this thesis was to develop a working experimental system to evaluate the efficacy of biocides on a sulfate-reducing bacterial biofilm. Upon validation, the research objectives were more specifically tailored to address the following questions.

1. To what extent does transport limitation impede penetration of the biocide into the biofilm?
2. To what degree does the biofilm react with the biocide?
3. Does repetitive dosing with a non-oxidizing anti-microbial elicit an adaptive response?

EXPERIMENTAL MATERIALS AND METHODS

The goal of the experimental design was to enrich an undefined produced water sample while possessing conditions similar to an oilfield setting. Temperature, pH, and anoxic conditions were monitored and maintained during the experiments. An oxygen-free Postgate C medium with a 1.5% salinity level was utilized for enrichment, preservation, and experimental analysis. Water from a produced water facility was enriched for sulfate-reducing bacteria and used as the inoculum for the biocide efficacy studies.

The metabolic activity of these organisms was monitored by the production of sulfide as compared to the corresponding uptake of sulfate. The sulfide concentrations were determined by a modified methylene blue method (Cline, 1969) while the sulfate levels were measured via ion chromatography. Biomass was quantified by total organic carbon analysis with a biofilm thickness measurement determined by cryoembedding and cryosectioning procedures. The concentrations of glutaraldehyde were assayed by gas chromatography.

Microorganisms

Samples of produced water from the Chevron Lost Hills oilfield in California were used as the source of a microbial consortium for the planktonic and biofilm studies. A water analysis indicated low levels of carbon and energy sources available to the microorganisms although evidence of microbial colonization was apparent downstream. Produced water was aliquoted into four 1-L glass jars with oil added to the top of the containers to minimize aeration. Upon receipt in the laboratory, 200-mL of one of the 1-L containers was transferred in 50-mL aliquots to 100-mL sterile and anoxic vials. These vials and the remaining 1-L containers were stored at 40°F.

The growth culture medium for these sulfidogenic bacteria was a modified Postgate C medium (Postgate, 1984) with a 1.5% salinity level. The composition and concentrations are denoted in Table 1. The appropriate levels of the sodium sulfate and sodium dithionite solutions were obtained from Pfennig *et al.* (1986).

The Postgate C recipe provided a composite sulfate concentration of 1.024 g-S/L with a 60% (w/w) sodium lactate salt (Sigma-Aldrich *D*-lactic acid, L-1375) serving as the primary organic substrate.

Table 1. Medium Composition

Constituent	Concentration (g/L)
KH_2PO_4	0.5
NH_4Cl	1
Na_2SO_4	4.5
$\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$	0.06
$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	0.06
Sodium lactate	6
Yeast extract	1
$\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$	0.004
Sodium citrate $\cdot 2 \text{H}_2\text{O}$	0.3
NaCl	15
$\text{Na}_2\text{S} \cdot 9 \text{H}_2\text{O}$	3-mL of 2.5g/50-mL stock
Sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$)	1-mL of a 1.5g/50-mL stock
0.1% Resazurin	1-mL

Enrichment and Inoculum Preservation

The base medium was prepared using the Hungate Method (Ljungdahl and Wiegel, 1986; Miller and Wolin, 1974) with the reducing agents of sodium sulfide and sodium dithionite. The sulfide solution was processed by adding 50-mL of ultrapure water to a 250-mL Corning Pyrex autoclavable media bottle (Fisher #06-414-1B) that incorporated a #6 size butyl-rubber stopper. The stopper was drilled to accommodate a Bellco anaerobic test tube (#2048-18150) equipped with a butyl rubber septum-type stopper and aluminum crimp ring. This facilitated anoxic and sterile transfers. While the headspace of the 250-mL bottle was being sparged

with high-purity nitrogen (99.99%), the sodium sulfide crystals were rinsed with ultrapure water to remove the inherent oxide layer, weighed, and quickly added to the bottle. The vessel was immediately sealed to limit any loss of sulfide to the gas phase. The high-purity nitrogen gas was introduced into a Lindberg/Blue M oven (Model #TF55035A) that contained a reducing column composed of copper wire maintained at 400°C. The gas was delivered to the appropriate vessels with an in-line 0.3 μm hydrophobic glass fiber bacterial air vent (Gelman Sciences #4210) and a sterile point-of-use 0.2 μm cellulose acetate syringe filter (Corning #21052-25). To further provide an anoxic environment, the sulfide bottle underwent a series of nitrogen and vacuum cycles via the rubber septum and a 25-ga. needle. This solution was subsequently autoclaved at 121°C for 30 minutes.

In a similar manner, the sodium dithionite solution was prepared by adding the appropriate amount of crystals to 50-mL of sterile oxygen-free ultrapure water in a 100-mL batch vial. The environment was made oxygen-free by performing the vacuum and nitrogen purge sequence three times. The final state of the vial headspace was maintained with a nitrogen positive pressure.

The 50-mL serum vials for enrichment and culturing were pre-assembled with a butyl rubber septum-type stopper and aluminum crimp ring followed by headspace evacuation with the nitrogen and vacuum sequence mentioned above. These vials remained under a state of vacuum prior to introduction with the medium.

To adequately provide an anoxic transfer of the Postgate C medium to the 50-mL serum vials, a 2-L Erlenmeyer flask was equipped with a butyl rubber stopper that included two process lines, one for a nitrogen purge and one for medium removal. The medium was boiled and cooled under a blanket of nitrogen per the Hungate method (Ljungdahl and Wiegel, 1986; Miller and Wolin, 1974). Once the medium cooled to room temperature, a positive pressure was supplied to the flask. Three-mL of the sodium sulfide and 1-mL of the 0.1% resazurin solutions were added to the mixture. Prior to these additions, the syringes were displaced of air via the high-purity nitrogen. A 60-mL syringe equipped with a 25-ga. needle was also purged with nitrogen prior to the aqueous transfers of the medium to the batch vials. The nitrogen blanket within the 2-L flask was periodically replenished as the headspace pressure diminished during the transfers.

Upon completion of the medium transfer, the individual batch vials were supplied with a nitrogen headspace and subsequently autoclaved for 30 min at 121°C. As the vials cooled to room temperature, 1-mL of filter-sterilized sodium dithionite was added to each vial. The pH of the base medium was initially adjusted to 8.0 using sterile 6N NaOH. This rendered a final pH = 7.0-7.5 (post sodium sulfide addition, autoclaving, and sodium dithionite addition). Periodic sampling of uncultured vials denoted a pH in the range of 7.2 to 7.3. The vials were stored at 40°F.

The initial enrichment of the preserved Lost Hills produced water was performed in duplicate. Vials were incubated at 35°C using a Lab-Line Imperial III

incubator, and assayed qualitatively via turbidity. Five-mL aliquots of these preserved produced waters were aseptically added to oxygen-free sterilized vials containing 30-mL of the modified Postgate C medium. As the vials became turbid, a sub-enrichment was prepared by transferring 1-mL to each of the five vials containing fresh Postgate C medium. It was during this portion of the culturing that the sulfate and microbially produced sulfide levels were monitored every two hours in an effort to capture a pseudo growth profile.

The resulting sulfide profile provided an optimum window of growth in which to perform the preservation of the inoculum. Hence, the frozen culture enrichments were permitted to grow into the mid to upper log phase prior to preservation with 20% (v/v) glycerol. The cryo-preservation vials were stored at -70°C . This enriched culture exhibited primarily Gram-negative staining. Motility and a curved rod-shaped morphology were noted under microscopic observation. Our population was not identified for specific sulfate-reducing bacteria even though other researchers have done so by 16S RNA methodologies (Amann *et al.*, 1991).

Planktonic Experiments

The efficacy of glutaraldehyde (the model biocide for our analysis and a benchmark in oilfield operations) for controlling suspended sulfate-reducing bacteria was determined in batch experiments using 50-mL batch vials. Various concentrations of the biocide were administered to determine a lethal dose for a

complete kill of planktonic organisms. The delivery of the biocide was designed to mimic the protocol for the annular reactor biofilm experiments (See Table 6 and Figure 10).

The biocide was administered in the late exponential to early stationary phase so that potentially toxic levels of sulfide did not inhibit the metabolism of the sulfate-reducing bacteria. In order to determine this optimum range, a confirmatory growth curve was generated to capture the metabolic activity of the microorganisms. Sulfide and sulfate levels were monitored over a 36-hour period.

For the biocide evaluation, the microorganisms were subjected to the dosing protocol as they entered the stationary phase. All portions of the protocol were carried out at 35°C. A 1.5-mL frozen culture was added to 30-mL of sterile and anoxic Postgate C media in a 50-mL batch vial. After approximately 30 hours (late exponential to early stationary phase), a 1-mL aliquot was transferred to a sterile, oxygen-free, and medium-free environment of 100-mL of 1.5% NaCl in a 250-mL Corning Pyrex autoclavable medium bottle equipped with a Bellco anaerobic test tube and butyl rubber septum-type stopper. The solution was incubated for a period of 6.5 hours. This time is analogous to the nutrient medium flush time in the biofilm experiments. At t=6 hours, the bottles were sampled for sulfide and sulfate. Upon completion of this "flush" stage, a 1-mL aliquot was transferred to 100-mL batch vials containing 60-mL of sterile and anoxic 1.5% NaCl at various glutaraldehyde concentrations, i.e. 0, 35, 50, 65, 100, 350, and 500 mg/L. The vials were incubated at 35°C for a period of 7 hours. The working concentrations

were made from a stock standard using the Union Carbide Uracide 250, a 50% (v/v), glutaraldehyde source.

At the end of the 7-hour contact period, the solutions were centrifuged to decant the unwanted aqueous component that contained the glutaraldehyde. A Sorvall Instruments Model RC5C centrifuge was operated at 10,000 rpm and 4°C for 10 minutes using the SS-34 rotor. The 60-mL centrifuge tubes were sparged with nitrogen for 5–10 minutes prior to sample transfer. Immediately after centrifuging and decanting the aqueous portion, a sterile and anoxic volume of 30-mL of Postgate C medium was added to re-suspend the pellet. The tube was vortexed for 2 minutes. These suspensions were then transferred to sterile and oxygen-free vials via a filter and syringe, which was followed by the addition of a nitrogen headspace. The ability of the planktonic sulfate-reducing bacteria to recover from the biocidal activity was quantified by sampling and analyzing the levels of sulfate and biogenic sulfide as they incubated at 35°C. The incubation period varied according to the biocide concentration and therefore ranged from 5 to 20 days. The "zero" concentration vial served as the control to the biocide treatments. This vial also accounted for any potential side effects as a result of the centrifugation and resuspension processes. In order to not deplete the available volume of nutrients within the vials, sulfate sampling did not occur until the sulfide concentration exceeded 10 mg S/L.

Biofilm Experiments

Assessment and screening of biocides has been educational and at times an overwhelming assignment. Early researchers performed screening operations using suspended organisms with the subsequent realization that the given concentrations were less effective in the field. Hence, the initial screening protocol was limited to batch vials with sessile efficacy evaluations being performed in laboratory pipeloops. While the laboratory loop was designed to mirror field criteria, its operation was cumbersome and time consuming. These combined qualities gave rise to the advent of smaller and quick turn around laboratory reactors, e.g. annular reactor, porous media columns, rotating disk reactors, and artificial biofilms (McIlwaine, 1998; Chen and Reinsel, 1996; Sanderson, 1996; Stewart *et al.*, 1998; Whitham and Gilbert, 1993; Pitts *et al.*, 1998).

A continuous flow annular reactor (BioSurface Technologies Corporation Inc., Model 920 with a digital rpm display) was chosen to evaluate the efficacy of glutaraldehyde for controlling sulfate-reducing bacteria. This type of reactor was selected due to a parallel but different study being performed by Chevron Inc. at their Lost Hills field site. The operating parameters listed in Table 2 mimic some of the field reactor configurations.

Table 2. Annular Reactor Operating Conditions

Parameter	Value	Units
Inner drum rotation	150	Rpm
Dilution rate	0.5	hr ⁻¹
Media flow rate	0.79	mL/min
1.5% NaCl flow rate	7.13	mL/min
Bulk liquid temperature	36	°C
Reactor volume	1	L
Nitrogen sparge flow rate @ 15 psi	6.1-8.6	mL/min

The BST Model 920 biofilm annular reactor consists of two concentric cylinders, a rotating inner cylinder and stationary outer cylinder. There is a provision for temperature control of the reactor environment via a water jacket adjacent to the stationary cylinder. Four vertical draft tubes on the inner cylinder ensures complete mixing of the reactor liquid phase. The annular reactor mixing characteristics were verified by a tracer study with the procedure listed in a forthcoming section. The rotating inner cylinder accommodates twenty removable coupon sites on the outer wall (Figure 1). Polycarbonate slides were used as the biofilm coupons. Each slide had an area of approximately 22 cm². The annular reactor provided an approximate wetted surface area to volume ratio of 260 m⁻¹. A detailed listing of the reactor component specifications is listed in Appendix A.

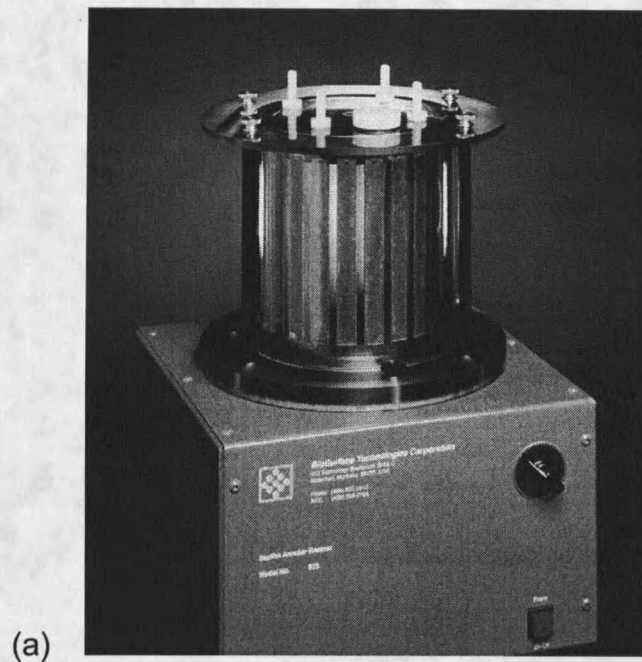
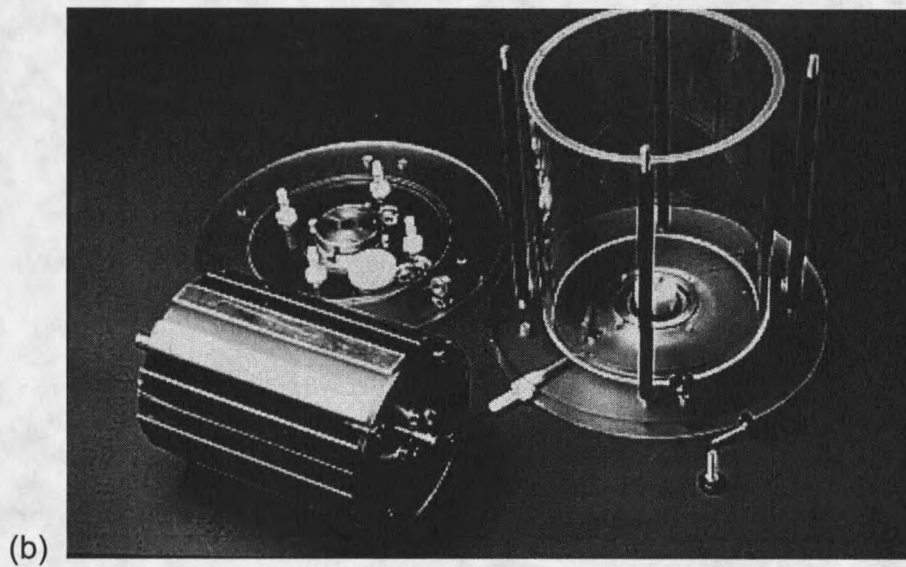


Figure 1. BioSurface Technologies Model 920 biofilm annular reactor

Annular Reactor Mixing Study

Prior to culturing a biofilm in the annular reactor, the reactor fluid residence time was characterized. A tracer study was performed with red food coloring to evaluate the mixing behavior within the reactor. The experimental setup is portrayed in Figure 2.

The effluent from the annular reactor was fed to the flow-through cell of a HACH DR2000 spectrophotometer. The distance between the effluent port and the spectrophotometer was minimized to alleviate any additional liquid volume and more importantly, to capture real-time changes within the reactor. Changes in the effluent concentration over time were recorded by downloading the absorbance readings via a RS232 cable (HACH #49670-00) to a Pentium®60 computer using HachLink data acquisition software (HACH #49665-00). This information provided an approximation to the hydraulic residence time distribution of non-reactive species moving through the reactor. The data ultimately aids in the interpretation of biocide reaction kinetics as the agents are displaced from the annular reactor.

Sampling intervals varied during the course of the experiment; every 10 seconds for the first 70 minutes followed by every minute for approximately 85 minutes, and then every 10 minutes for the remaining duration of the experiment, a period of 9 to 11 hours. The combined influent flow rate was set to 7.9 mL/min

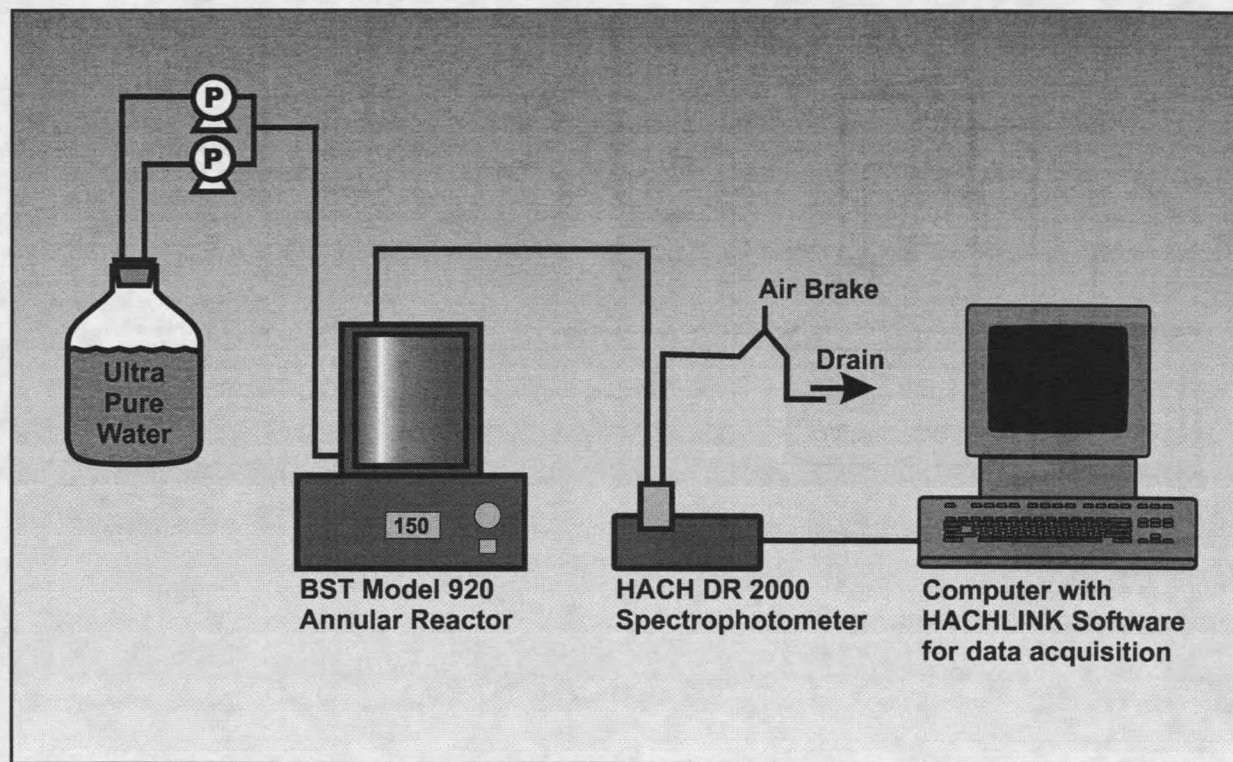


Figure 2. Hydraulic residence time tracer study schematic

using the medium and dilution water Masterflex feed pumps with the inner cylinder rotating at 150 rpm. The inlet was chosen to be at the bottom port of the reactor to eliminate the necessity for purging the reactor headspace during the anoxic experiments. The reactor was filled with approximately 1-L of deionized water and any entrained air was displaced with the aid of the feed water. A glass-Y serving as an air break was included on the downstream side of the flow-through cell to eliminate backpressure while providing a gravity feed to the drain. The maximum absorbance of the Schilling's red food coloring was determined by scanning the visible spectrum using a mixture comprised of one drop of the concentrated food coloring to 25-mL of deionized water. The maximum absorbance for this mixture was found to coincide at a wavelength of $\lambda = 500\text{nm}$. Data acquisition was initiated immediately prior to the 0.5-mL injection of the tracer at the inlet port.

Annular Reactor Start-up and Operation

The annular reactor and polycarbonate coupons were cleaned with hot water and a general-purpose laboratory tested cleaner (Fisherbrand Versa-Clean #04-343) followed by a cascading rinse with deionized water. Since an undefined oilfield inoculum served as our microbial consortium, the aseptic protocols were primarily confined to medium preparation and delivery. The focus of this effort was to eliminate contamination of the nutrient medium. Masterflex Tygon® food grade tubing (Cole-Palmer #06419) was used due to its durability upon autoclaving, transparency, and minimal gas permeability. A Masterflex Digital Console L/S

Drive (Cole-Palmer #07523-30) with Masterflex L/S size #13 tubing pump head (Cole-Palmer #07013-52) consisting of a polyphenylene sulfide housing and stainless steel rotor was used to deliver the medium to the reactor. A Masterflex Variable-Speed Console L/S Drive (Cole-Palmer #07521-50) and L/S size #16 tubing pump head (Cole-Palmer #07016-52) supplied the 1.5% NaCl solution. A glass flow break was included in the influent line to impede microbial migration from the biofilm reactor to the medium source. Process lines were pre-assembled and disinfected by pumping a 20 mg/L sodium hypochlorite solution for 10 minutes followed by a 2 hour rinse with sterile ultrapure water. It was during this period that the flow rates were verified and if necessary, adjusted accordingly. Once the reactor was filled with nutrient medium, an additional 3 hours of nutrient medium were processed through the reactor to counteract any residual sodium hypochlorite.

The Postgate C culture medium was prepared in 10-L quantities using a 13-L Pyrex® glass carboy. The initial annular reactor verification experiment included the sodium sulfide and sodium dithionite reducing agents. It was later determined that these constituents were unnecessary due to the intrinsic behavior of the mixed population in providing conditions favorable to the metabolism of sulfate-reducing bacteria. Henceforth, these reducing agents were no longer included.

The medium carboy was fitted with a butyl rubber stopper that accommodated ports for liquid transfer to the reactor, nitrogen sparging using a solvent reservoir filter (Waters #WAT025531), and a vent with an in-line 0.3 µm hydrophobic glass

fiber bacterial air vent (Gelman Sciences #4210). The delivery of high-purity nitrogen was set to 6.1-8.6 mL/min at 15 psi using a Cole-Palmer (#03216-04) flowmeter based upon dissolved oxygen levels. The pH of the medium was adjusted to 8.4-8.5 using sterile 6N NaOH and autoclaved for 3 hours at 121°C. Post-autoclaving, the medium was continuously purged with high-purity nitrogen to displace any absorbed oxygen during the cooling process. This purging sequence was maintained for a period of approximately 12 hours. A reducing column containing copper wire and maintained at 400°C removed traces of oxygen in the nitrogen feed. After cooling of the nutrient medium, aseptic and anoxic samples were obtained from the media for pH adjustment. The pH was adjusted to 7.1-7.3 using sterile 6 N NaOH via a syringe and 0.2 µm cellulose acetate syringe filter.

The 1.5% NaCl dilution water was prepared in 19-L quantities with ultrapure water using a 19-L Pyrex® glass carboy. The carboys were equipped in the same fashion as the medium containers. The solution was continuously mixed using a stir plate and purged with nitrogen for a 12-hour period prior to bringing the solution on-line. Oxygen levels were recorded using a CHEMet 0-1 ppm dissolved oxygen kit (Fisher Scientific #13-299-201).

Once the process lines were disinfected and flushed, the medium and dilution water carboys were placed on-line and used to fill the reactor with a 1/10 strength Postgate C medium (150-170 mg C/L and 102 mg S/L of sulfate). After filling the reactor, any entrained air was displaced with the continuous addition of the medium and dilution water. The flows were maintained for an additional 3 hours to

neutralize and/or flush any remaining reactive species of the disinfectant. The annular reactor was maintained at 36°C by means of a Neslab RTE-221 thermostat controlled water bath recirculating through the water jacket.

Aqueous samples from the rotating annular reactor were anoxically retrieved from the bulk liquid phase through a septum sampling port. The sampling port was fitted at the top of the reactor with a ¼" Teflon® male connector and 0.5" diameter Teflon® faced septum (Supelco#2-2657). In a similar fashion, a ¼" Teflon® union tee and septum was installed on the influent line. A sterile and reusable two-inch 22-ga. deflexive noncoring septa penetration needle (Fisher Scientific #14-825-15L) was used to extract the sample. A schematic rendition and photograph as illustrated in Figures 3 and 4, respectively, depicts the experimental system.

Inoculation was accomplished by injecting 0.75-mL of a frozen stock culture into the reactor and letting it operate in batch mode for 2 hours. Continuous flow was then initiated to support the proliferation of aerobic organisms. The remaining 0.75-mL was used to inoculate a 30-mL Postgate C batch vial and enriched for approximately a 14-hour period. At this point, the reactor was returned to batch mode, inoculated with 30-mL of viable, sulfide-producing organisms and maintained for additional 48 hours. Sulfide levels rose to approximately 50 mg S/L during this time. Continuous flow was re-initiated at a flow rate of 7.9 mL/min. The influent process line and the bulk liquid phase were sampled for sulfate and sulfide. The polycarbonate biofilm coupons were systematically removed during

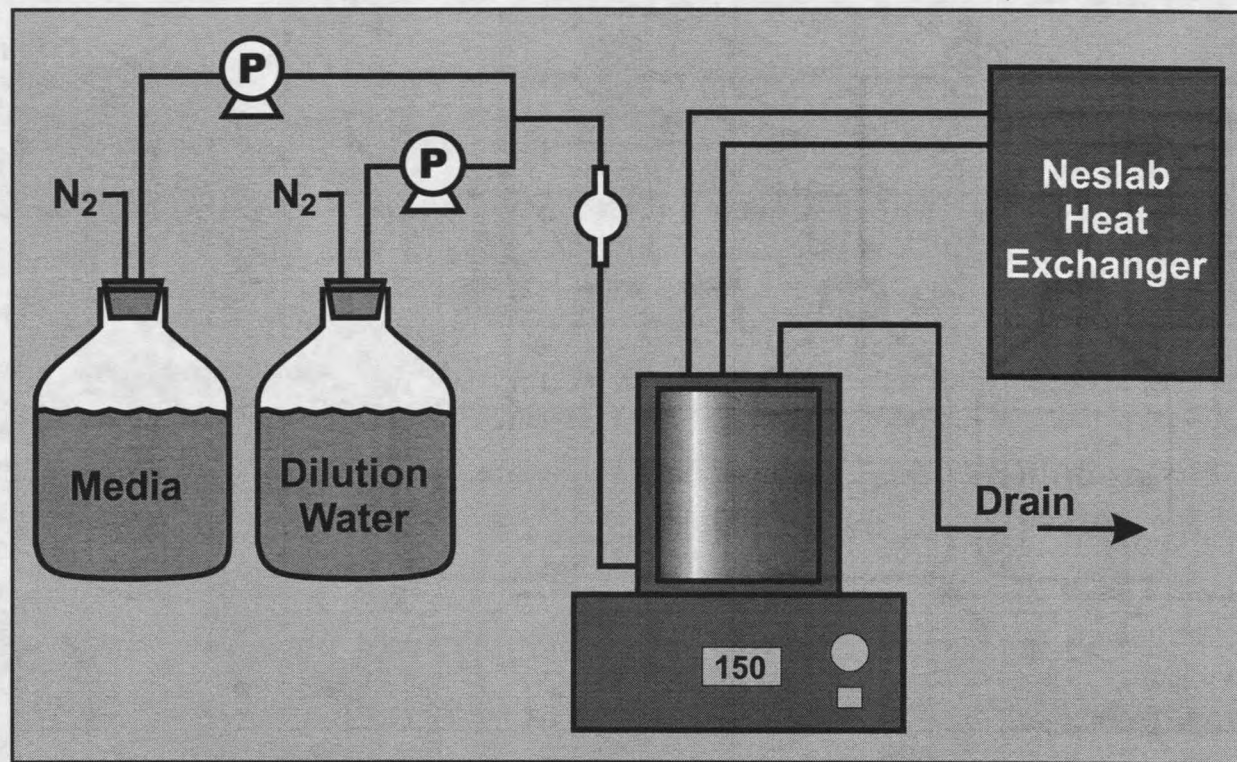


Figure 3. Schematic of biofilm annular reactor experimental system.

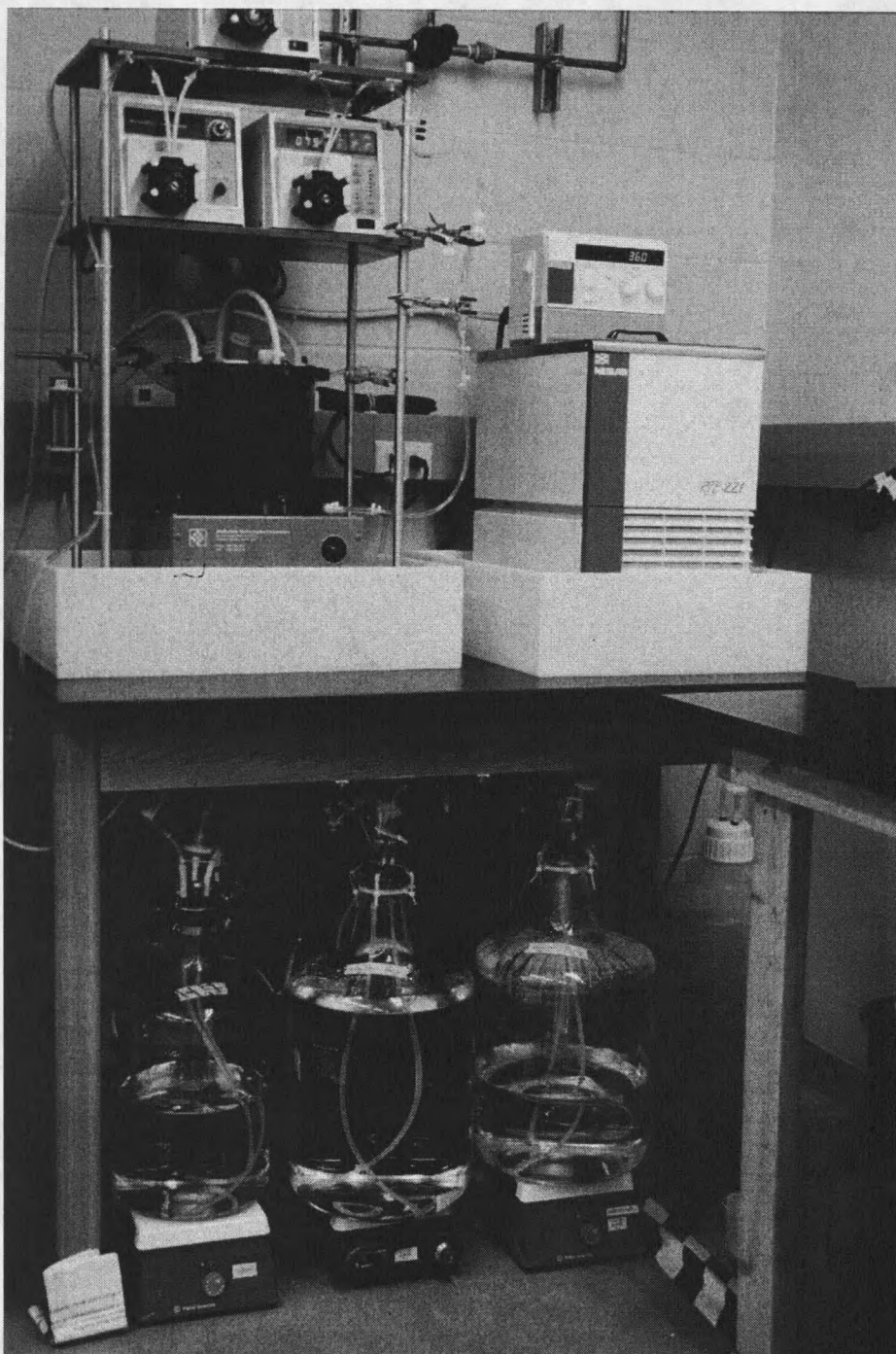


Figure 4. Photo of biofilm annular reactor laboratory system

the course of an experiment for the quantification of total organic carbon and biofilm thickness.

Biocide Addition

A series of batch vial experiments were performed at 35°C to investigate potential reactions of glutaraldehyde with the individual components of the nutrient medium. The glutaraldehyde was determined to be stable except in the presence of yeast extract. To prevent neutralization of glutaraldehyde by the nutrient medium, the Postgate C medium flow was turned off for 6.5 hours in advance of the biocide dose. The 1.5% NaCl dilution water continued to flow and displaced any residual medium from the reactor. A 1-mL dose of 50% glutaraldehyde was injected through the influent line to the 1-L reactor volume. A 1-mL sample from the bulk liquid was immediately taken for glutaraldehyde analysis. Sampling continued every 15 minutes for the next 7 hours. Approximately 0.6-mL of the sample was discarded as waste when it was filtered through a 0.2 µm cellulose acetate syringe filter. The remaining volume was filtered and dispensed into a 2-mL, 12 x 32 mm Target silanized screw thread vial (Fisher P/N 03-375-11AA or C4010-S2W). The concentration of glutaraldehyde was determined using a Hewlett-Packard 5890 Series II Gas Chromatograph. The flow of nutrient medium was reinstated at the completion of the 7-hour biocide treatment. Bulk liquid sulfate and sulfide concentrations were again monitored to capture the recovery of the sulfate-reducing bacteria.

Nitrite Addition

An additional biofilm study was performed in the annular reactor to investigate the effectiveness of nitrite as an inhibitor to biogenic sulfide by sulfate-reducing bacteria. A 24-hour continuous dose of approximately 110 mg N/L of sodium nitrite (VWR #SX0665-1, ACS grade) was delivered to the reactor. The nitrite solution was prepared anoxically in conjunction with the 1.5% NaCl dilution water. The flow of the nutrient medium was maintained to the reactor for the monitoring of metabolic activity. Nitrite levels were sampled from both the influent and bulk fluid ports with the latter at a frequency of every thirty minutes. Sulfide and sulfate concentrations were also obtained at these intervals. At the end of the 24-hour period, the nitrite/NaCl carboy was taken off-line and replaced with an anoxic carboy of 1.5% NaCl. Sampling continued as the nitrite was displaced from the reactor. Sulfide and sulfate levels were continually monitored to investigate any lasting suppression of sulfide generation by the sulfate-reducing bacteria.

Analytical Methods

Aqueous samples from the biofilm annular reactor were anoxically retrieved through a sampling port fitted with a 0.5" diameter Teflon[®] faced septum using sterile and reusable two-inch long 22-ga. deflective noncoring septum penetration needles and sterile syringes that were purged with nitrogen. Influent samples were

taken in a similar manner. A 1-mL sample was removed with 0.1 to 0.5 mL allotted for immediate sulfide analysis while the remaining volume was reserved for determination of sulfate concentration. If sampling point was to be performed in triplicate, three separate syringes were used to retrieve the samples.

While Rosser and Hamilton (1983) documented the simplicity of using a radiorespirometric assay in determining sulfate reduction (Maxwell and Hamilton, 1986; Rosser and Hamilton, 1983), sulfide concentrations were analyzed using the spectrophotometric assay described by Cline (1969). Once the sample was obtained from the annular reactor, it was immediately fixed in zinc acetate. In the case of a 50:1 dilution, the needle was immersed in 4.9-mL of 1% ZnAc and 0.1-mL of the sample was expelled into the solution. Five-mL of the 1% ZnAc served as the spectrophotometer blank. After the sample was placed in the zinc acetate to fix the soluble sulfide, 0.4-mL of the Diamine-R reagent was added and allowed to react for a minimum of 30 minutes. The absorbance was read at a wavelength of 670 nm using the Spectronic Instruments Genesys 5 Spectrophotometer.

The remaining 0.5-mL of the sample was diluted ten-fold with ultrapure water for determination of sulfate levels. These samples were stored in a freezer to inhibit metabolic activity until the sample could be processed. For sulfate analysis, the samples were pretreated with an OnGuard-Ag cartridge (Dionex #039637) in series with a 0.2 μm ion chromatography acrodisc (Fisher Scientific #09-730-257) due to the high levels of sodium chloride present in the samples. The 0.2 μm acrodisc was used to remove any suspended biomass. The first 2.5-mL from a 3-

mL aliquot were discarded post filtration with the remaining 0.5-mL dispensed into Dionex autosampler polyvials with filter caps (#38142). The samples were analyzed by ion chromatography using a Dionex IC DX300 configured with a 2-mm AS4A-SC anion column (#43125), a IonPac AG4A-SC guard column (#43126), and a 15 μ L sample loop. The mobile phase for the anion separation was a mixture of 1.7 mM sodium bicarbonate and 1.8 mM sodium carbonate delivered at 1 mL/min.

For the single nitrite experiment, nitrite levels were assessed using a spectrophotometric HACH Instruments Nitrite Test Kit (#20596-00). A 0.1-mL sample that was obtained with the same syringe for the sulfide and sulfate analyses was dispensed into 4.9-mL of ultrapure water. The HACH NitrVer 3 nitrite reagent pillow packet was added to the solution and vortexed for 1 minute. Absorbance readings were recorded at a wavelength of 546 nm using the Spectronic Instruments Genesys 5 Spectrophotometer. The absorbance values for the samples were consistently recorded ten minutes after adding the pillow packet due to a continuous reaction between the sample and the reagent. The samples were vortexed for an additional 50 seconds prior to the absorbance reading. Generation of the nitrite standard curve (Appendix D) was performed using an ACS grade of NaNO_2 with the same sample volumes listed above.

As mentioned in the Biocide Addition section, aqueous samples were also retrieved to assay for glutaraldehyde concentration by the means of gas chromatography. The reactor was sampled every 15 minutes for 7 hours (biocide

contact time). Filtered samples were immediately injected into the Hewlett-Packard 5890 Series II Gas Chromatograph that was equipped with a flame ionization detector (FID) and a 4ft. x 2mm ID glass packed column with 80/100 Porapak PS (Supelco P/N 2-0346). Three- μ L injections were made using a 10- μ L Hamilton Model 701 syringe (Alltech #803300). Some of the glutaraldehyde process parameters are listed in Table 3.

Glutaraldehyde standard curves were generated the day of treatment using a nominal 50% glutaraldehyde standard (1,5-pentanedial, Union Carbide). These curves are depicted in Appendix D.

Table 3. HP 5890 Series II Operating Conditions

Parameter	Value
Injector temperature	190°C
Detector temperature	250°C
Oven temperature	185°C
Column head pressure	33 psi
Carrier flow rate	20-21 ml/min
Helium pressure	60 psi
Air pressure	40 psi
Hydrogen pressure	20 psi
Detector type	FID
Run time	8 min

Biofilm Sampling: Areal Carbon Density and Thickness

Biofilm covered slides were removed through the threaded coupon port on top of the annular reactor. Sampling was performed by disinfecting this area with ethanol while flooding the port with a nitrogen blanket. The nutrient medium and dilution water flows were ceased with the influent and effluent lines pinched off. A screwdriver type of device fitted with a hook on the end was used to extract the polycarbonate coupons. Once a coupon was removed from the reactor environment, it was immediately replaced with a new one. Coupon selection throughout the different phases of an experiment was based upon a random number generator at the onset of the study. Care was taken to minimize the amount of time that the coupon port was open to the atmosphere. The retrieval and replacement of three coupons took approximately 2-3 minutes.

At least two coupons were sampled to determine biofilm carbon areal density. Upon removal, the coupons were individually placed into ~950-mL of sterile 1.5% NaCl for 5 minutes. This step was included to dilute any background carbon that might be associated with the nutrient medium as opposed to the intrinsic biomass carbon. Understandably, this protocol presents the opportunity for biofilm carbon loss. No sloughing of the biomass was observed during the rinsing step.

While carefully holding the coupon by the side edges, the biomass was scraped into a 100-mL beaker using a razor blade. The coupon was rinsed with 8-

mL of sterile 1.5% NaCl in 1-mL aliquots using a P1000 pipetman. Two additional milliliters were used to rinse the contents of the beaker into the dilution tube. The contents of the dilution tube were then homogenized for 1 minute with a Janke & Kunkel IKA-Labortechnik Ultra-Turrax T25 Homogenizer and an S25N-8G tip. The tip was autoclaved prior to use but flame sterilized and rinsed with ultrapure water between samples. For determination of total organic carbon, the samples were vortexed for 1 minute and processed using a Dohrman DC-80 Total Organic Carbon analyzer.

Enumeration with Epifluorescence Microscopy

Biofilm thickness was quantified by microscopic examination of frozen biofilm cross sections (Huang *et al.*, 1995; Yu *et al.*, 1994). The polycarbonate coupons were removed from the reactor by the same methodology described above and at the same interval as the areal carbon density coupons. As the coupon was removed from the annular reactor, it was turned on its edge to wick away any excess moisture. A layer of Tissue-Tek® O.C.T. Compound #4583 (10.24% w/w polyvinyl alcohol; 4.26% w/w polyethylene glycol; 85.5% w/w nonreactive ingredients) was placed on the lower and upper third portions of the coupon. The coupon was immediately placed on dry ice. Once the O.C.T. turned opaque white, which indicated solidification, the biofilm coupon was carefully flexed to remove the embedded specimen. The separated sample was then oriented with the substratum side up and placed back onto the dry ice with sterile tweezers.

Another layer of O.C.T. was added to cover this surface. When the specimen was fully encapsulated with the frozen O.C.T. matrix, the substratum was labeled with an indelible marker, wrapped in aluminum foil, and stored at -70°C prior to cryosectioning. The frozen biofilm sections were processed with the Leica CM 1800 Cryostat at a 5 µm thickness and subsequently transferred to polylysine coated glass slides which possessed a positively charged surface (Fisher Scientific #12-550-17: 75mm x 25mm Colorfrost Plus microscope slides). Figure 5 illustrates the cryoembedding and cryosectioning procedures.

To effectively quantify biofilm thickness, the embedded sections were stained with a 1µg/mL concentration of DAPI (4', 6-diamidino-2-phenylindole) by dispensing 5 µl drops with a RANIN EDP2 100 ml pipetman to cover the surface area of the sample. The slides were placed in the dark and air-dried for a period of 3 minutes. Tilting the slide on its side while adjacent to a laboratory napkin displaced the excess DAPI. Any residual stain was then carefully removed by blotting each section with Kimwipe tissue paper (Kimberly Clark, Fisher Scientific). The samples for all the experiments were stained on one occasion and stored at 40°F until microscopic examination.

The sectioned samples were examined using a Nikon Eclipse E800 microscope with epifluorescence illumination (100 watt mercury lamp). The microscope was equipped with a DAPI/UV cube unit with an excitation filter (340-380 nm) and a barrier filter (435-495 nm). Images were captured using a 40x oil

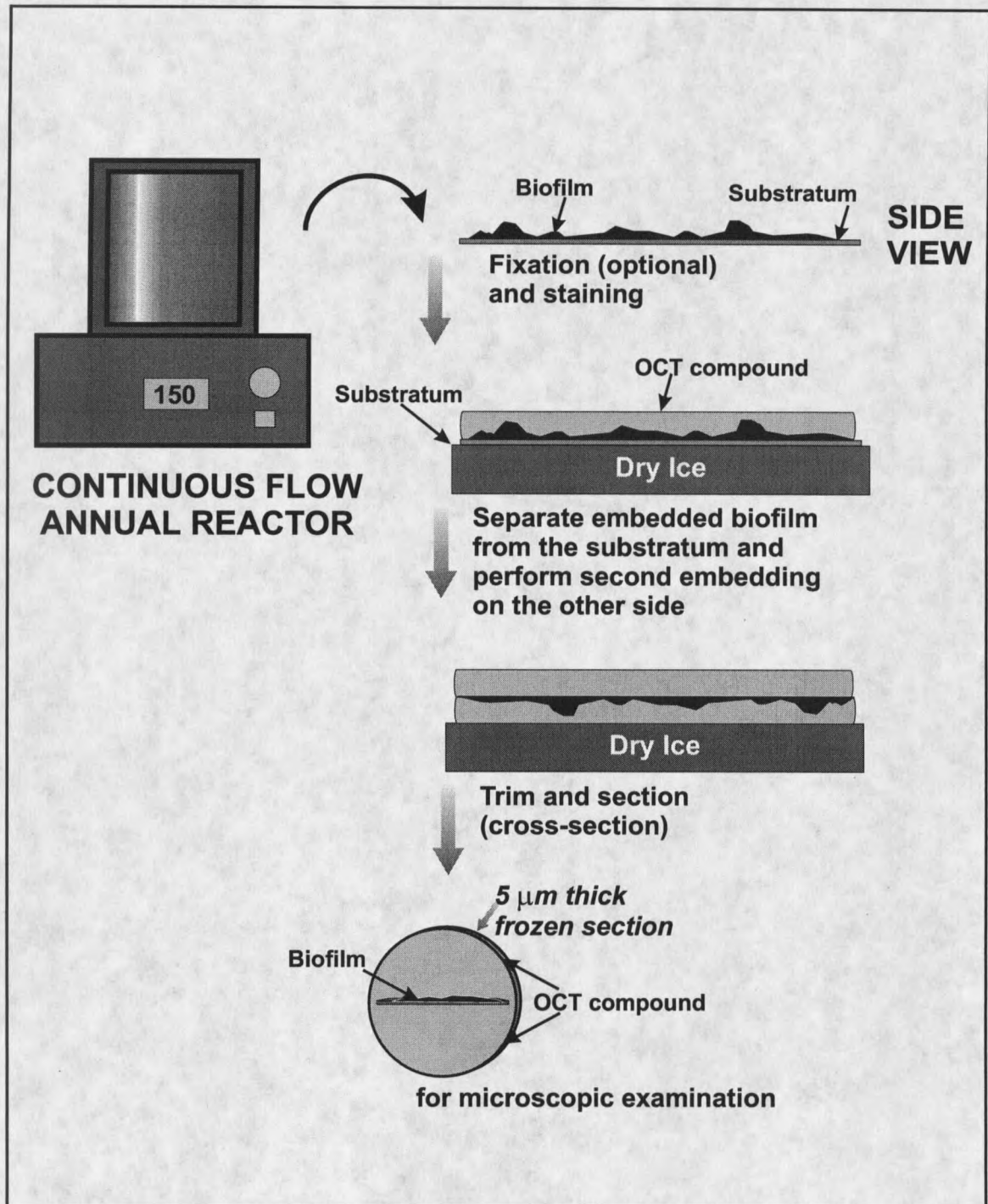


Figure 5. Diagram of cyroembedding and cyrosectioning procedures

immersion objective in conjunction with an Optronics Engineering CCD camera (Model No. DEI-470F) and color video monitor (Sony Corporation Model No. PVM-1342Q). An image of the stage micrometer was taken at these microscope settings to quantify biofilm thickness. The images were converted to a TIF file format and imported to *ImageTool*, (Version 2.0). Analysis was performed on a Digital Pentium® 133Mhz computer using the UTHSCSA *ImageTool* program (developed at the University of Texas Health Science Center at San Antonio, Texas and available from the Internet by anonymous FTP from maxrad6.uthscsa.edu with an Internet web address of <http://ddsdx.uthscsa.edu/dig/itdesc.html>). Twenty-one regions were arbitrarily chosen for measurement of biofilm thickness along the length of a sectioned sample. The sample was scanned a second time to acquire the minimum and maximum thickness. This software allows the user to define the substratum with a line and then perpendicularly move to the outer fringe of the biofilm. The measurement is displayed and stored in a spreadsheet format.

RESULTS

This section presents the results of experimental investigation into the efficacy of the antimicrobial agent, glutaraldehyde, and inhibitory agent, nitrite, on an indigenous population of sulfidogenic bacteria that was obtained from an oilfield produced water facility. Glutaraldehyde microbial experiments were conducted with both planktonic and biofilm cultures while the nitrite study was limited to the biofilm setting.

Planktonic Experiments

The undefined inoculum from the Lost Hills produced water facility was cultured for lactate utilizing sulfate-reducing bacteria using the Postgate C medium. The generation of sulfide and the corresponding uptake of sulfate by these microorganisms in batch vials over a 36-hour period are reported in Figure 6. Sulfide and sulfate concentrations are expressed in terms of mg-Sulfur/liter, e.g. 50 mg S²⁻ as S/L and henceforth are represented as mg-S/L. Sulfate levels were reduced by approximately 50% from their initial values. Using the same November 18, 1998 data and plotting the change in sulfide concentration versus change in sulfate concentration yielded a linear relationship with a correlation coefficient of 0.988 (Appendix E). As a precursor to the planktonic biocide experiments, batch vial studies were performed to document and address any potential changes in

activity or growth by monitoring the concentrations of sulfate and sulfide. Three pseudo-growth curves were performed and they are listed accordingly in Appendix E. An apparent specific growth rate was calculated from each of the sulfide curves, which ranged from 0.26 to 0.27 hr⁻¹. These rates were determined under growth conditions using the full strength Postgate C medium.

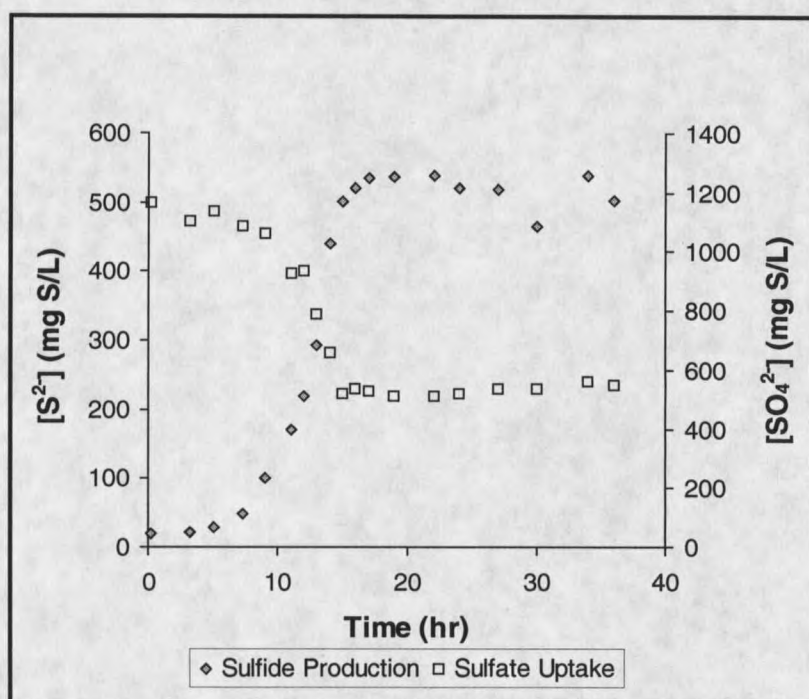


Figure 6. Sulfide production and sulfate uptake for a planktonic suspension enriched with Postgate C medium at 36°C (November 18, 1998 data).

The effectiveness of glutaraldehyde on planktonic SRB suspensions was determined by evaluating various concentrations of the biocide (0, 50, 100, 350, and 500 mg/L) on a viable 16-hour culture. Treatment efficacy was assessed by the period of sulfide inhibition. The delivery of the biocide coincided with the

microorganisms departure from the exponential phase of their growth.

Figure 7 depicts the recovery in the microorganisms ability to reduce sulfate as a function of glutaraldehyde concentration. As can be observed, concentrations of ≥ 100 mg/L were lethal to the planktonic suspensions. A portion of the exponential growth phase for the 50 mg/L vial was unfortunately not captured due to the abrupt resumption in sulfide production.

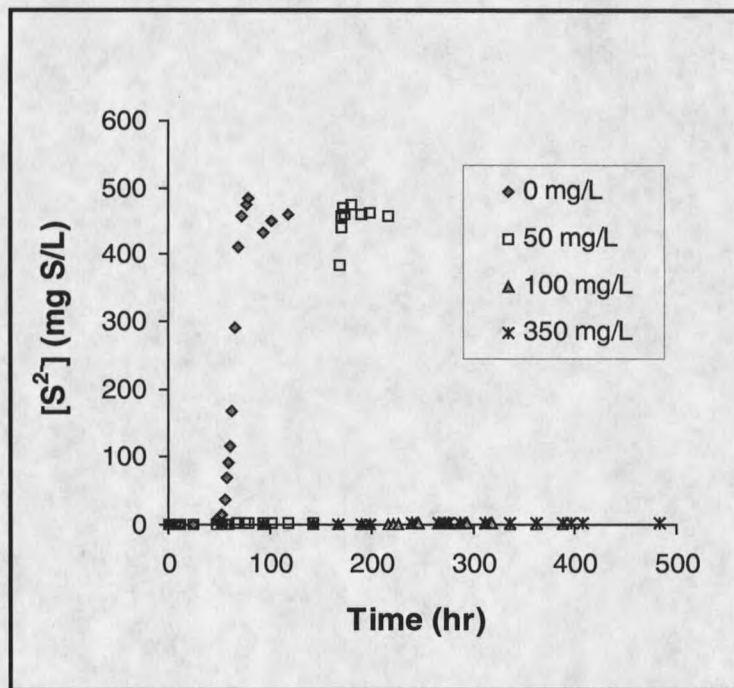


Figure 7. Effect of glutaraldehyde on the activity of planktonic sulfate-reducing bacteria. (Planktonic Experiment #1)

The results presented in Figure 7 were also interpreted in terms of two measurable parameters. The first entity was the amount of time metabolic activity was suppressed below 10 mg-S/L of produced sulfide ($t_{<10 \text{ mg-S/L}}$). A similar quantitative analysis was expressed in terms of the time required to attain 90% of pretreatment steady-state sulfide levels ($t_{90\% \text{ S-S}}$). The respective quantities for these measurables from the planktonic experiments are listed in Table 4.

Table 4. Planktonic recovery time in response to glutaraldehyde treatment. Recovery indices are expressed in terms of the time sulfide production is suppressed below 10 mg-S/L and the time required to reach 90% of steady-state sulfide levels. Recovery time was measured beginning at the time when fresh nutrients were supplied to the vials.

Glutaraldehyde Concentration	$t_{<10 \text{ mg-S/L}}$ (hr)	$t_{90\% \text{ S-S}}$ (hr)
0 mg/L #1	49	69
0 mg/L #2	49	65
0 mg/L #3	42	57
50 mg/L	143	169
100 mg/L	>385	>385

The 50 mg/L level of glutaraldehyde rendered approximately a 100-hr delay as compared to the control vial (0 mg/L glutaraldehyde) for both the time below 10 mg-S/L of sulfide and the time in obtaining 90% of steady-state sulfide levels. Activity in the 100 mg/L was monitored in excess of 385 hours with no evidence of recovery in sulfide production. The planktonic laboratory data and sulfide recovery times are listed in Appendices E and I, respectively.

Biofilm Experiments

Biofilm Reactor Mixing Characteristics

The hydraulic character of a process reactor can be defined by the residence time distribution of individual particles of liquid flowing through the tank (Viessman and Hammer, 1993). The ultimate goal in defining the hydraulic characteristics of the annular reactor was to provide a better approximation for predicting reactive behavior of constituents in the reactor. Knowing the hydraulic residence time distribution (HRT) of a reactor and its degree of dispersion allows for the basis of developing a kinetic description of a process reaction.

A tracer study was used to evaluate the HRT in the rotating annular reactor by introducing a slug of red food coloring into the reactor influent while measuring its effluent concentration over time. This pulsed injection of the non-reactive tracer study was repeated four times with the BioSurface Technologies Model 920 Biofilm Annular Reactor. Two of these evaluations addressed mixing differences as a function of the influent port orientation, i.e. top versus bottom feed, with minimal discrepancies between the studies. The remaining experiments were duplicate bottom feed studies operated at an inner cylinder rotational speed of 150 rpm and an influent flow rate of 7.9 ml/min. The compiled parameter analyses for the bottom feed experiment data sets are listed in Table 5 where Q is the flow rate

(mL/min), V_R the reactor volume (mL), D the dilution rate (hr^{-1}), and t_m the mean hydraulic residence time (hr).

Table 5. Hydraulic characteristics of non-reactive tracer studies.

Parameter	Bottom Feed Experiments		
	Theoretical	Tracer #3	Tracer #4
Q (mL/min)	7.9	7.9	7.9
V_R (mL)	1000	985	1030
D (hr^{-1})	0.5	0.47 ± 0.001	0.43 ± 0.002
t_m (min)	120	125	130

The effluent concentration profile (C curve) in Figure 8 illustrates the effluent response to the pulsed influent stimulus.

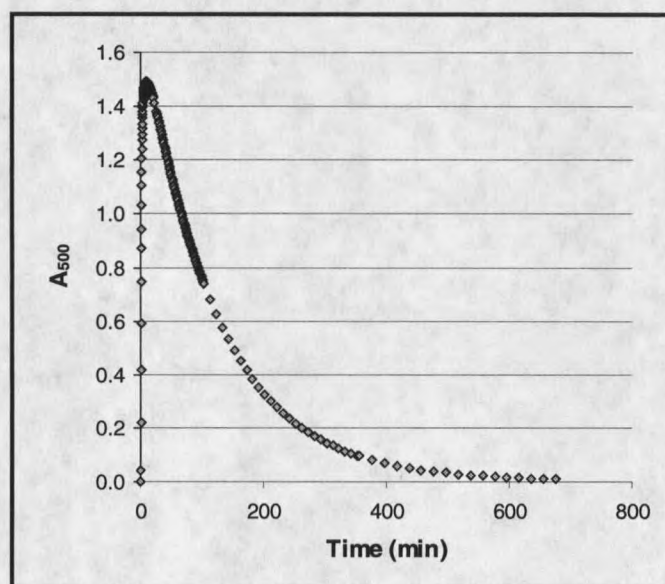


Figure 8. Effluent concentration profile from Tracer #3 for a bottom feed to the annular reactor. The reactor was operated at 150 rpm with an influent flow rate of 7.9 mL/min.

The washout behavior in an ideal CSTR can be modeled by an exponential decay function or by performing a natural logarithm transformation of the effluent concentration and plotting versus time. The slope of the linear regression represents the dilution rate for the reactor in the absence of reactive constituents. This concept is further illustrated in Figure 9 with all the associated analyses and experimental data listed in Appendices B and C.

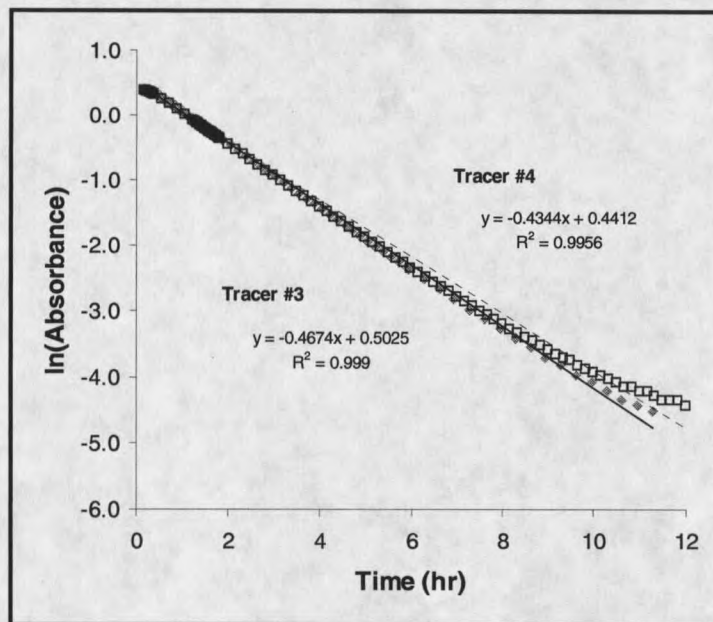


Figure 9. Washout behavior (slope = dilution rate, D) of the non-reactive tracer in the BST Model 920 biofilm annular reactor.

Sequence of Events for Biofilm Experiments

One of the mechanisms for glutaraldehyde's efficacious nature with microorganisms is the biocide interaction with membrane bound protein (Scott and

Gorman, 1991). Yeast extract was one of the Postgate C medium components that obviously possess inherent cellular components. To prevent neutralization of the glutaraldehyde by the Postgate C medium, the medium was "flushed" from the reactor prior to administering the biocide. Table 6 categorizes the sequence of events required for the dosing with glutaraldehyde. This protocol is portrayed in Figure 10 for a given biofilm annular reactor experiment through the aid of the biogenic sulfide profile. The data points are connected to assist the reader with interval transitions.

The rotating annular reactor was operated in batch mode until a steady-state sulfide level of approximately 50 mg-S/L was achieved. As the reactor was transferred from batch to continuous flow, the produced sulfide was displaced from the reactor. Microbial sulfide production resumed as the microorganisms began to metabolize the replenished carbon and energy sources. Washout of sulfide and sulfate occurred during the medium "flush" stage of the protocol to diminish the neutralization effects of the medium with the biocide. The nutrient amendment was reinstated after the biocide treatment period. Metabolic activity was again evident through the assimilation of sulfate and the corresponding production of sulfide.

Table 6. Biofilm annular reactor biocide dosing sequence protocol

Sequence	Event	Description
1	Batch Mode	~48 hours to steady-state $[S^{2-}] = 50$ mg-S/L
2	CSTR Mode	Initiate continuous flow, washout, and recovery
3	Steady state	$[S^{2-}] = 50+$ mg-S/L
4	Medium flush	Elimination of abiotic reaction of biocide and medium
5	Biocide treatment	Pulse dose injection of concentrated biocide
6	Contact period	6.75 hour monitoring of biocide and metabolic activity
7	Resume medium flow	Nutrient flow restarted; sulfate replenished and sulfide levels monitored
8	Steady-state	Sulfide levels return to approximately 50 mg-S/L

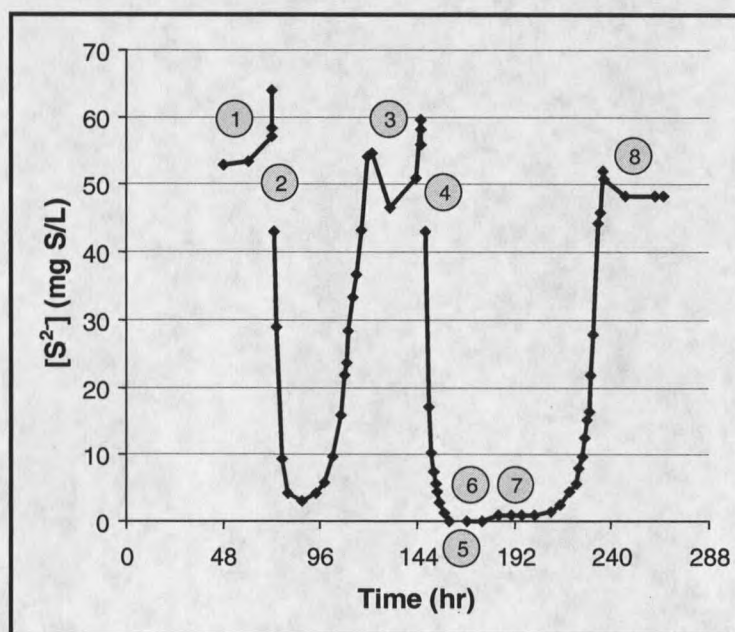


Figure 10. Sequence of events during a biofilm experiment (Experiment #8). The numbers correspond to the steps summarized in Table 6.

Control Experiments

The control experiments proceeded in the same fashion as the biocide studies but with one important difference. The applied dose was a 1-mL pulse injection of ultrapure water. The nutrient medium "flush" stages commenced after approximately 24-30 hours of pretreatment steady-state sulfide levels. Figure 11 exemplifies the sulfide and sulfate profiles for a typical repetitive control dose experiment.

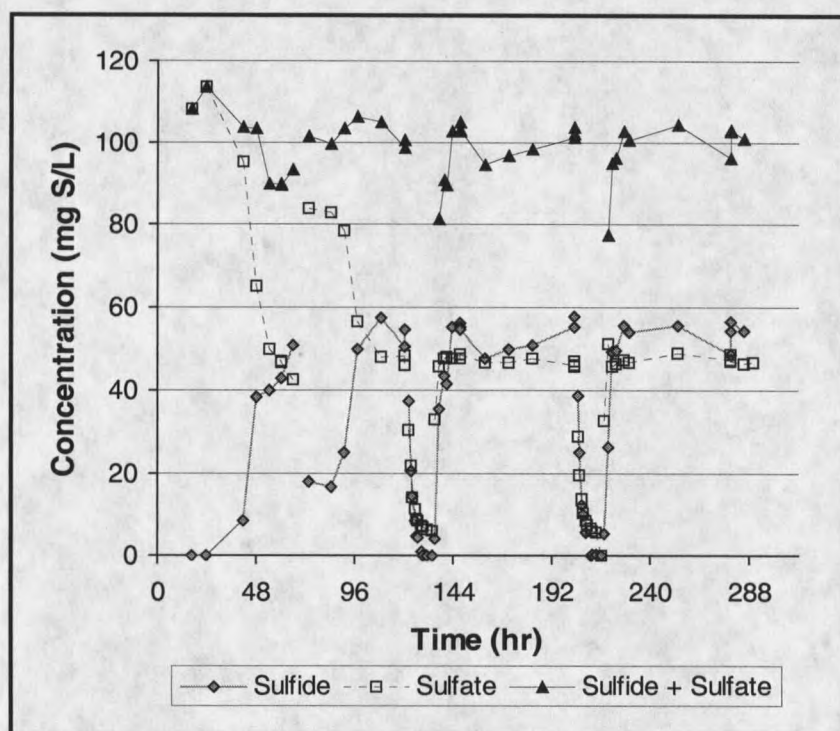


Figure 11. Typical annular reactor control experiment. Concentration profiles for sulfide and sulfate in response to a repetitive dose with ultrapure water. See Table 6 and Figure 10 for experimental protocol. (Control #3 data)

The plot of the total sulfur (sulfide plus sulfate) for the system qualitatively illustrated near closure of the mass balance. It was hypothesized the remaining

sulfur was bound in terms of a FeS precipitate. The sulfide profile portrayed the sequence outlined in the preceding section. As the nutrient flow was returned to the reactor environment, a similar and immediate response in sulfate reduction was observed for two independent doses.

Comparison of Control Experiments

Two control experiments were performed in the annular reactor. One application was a single dose while the second study investigated the response to a repetitive dose of ultrapure water. The 1/10th strength Postgate C medium was delivered to the reactor, which provided an available electron donor concentration of approximately 100 mg-S/L as sulfate. These two experiments were performed to demonstrate the reproducibility of the annular reactor while obtaining a baseline of microbial activity. Since these experiments were not subjected to any inhibitory or biocidal agents, the combined profiles from the two experiments were interpreted as a triplicate of single ultrapure water doses. The complementary profiles of sulfide and sulfate for the three individual dosing periods are shown in Figure 12. These profiles exemplified good repeatability between experiments. In the case of the sulfate curves, the sulfate-reducing bacteria from Control #2 displayed a 3-hour delay in metabolizing the sulfate as compared to the microorganisms for the two profiles from Control #3. The last sampling point of the steady-state intervals was performed in either duplicate or triplicate to document potential sampling variation. The experimental data for these studies

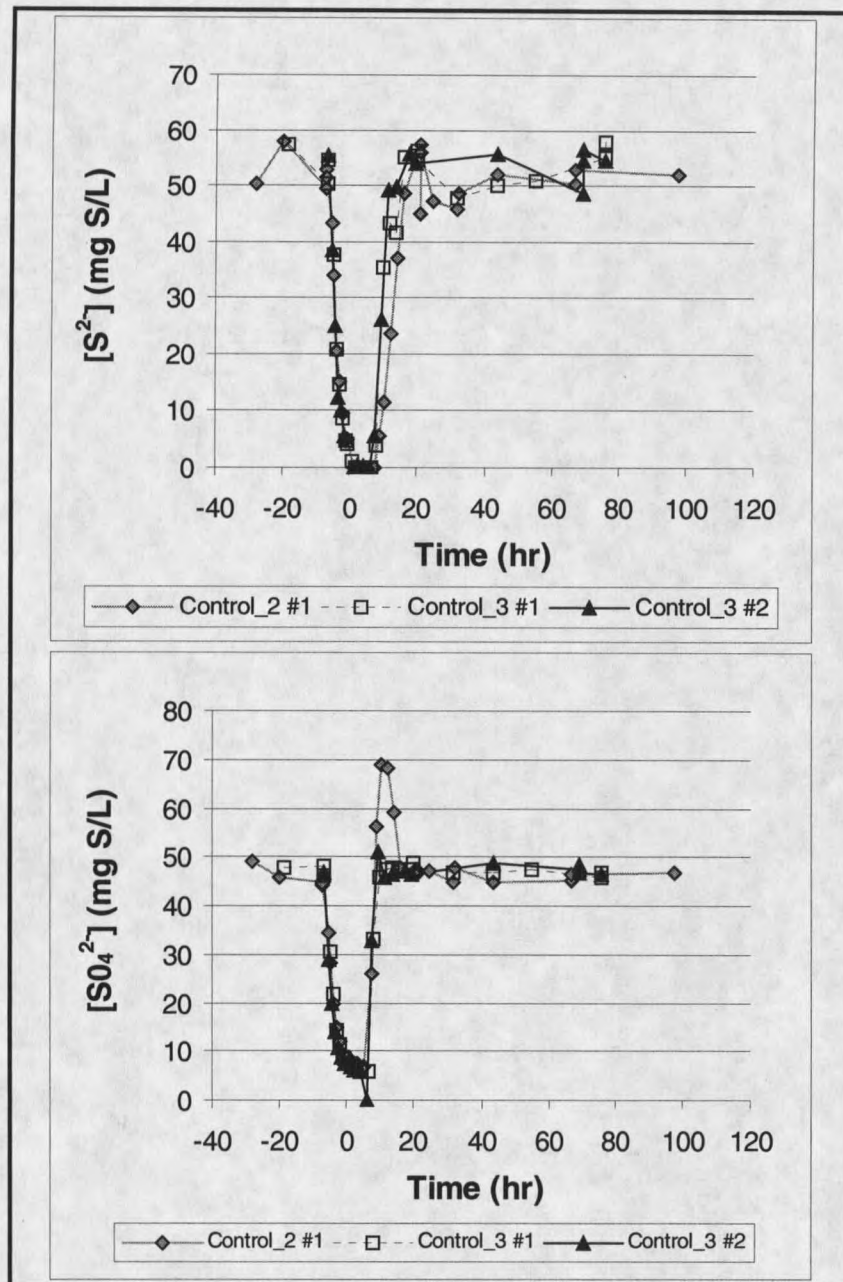


Figure 12. Sulfide and sulfate profile comparison for annular reactor control experiments administered with a 1-mL dose of ultrapure water. The notation #1 implies dose #1 and #2, dose #2. Time zero denotes the pulse injection of ultrapure water; -6.5hr the time nutrient amendment ceased; and +7hr the time nutrient amendment was reinitiated.

are listed in Appendix F.

Biocide Experiments

A 1-mL pulse injection of concentrated glutaraldehyde was administered to provide a calculated peak concentration of 500 mg/L. Three separate experiments were performed: one single dose and two repetitive dose studies. This provided an overall comparison of three single doses with a similar evaluation between the two repetitive dose profiles. The raw data for the biofilm experiments are listed in Appendices F through I.

Figure 13 demonstrates the changes in the metabolic activity of the sulfate-reducing bacteria as a result of a repetitive biocide dosing scheme in the biofilm annular reactor. The figure illustrates the complimentary behavior between the production of sulfide and sulfate consumption during the respective periods of inhibition and recovery. Figure 14 portrays the sulfide profile comparison for the three separate experiments with time zero indicating the pulse injection of the biocide.

Sulfide production was inhibited for similar periods in each of the glutaraldehyde dosing experiments. At first glance, Experiment #4 appears to return to steady-state values that are lower than the pretreatment sulfide levels. Further review of the raw data revealed the average pre- and post-dose concentrations are within 2%. The pre-dose interval displayed two initial sampling

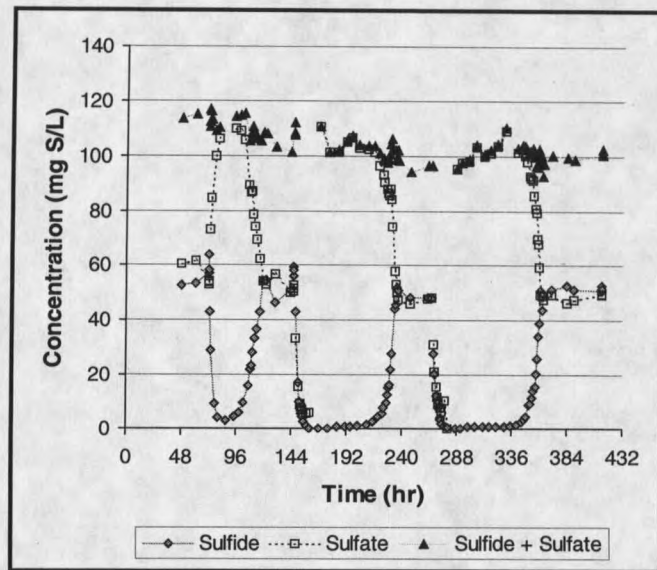


Figure 13. Sulfide and sulfate concentration profiles in response to repetitive pulse injections of 500 mg/L glutaraldehyde. See Table 6 and Figure 10 for experimental protocol. (Annular reactor Experiment #8 data)

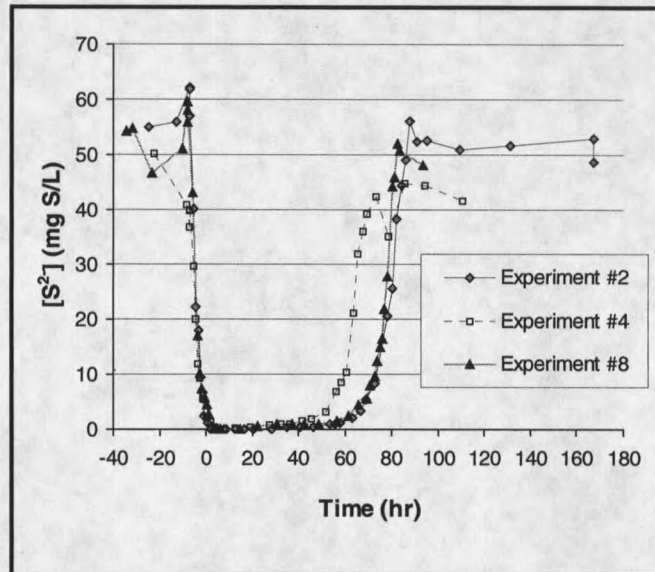


Figure 14. Sulfide profile comparison for three separate annular reactor experiments that were subjected to a single dose of 500 mg/L glutaraldehyde. Time zero denotes the pulse injection of glutaraldehyde; -6.5hr the nutrient ceased; and $+7\text{hr}$ was reinitiated.

points that exceeded 50 mg-S/L of sulfide with four additional samples that bordered concentrations of 40 mg-S/L.

Polycarbonate coupons were sampled at discrete intervals to ascertain biofilm thickness and areal carbon density. The corresponding sets of coupon data are listed in Appendices G and H, respectively, with the mean values and standard deviations similarly reported in Tables 12 and 13 of the Biofilm Thickness and Areal Carbon Density sections.

Glutaraldehyde and Control Dosing Comparison

The sulfide profiles in Figure 15 depict the behavior consistent with washout due to medium removal, suppression during the dosing interval, and obvious resurgence of the sulfate-reducing bacteria. It was apparent that the Experiment #2 profile (glutaraldehyde treatment) experienced a period of suppressed sulfide production after resumed nutrient flow when compared to the control.

An alternative representation of glutaraldehyde efficacy was summarized in Table 7. The associated raw data are located in Appendix I. As illustrated, the Control biofilms were restored to 90% of their pretreatment steady-state sulfide levels in fewer than 10 hours. The biofilms subjected to the glutaraldehyde treatments were consistently suppressed below 10 mg-S/L of sulfide for a period of 60.8 ± 10.8 hours post continuation of nutrient flow. These treated biofilms achieved 90% of steady-state in an average of 72.6 ± 8.7 hours in contrast to

7.3 ± 2.1 hours for the Control studies. Both the control and treatment experiments exemplified a more rapid resurgence when compared to their initial continuous flow profile.

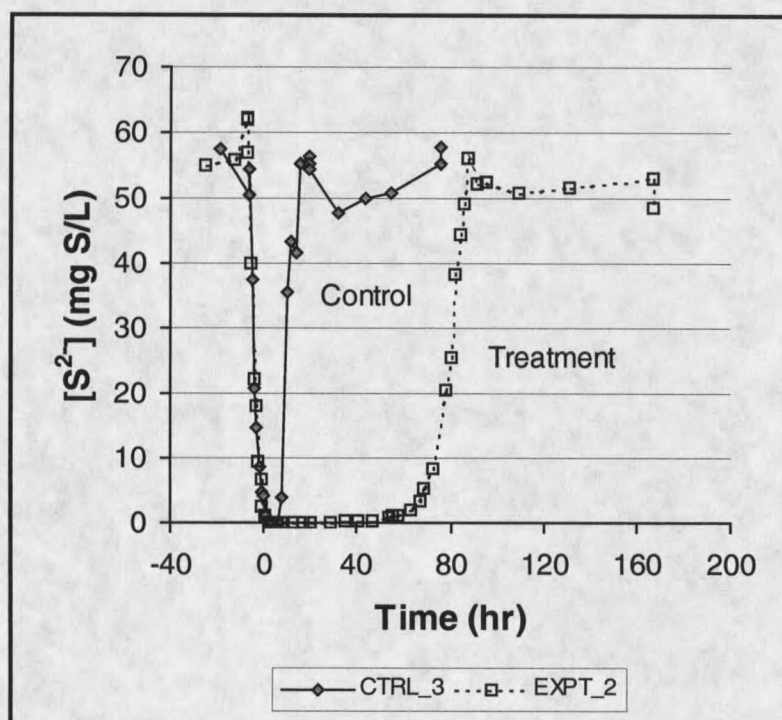


Figure 15. Sulfide profile comparison between a control experiment with ultrapure water versus a dose of 500 mg/L glutaraldehyde. Time zero denotes the pulse injection of glutaraldehyde; $-6.5hr$ the time nutrient amendment ceased; and $+7hr$ the time nutrient amendment was reinitiated. (Annular reactor data from Control #3 and Experiment #2)

Table 7. Sulfate-reducing bacterial biofilm recovery time in response to glutaraldehyde treatments. Recovery indices are expressed in terms of the time sulfide production is suppressed below 10 mg-S/L and the time required to reach 90% of steady-state sulfide levels. Recovery time was measured beginning at the time when the nutrient amendment was reinitiated.

Annular Reactor Experiment	$t_{<10 \text{ mg-S/L}}$ (hr)	$t_{90\% \text{ S-S}}$ (hr)
Control_2	3	9
Control_3 #1	1	8
Control_3 #2	1	5
Experiment_2	67	80
Experiment_4 #1	53	65
Experiment_4 #2	46	62
Experiment_8 #1	66	75
Experiment_8 #2	72	81

Glutaraldehyde Reaction Rates

Retrieval of aqueous samples for the quantification of glutaraldehyde levels within the annular reactor commenced one minute following biocide injection. Samples were acquired every fifteen minutes during the 6.75-hour contact period. The bulk liquid biocide concentration for the dosing cycle followed an exponential decay profile similar to the effluent profiles for the non-reactive tracer studies.

An overall objective of the biocide studies was to gain insight to the behavior of the biofilm when it was subjected to the dosing of the antimicrobial agent, glutaraldehyde. Therefore, it was of interest to examine the possibility and to what

extent the biofilm would react with the biocide. A conventional reaction kinetics approach to such an interaction is to initially perform a natural logarithm transformation of the concentration data and plot these values versus time. This analysis is portrayed in Figure 16 for the individual doses of glutaraldehyde. The distribution of the data exhibit similar linear transformations. Values of the correlation coefficient for the linear fit to the data sets exceed 0.99. The corresponding transformations and regression analyses are listed in Appendix J.

The slope of these curves is a composite first-order reaction rate coefficient, K , which is characterized by reactor hydraulics and the interaction of the biocide with the biofilm. The values are listed in Table 8. The mean value of this rate coefficient for the five independent doses is 0.43 hr^{-1} . Embedded within the value of slope are the dilution rate, D , and the biocide reaction rate coefficient, k .

Performing a glutaraldehyde mass balance with respect to the annular reactor control volume provides a mechanism for determining the reaction rate coefficient. For the bulk fluid phase of interest, the mass balance incorporates accumulation, bulk flow in and out of the reactor, and the surface reaction of the biocide with the biomass within the reactor. The governing mass balance proceeds as follows.

$$\text{Accumulation} = \text{Flow In} - \text{Flow Out} - \text{Reaction} \quad (1)$$

Since glutaraldehyde was not a component of the influent feed to the reactor, the second term reduces to zero. Substituting the parameters that describe the remaining processes results in equation (2).

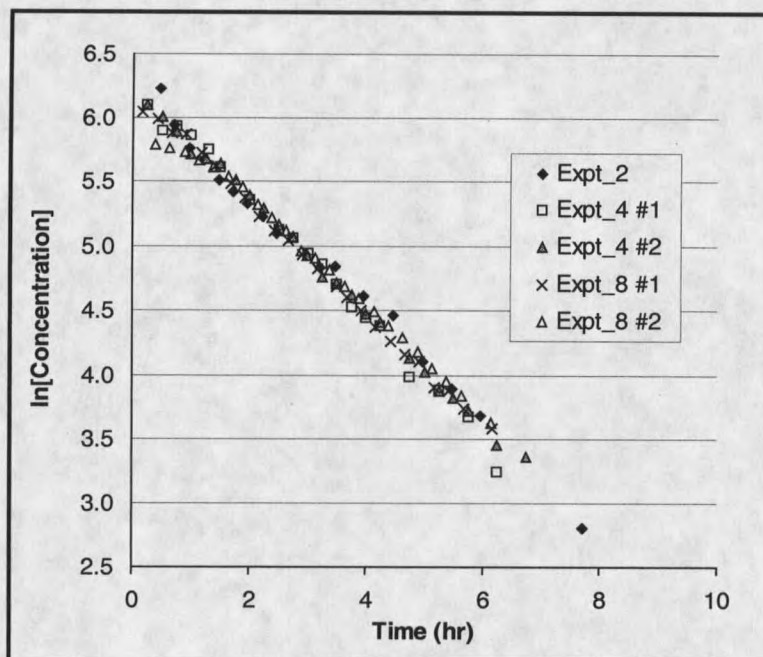


Figure 16. Natural logarithm of the glutaraldehyde concentration versus time for all glutaraldehyde annular reactor experiments. The slope of these curves provides the first order reaction rate coefficients of the glutaraldehyde.

Table 8. Composite first-order reaction rate coefficients for the glutaraldehyde annular reactor experiments.

Glutaraldehyde Study	$K \text{ (hr}^{-1}\text{)}$
Experiment_2	0.43
Experiment_4 #1	0.46
Experiment_4 #2	0.44
Experiment_8 #1	0.43
Experiment_8 #2	0.40

$$V \frac{dC_B}{dt} = -QC_B - r_B A_b L_f \quad (2)$$

where V is the reactor volume (m^3), C_B the biocide concentration (mg/m^3), Q the flow rate (m^3/sec), r_B the reaction rate of glutaraldehyde with the biofilm ($\text{mg}/\text{m}^3\text{hr}$), A_b the biofilm surface area (m^2), and L_f the biofilm thickness (m). Since the natural logarithm transformation of the biocide concentration versus time data yields a first-order dependence; the reaction rate was defined as

$$r_B = kC_B \quad (3)$$

with k termed the first-order reaction rate coefficient (hr^{-1}). Substituting (3) into (2) and rearranging the relationship results in the expression described by equation (4).

$$\frac{dC_B}{C_B} = - \left[\frac{Q}{V} + \frac{kA_b L_f}{V} \right] \quad (4)$$

The term Q/V is defined as the dilution rate, D . Integration of equation (4) produces

$$\ln \left(\frac{C_B}{C_{Bi}} \right) = e^{- \left[D + \frac{kA_b L_f}{V} \right]} \quad (5)$$

where C_{Bi} is the initial biocide concentration and the exponent is described by the slope of the $\ln C_B$ vs. time curve (previously defined as the composite reaction rate coefficient, K). Solving for the reaction rate coefficient results in equation (6).

$$k = \frac{V}{A_b L_f} (K - D) \quad (6)$$

The individual reaction rate coefficients are summarized in Table 9. The calculations utilized an average biofilm thickness (post biocide dose, Table 11), a reactor surface area of 0.26 m² (Appendix A), a dilution rate of 0.43 hr⁻¹, and a nominal reactor volume of 1-L. The dilution rate was lower than the tracer study values because the overall flow rate was reduced in the absence of the nutrient medium flow to the reactor.

Table 9. First-order reaction rate coefficients for the glutaraldehyde annular reactor experiments.

Glutaraldehyde Study	$L_{f\text{ avg}}$ (μm)	K (hr ⁻¹)	k_{glut} (hr ⁻¹)
Experiment_2	5.1	0.43	0
Experiment_4 #1	5.7	0.46	20
Experiment_4 #2	4.4	0.44	8.7
Experiment_8 #1	3.1	0.43	0
Experiment_8 #2	5.8	0.40	0

It was stated previously that the tracer studies elucidated the behavior of a non-reactive species traveling through the reactor, i.e. the dilution rate. The maximum difference between the dilution rate for the two tracer studies and the lowest composite glutaraldehyde reaction rate coefficient was 0.03 hr⁻¹.

Nitrite Addition

Inhibition of microbial produced sulfide was also investigated by introducing a 24-hour continuous dose of approximately 110 mg-N/L of nitrite to the rotating annular reactor. The time course of sulfide production proceeded with a similar protocol to the glutaraldehyde studies except the nutrient flow was maintained during the delivery of the nitrite to potentially elicit a sulfide production response.

The nitrite was added with the 1.5% NaCl dilution water after 30 hours of steady-state sulfide levels. Within 30 minutes and as long the nitrite levels exceeded 15 mg-N/L; the produced sulfide levels started to decrease. The nitrite concentration attained 100 mg-N/L in the bulk liquid after 6-hours of continuous feed. This coincided with a sulfide level of approximately 4 mg-S/L. The sulfate concentrations during this dosing period corroborated the sulfide levels, which indicated inhibition of SRB metabolism. The changes in nitrite, sulfide and sulfate concentrations are illustrated in Figure 17 for the 110 mg-N/L nitrite repetitive dose. The experimental data for this study are listed in Appendix F. The time to recovery commenced when the nitrite flow was removed from the biofilm reactor. Four hours after the nitrite delivery was terminated, the bulk fluid nitrite levels were less than 15 mg-N/L and resuscitation in sulfide production was noted. As residual nitrite was flushed from the reactor, microbial produced sulfide was no longer suppressed.

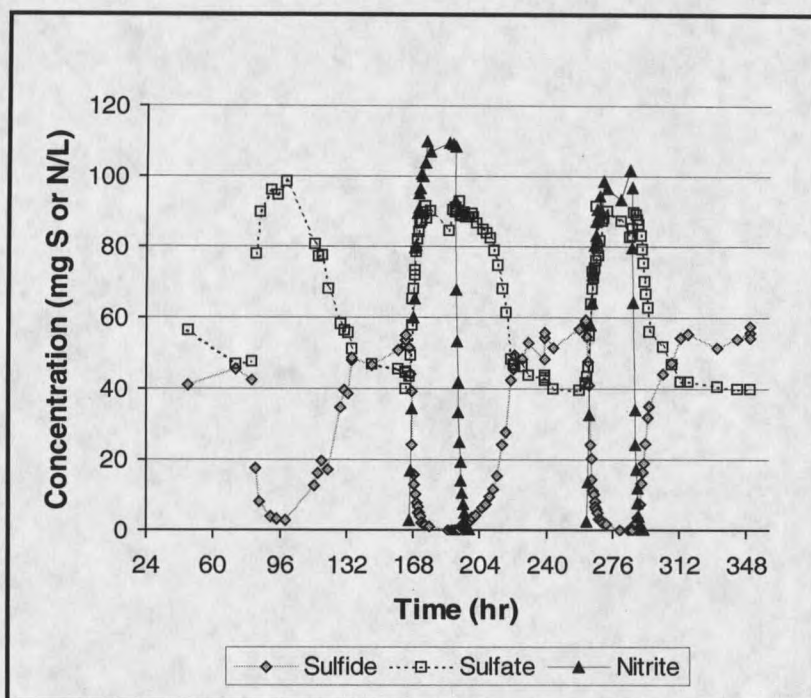


Figure 17. Sulfide and sulfate concentration profiles in response to two 24-hour continuous feeds of 110 mg-N/L of nitrite. See Table 6 and Figure 10 for experimental protocol. (Annular reactor Experiment #6 data)

The repetitive dose rendered similar results: repressed sulfide levels after 30 minutes of exposure to the nitrite (bulk liquid nitrite concentration of 14 mg-N/L). Sulfide levels decreased to 4-5 mg-S/L with a bulk fluid nitrite concentration of 90 mg-N/L as compared to 100 mg-N/L for the first dose. Sulfate levels confirmed the inhibition of sulfide with the bulk liquid concentration being equal to the influent levels. The recovery of sulfide production occurred earlier with the second dose, Table 10, as evident by sulfide levels approaching 2 mg-S/L only two hours after terminating the nitrite delivery. This was coincident with 33 mg-N/L nitrite in the

bulk fluid. It was also noted that the bulk fluid nitrite concentration during the second dosing interval never exceeded 102 mg-N/L as compared to 110 mg-N/L for the first application. The influent nitrite concentration throughout the individual doses averaged 112 mg-N/L.

Table 10. Sulfate-reducing bacterial biofilm recovery time in response to nitrite addition. Recovery indices are expressed in terms of the time sulfide production is suppressed below 10 mg-S/L and the time required to reach 90% of steady-state sulfide levels. Recovery time was measured beginning at the time when the nutrient amendment was reinitiated.

Nitrite Experiment & Dose	$t_{<10 \text{ mg-S/L}}$ (hr)	$t_{90\% \text{ S-S}}$ (hr)
Experiment_6 #1	19	32
Experiment_6 #2	5	24

The washout behavior of the nitrite from the rotating annular reactor for the two doses is illustrated in Figure 18. Qualitatively, the nitrite washout profiles appear to be nearly identical. Further examination revealed the residual from the second dosing period was removed 1.5 hours earlier than the first dose. These two curves demonstrate a faster departure from the reactor when compared with exit age distribution for one of the glutaraldehyde experiments.

As with the analysis of the glutaraldehyde data, a natural logarithm transformation of the concentration versus time data was made to gain insight to the reaction kinetics between the nitrite and biofilm during the washout phase. The converted data sets are plotted in Figure 19. Transformations were not performed

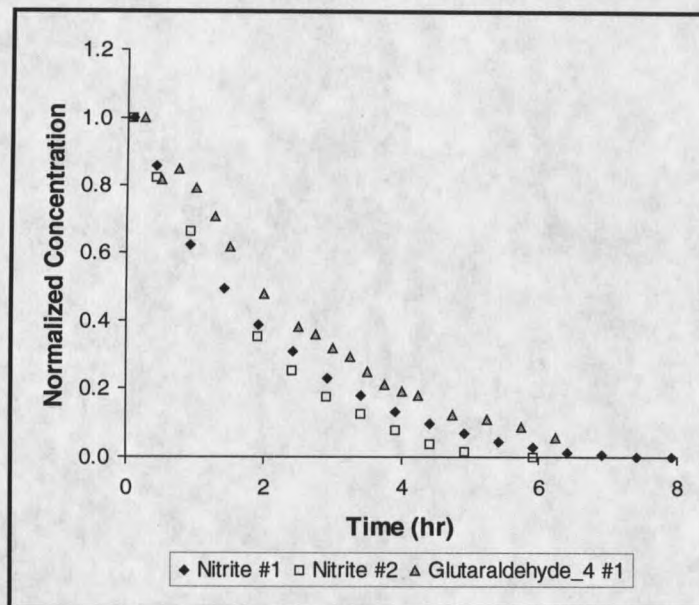


Figure 18. Comparison of nitrite and glutaraldehyde washout profiles from the biofilm annular reactor.

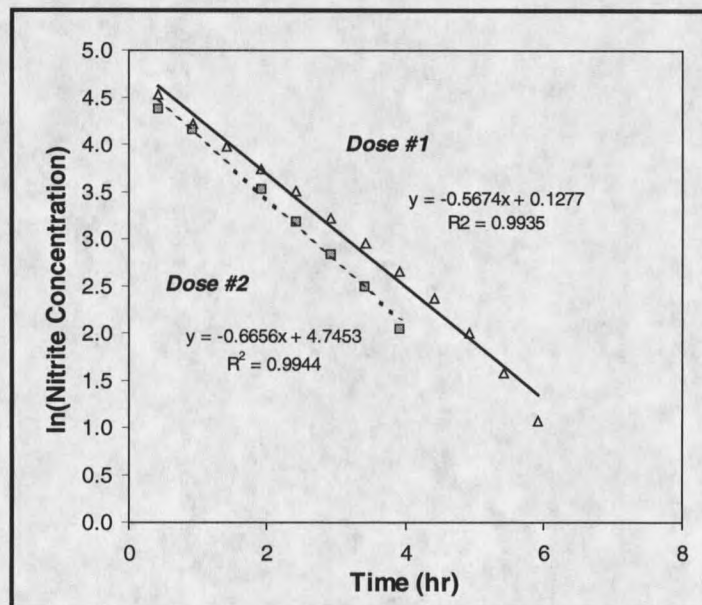


Figure 19. Natural logarithm of nitrite concentration versus time for two separate doses in annular reactor Experiment #6. The slope of these curves provides the first-order reaction rate coefficients during washout.

for nitrite concentrations that were less than 1 mg-N/L. There was a good correlation of the linear fit to the data with r^2 values exceeding 0.99. Correlation by the use of a linear transformation implied a first-order reaction rate coefficient, which was designated by the slope of the curve. The reaction rate coefficients for the effluent portion of the dosing cycle were 0.57 and 0.67 hr^{-1} , respectively for the first and second doses. The coefficients were greater than the dilution rate of the reactor (0.5 hr^{-1}) which indicated a reaction between nitrite and the microorganisms as nitrite was displaced from the reactor environment.

A more important aspect was the reaction rate of the nitrite during the dosing cycle. The material balance again provides a phenomenological approach in determining the observable reaction rate. The balance for the bulk liquid phase begins with the processes of accumulation, flow in, flow out, and the reactive component. The accumulation term was reduced to zero since the analysis was concurrent with steady-state influent and bulk liquid levels of nitrite. Equation (7) portrays the simplified material balance for the nitrite.

$$Q(C_{in,avg} - C_{out,avg}) = R_{obs}A_bL_f \quad (7)$$

The observed reaction rate was therefore described by

$$R_{obs} = \frac{Q(C_{in,avg} - C_{out,avg})}{A_bL_f} \quad (8)$$

The steady-state nitrite concentrations were averaged over the last five sampling points of the interval and the reactor flow rate was operated at 7.9 mL/min. The

average thickness of the biofilm post the dosing periods was used in the evaluation of equation (8). Table 11 lists the observable rates for these two nitrite doses.

Table 11. Observable reaction rate for nitrite doses.

Nitrite Dose	$C_{in,avg}$ (mg/L)	$C_{out,avg}$ (mg/L)	$L_{f,avg}$ (μm)	R_{obs} (mg/L*min)
1	112.4	107.9	4.7	29.3
2	112.5	95.7	9.9	51.7

Biofilm Coupon Analyses

Biofilm Thickness

Utilization of the cryoembedding and cryosectioning techniques permitted assessment of biofilm cross-sections with resolution capability to individual bacterial cells. Epifluorescent microscopic examination of the embedded biofilm revealed a heterogeneous structure that consisted of microcolonies, potential water channels, and occasional aggregated structures. Biofilm thickness varied during the course of an experiment as a result of the different dosing protocol sequences. Forty-nine discrete measurements (7 measurements/image) were captured on a per coupon basis with the average thickness and standard deviation reported in Table 12. Data for the individual experiments are listed in Appendix I. The data in the table substantiate the qualitative observations made with regards

Table 12. Biofilm thickness, L_f

Experiment	Interval	L_f (μm)		
		Min	Max	$\bar{X} \pm \sigma$
<i>Control #2</i>	Steady-state	0.0	17.7	2.9 ± 2.1
	Before dose	0.0	23.7	2.4 ± 1.9
	After dose	0.0	15.9	3.6 ± 2.0
	Return to steady-state	3.8	10.9	4.0 ± 2.6
<i>Control #3</i>	Steady-state	0.0	32.4	4.5 ± 2.5
	Before dose #1	0.0	33.9	5.9 ± 6.5
	Return to steady-state	0.0	21.7	5.2 ± 2.0
	Before dose #2	0.0	14.0	6.2 ± 2.6
	Return to steady-state	0.0	24.2	8.0 ± 3.9
<i>Experiment #2</i>	Steady-state	1.7	25.4	8.2 ± 3.5
	After dose	0.0	17.3	5.1 ± 2.4
	Return to steady-state	0.0	23.5	7.4 ± 4.2
<i>Experiment #4</i>	Steady-state	0.0	19.6	2.4 ± 1.6
	After dose #1	0.0	26.9	5.7 ± 3.8
	Return to steady-state	0.0	25.0	5.9 ± 3.9
	After dose #2	0.0	18.3	4.4 ± 3.0
	Return to steady-state	0.0	34.8	9.8 ± 5.4
<i>Experiment #6</i>	Steady-state	0.0	22.3	4.5 ± 2.2
	After dose #1	0.0	12.3	4.7 ± 2.6
	Return to steady-state	0.0	31.2	6.8 ± 4.4
	After dose #2	0.0	35.1	9.9 ± 6.1
	Return to steady-state	0.0	41.1	10.0 ± 6.8
<i>Experiment #8</i>	Steady-state	0.0	49.1	6.8 ± 5.6
	After dose #1	0.0	31.7	3.1 ± 4.1
	Return to steady-state	0.0	26.2	5.8 ± 2.6
	After dose #2	0.0	32.6	5.8 ± 3.5
	Return to steady-state	0.0	40.9	10.2 ± 4.5

to biofilm heterogeneity. Variances within an image were fairly homogeneous with the most of the variation occurring from image to image. While the average thickness was consistently in the single digit range, biofilm clusters were documented five times as thick. These structures seemed recalcitrant to the detrimental effects of the introduced agents. A graph of the mean thickness, as tabulated in Table 12, versus experiment interval is illustrated in Figure 20.

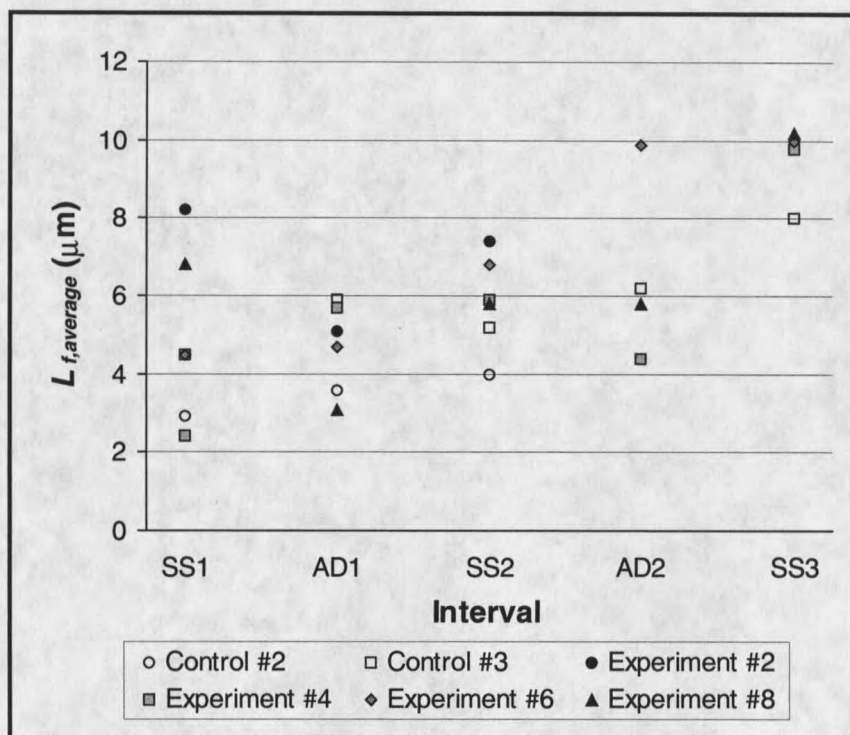


Figure 20. Progression of the average biofilm thickness during the annular reactor experiments. The acronyms imply dosing intervals, e.g. SS1 = Steady-State #1, AD1 = After Dose #1, etc. Control #2 and Experiment #2 were single dose studies while the remaining experiments experienced repetitive doses. Control experiments were subjected to dosing with ultrapure water. Experiments #2,4 and 8 were glutaraldehyde studies while Experiment #6 addressed the effects of nitrite addition.

The Control experiments were subjected to dosing with ultrapure water while Experiment #6 experienced the 24-hour continuous dose of nitrite. All the remaining experiments (2,4,8) were glutaraldehyde studies. There was an overall trend of increasing biofilm thickness during the course of the experiments. Even in the absence of the antimicrobial and inhibitory agents, the control studies did not attain as thick of biofilms when compared to the treated experiments. The glutaraldehyde treated biofilms exhibited an apparent reduction in thickness after the doses due to the biocidal activity, but proved to be resilient and surpassed the previous steady-state thickness. Exposure of nitrite to the sulfate-reducing bacterial biofilm provided periods of bacteriostasis but the overall progression in development and growth was unimpeded. The biofilm did, however, appear to attain a stationary phase by maintaining nearly the same overall thickness for the intervals After Dose #2 and Steady-State #3.

Biofilm Areal Density

Biological mass was assessed by quantifying the carbon content within the biofilm at intervals coincident with the biofilm thickness measurements. Three coupons were randomly selected at these distinct periods of an experiment. Efforts were taken to remove background carbon interference from the Postgate C medium prior to sample injection in the total organic carbon (TOC) analyzer. The carbon content was quantified on a per coupon basis and reported as areal carbon density ($\mu\text{g C/cm}^2$). Two to three coupons were sampled on a per interval basis

Table 13. Biofilm areal density in terms of total organic carbon

Experiment	Interval	Average TOC ($\mu\text{g/mL}$)	Average Areal Density ($\mu\text{g/cm}^2$)
<i>Control #2</i>	Steady-state	86.5 ± 1.1	39.2 ± 0.5
	Before dose	69.5 ± 19.4	31.5 ± 8.8
	After dose	97.5 ± 36.6	44.2 ± 16.6
	Return to steady-state	170.4 ± 23.1	77.3 ± 10.5
<i>Control #3</i>	Steady-state	412.7 ± 45.5	187.3 ± 20.6
	Before dose #1	452.6 ± 372.6	205.4 ± 169.1
	Return to steady-state	581.1 ± 489.3	263.7 ± 222.0
	Before dose #2	89.3 ± 46.4	40.5 ± 21.1
	Return to steady-state	505.2 ± 295.4	229.3 ± 134.0
<i>Experiment #2</i>	Steady-state	62.9 ± 40.0	28.6 ± 18.2
	Before dose	79.7 ± 14.2	36.2 ± 6.5
	After dose	62.3 ± 55.4	28.3 ± 25.2
	Return to steady-state	123.9 ± 86.8	56.2 ± 39.4
<i>Experiment #4</i>	Steady-state	209.4 ± 89.7	95.0 ± 40.7
	After dose #1	132.5 ± 39.9	60.1 ± 18.1
	Return to steady-state	170.1 ± 36.9	77.2 ± 16.7
	After dose #2	172.7 ± 87.1	78.4 ± 39.5
	Return to steady-state	81.3 ± 21.1	36.9 ± 9.6
<i>Experiment #6</i>	Steady-state	58.7 ± 34.5	26.7 ± 15.6
	After dose #1	99.9 ± 63.7	45.3 ± 28.9
	Return to steady-state	92.6 ± 26.3	42.0 ± 11.9
	After dose #2	102.0 ± 8.5	46.3 ± 3.9
	Return to steady-state	130.0 ± 52.5	59.0 ± 23.8
<i>Experiment #8</i>	Steady-state	106.7 ± 10.1	48.4 ± 4.6
	After dose #1	144.9 ± 27.8	65.8 ± 12.6
	Return to steady-state	88.0 ± 26.0	39.9 ± 11.8
	After dose #2	197.5 ± 43.6	89.6 ± 19.8
	Return to steady-state	110.1 ± 43.8	50 ± 19.9

with each sample being injected twice on the TOC analyzer. Small differences were revealed between the two readings, but large differences were encountered amongst the coupons. The average areal carbon densities listed in Table 13 are plotted in Figure 21 as a function of the dosing cycle interval.

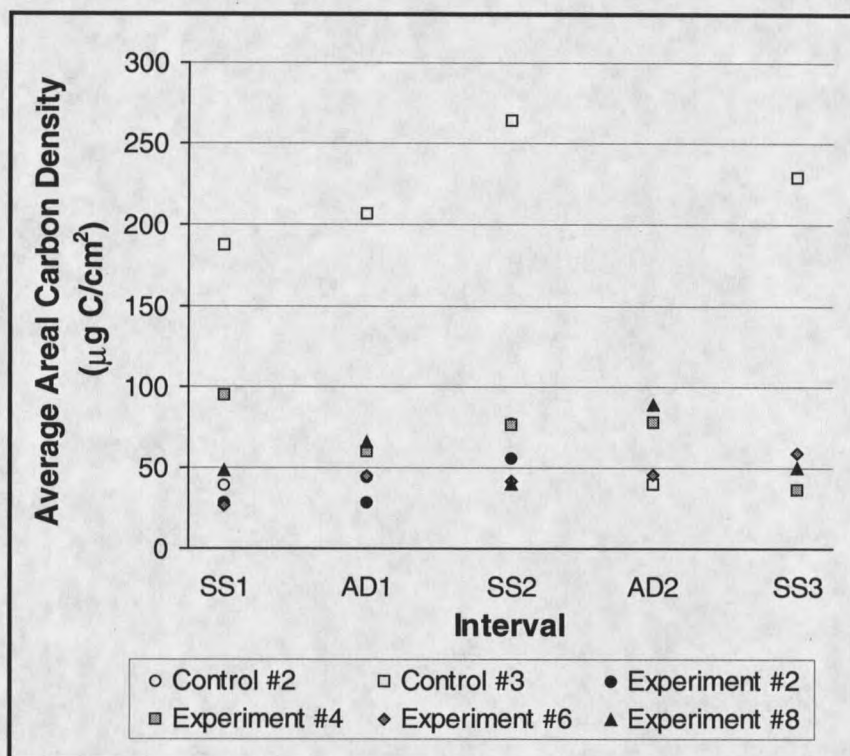


Figure 21. Changes in areal carbon density as a function of dosing interval during a biofilm experiment. The acronyms imply these intervals, e.g. SS1 = Steady-State #1, AD1 = After Dose #1, etc. Control #2 and Experiment #2 were single dose studies while the remaining experiments experienced repetitive doses. Control experiments were subjected to dosing with ultrapure water. Experiments #2,4 and 8 were glutaraldehyde studies while Experiment #6 addressed the effects of nitrite addition.

Aside from the outlying data set of Control #3, the overall trend in areal carbon density, albeit noisy, was the carbon content of the biofilm maintained a fairly

constant level of approximately $50 \mu\text{g C/cm}^2$ regardless of the dosing regime. The values for the Control #3 data set are four to five times higher than the other experiments. The trend of the Control #3 data mimic the other data sets with a relatively constant level of areal carbon density except for the After Dose #2 data point.

Microbiologically Influenced Corrosion

Sulfate-reducing bacteria (SRB), in addition to other physiological groups, have been implicated in the corrosion of metals and alloys (Little and Wagner, 1994). Their ability to reduce sulfate to hydrogen sulfide provides a mechanism for production of metal sulfides as corrosion products. The symptomatic by-products of the SRB metabolism include the blackening of waters, hydrogen sulfide odor, and the black sulfide corrosion products.

The exposed metal surfaces of the rotating annular reactor were manufactured from 316 stainless steel. The primary interface between the drive unit and the inner cylinder was a stub shaft bound by a bearing and two lips seals. Microbiologically influenced corrosion (MIC) has been characterized as localized corrosion that includes, but not limited to, pitting, under-deposit corrosion, and stress corrosion cracking. A comparison of the stub shaft and bearing prior to- and post- a glutaraldehyde experiment is shown in Figure 22. Damage to these surfaces was displayed by the pitted and blackened surfaces. Upon dismantling of

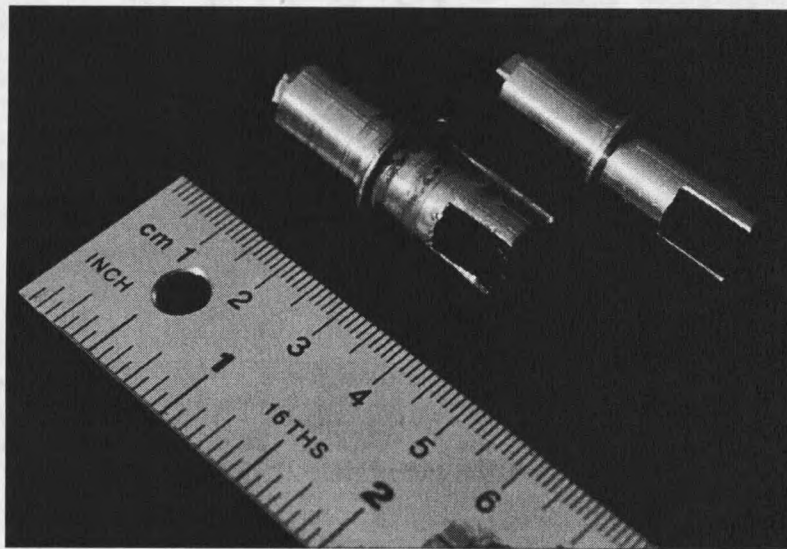
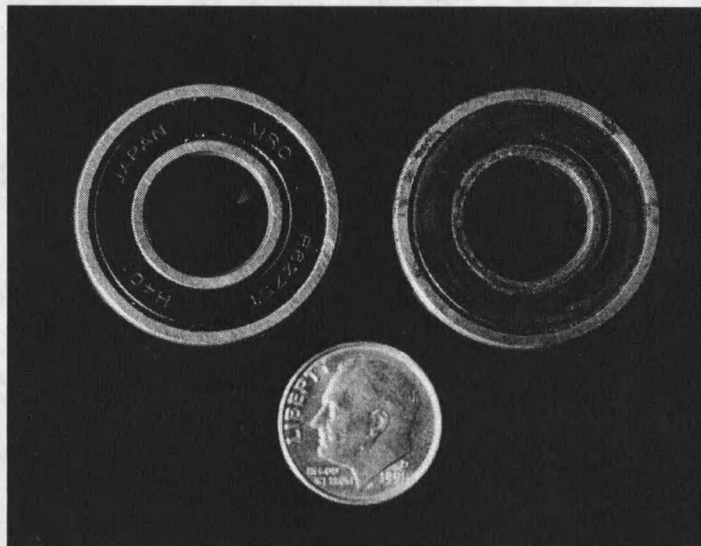


Figure 22. Stub shaft and bearing from the bottom plate of the biofilm annular reactor. Damage to these surface by the sulfate-reducing bacteria generated corrosion by-products are evident by the scored and blackened surfaces. A clean shaft and bearing are shown for comparison.

the reactor, the bearing was seized to the stub shaft and mechanically removed with the aid of a vise and hammer. A closer view of the stub shaft is presented in Figure 23.

Section A of the shaft reveals areas of stress-induced corrosion due to the mechanical interface with the inner cylinder. Migration of the bacteria was evident along the length of the shaft. Scoring of the shaft in Section B was marked by the regions of contact with the lip seal. The severity of the corrosion by-products was unmistakable and delineated by the bands that signify the upper and lower portions of the bearing in Section C. Loss of material was also obvious in this region due to the detrimental effects of sulfate reducing bacteria when in contact with metal surfaces.

Transport Limitation

Antimicrobial agent mass transfer resistance in the biofilm is an important aspect in determining the efficacy in suppressing microbial activity. This feature can be analyzed through the utilization of an observable modulus, Φ . The observable modulus is defined by (Bailey and Ollis, 1986) as

$$\Phi = \frac{R_{obs}}{D_{e,B} C_B} \left(\frac{V_b}{A_b} \right)^2 \quad (9)$$

where R_{obs} represents the observed biocide consumption rate by the biofilm, V_b

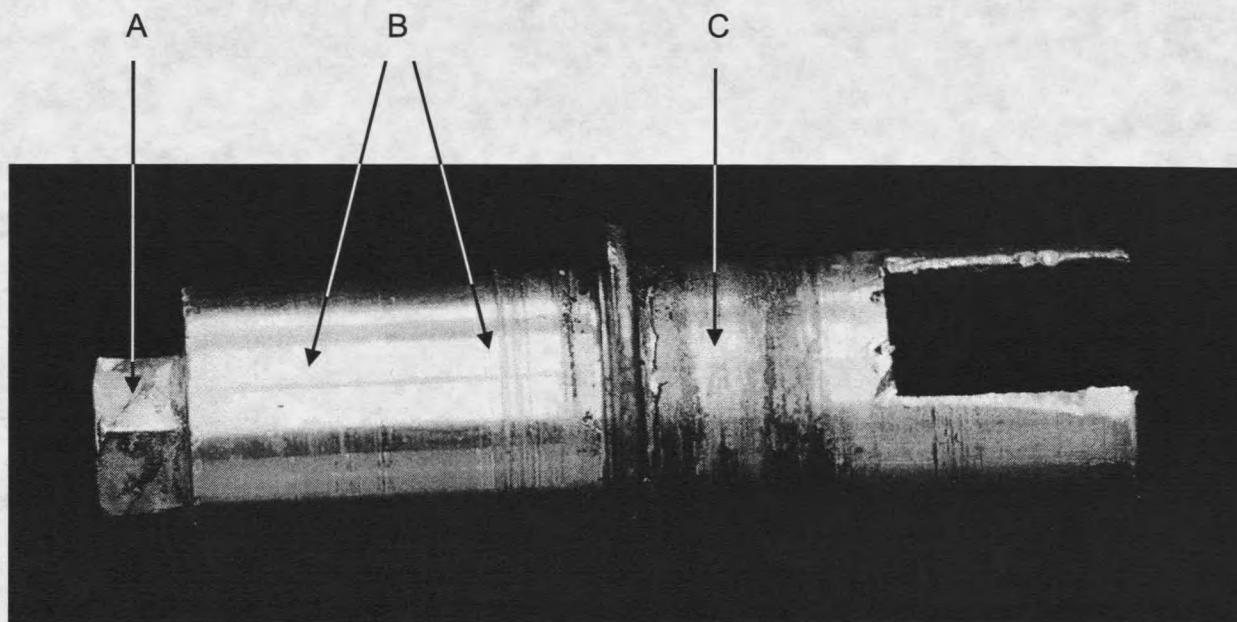


Figure 23. Microbially influenced corrosion by sulfate-reducing bacteria on the bottom plate stub shaft. This shaft was extracted after 17 days in the biofilm reactor.

the biofilm volume in the reactor, $D_{e,B}$ the effective diffusivity of the biocide in the biofilm, C_B the biocide concentration, and A_b the biofilm surface area. In the case of a slab biofilm ($V_b = A_b L_f$), equation (9) reduces to

$$\Phi = \frac{R_{obs} L_f^2}{D_B C_B} \quad (10)$$

Since the observable modulus depends on the measurable overall consumption rate, R_{obs} can be defined in the case of the antimicrobial, glutaraldehyde, as

$$R_{obs} = k C_B \quad (11)$$

with k representing the first-order reaction rate coefficient of the biocide with the biofilm. The derivation of the overall consumption rate for the interaction of the nitrite with the biofilm was outlined in the Nitrite Addition section by equation (8). The relationship is listed here for completeness.

$$R_{obs} = \frac{Q(C_{in,avg} - C_{out,avg})}{A_b L_f} \quad (12)$$

The surface area of the biofilm, A_b , was assumed to be comprised of the available annular reactor wetted surface area as calculated in Appendix A (0.26 m²). The effective diffusion coefficient of glutaraldehyde, D_{glut} , in the biofilm was assumed to be 0.4 times (Stewart, 1998) the effective diffusivity permeability, D_{aq} , of glutaraldehyde in water (see Appendix K, (Wilke and Chang, 1955)). The ratio of the biofilm diffusivity to the bulk liquid diffusivity (τ) of the inhibitory agent, nitrite, was assumed to be 0.5 (Stewart, 1998). These parameters were evaluated at a

reactor temperature of 36°C and are summarized in Table 14. Table 15 lists the required variables for evaluation of equation (10) as a function of experiment number. The overall consumption rate for the glutaraldehyde was purposely not

Table 14. Effective Diffusive Permeabilities and Diffusion Coefficients

Solute	D_{aq} (m ² /sec)	τ	D_e (m ² /sec)
Glutaraldehyde	9.66×10^{-10}	0.4	3.86×10^{-10}
Nitrite	1.56×10^{-9}	0.5	7.8×10^{-10}

Table 15. Observable Moduli for glutaraldehyde and nitrite biofilm experiments.

Experiment	$L_{f\text{ avg}}$ (μm)	k_{glut} (hr ⁻¹)	R_{obs} (mg/L*min)	Φ
Expt_4 #1 (glutaraldehyde)	5.7	20.24	---	4.7×10^{-4}
Expt_4 #2 (glutaraldehyde)	4.4	8.74	---	1.2×10^{-4}
Expt_6 #1 (nitrite)	4.7	---	29.3	1.3×10^{-4}
Expt_6 #2 (nitrite)	9.9	---	51.7	1.1×10^{-3}

listed in Table 15. By substituting the relationship defined by equation (11) into the observable modulus in (12), the observable modulus was concluded to be independent of biocide concentration.

As defined by Bailey and Ollis (1955), when Φ is sufficiently large, $\Phi > 3$, diffusion of the solute was slow relative to the reaction with the biofilm. However, if $\Phi < 0.3$ then transport limitation was not the predominate factor. As is clear from Table 15, diffusion of the biocidal and bacteriostatic agents was not a significant factor in these experiments.

DISCUSSION

The goal of this research was to measure aspects of biocide action against sulfate-reducing bacterial biofilms. The principal objectives were to: (1) establish a methodology for assessing the efficacy of antimicrobial agents under conditions similar to an oilfield environment, (2) investigate reaction and transport limitations associated with the delivery of a biocide, and (3) evaluate the plausibility of microbial adaptation when subjected to recurring biocides. The experimentally measured parameters chosen to address these questions were sulfate utilization, sulfide production, quantification of areal carbon density and biofilm thickness, and antimicrobial agent concentration.

Experimental System

The undefined inoculum obtained from the Lost Hills produced water facility was enriched for sulfate-reducing bacteria (SRB) as the signature microbe of interest. SRB's were chosen because of the symptomatic problems (souring of oil, metal sulfides, etc.) that arise from their metabolism. Grab (1993) stressed the importance of investigating intact biofilms with biocides under appropriate field conditions (temperature, pH, relevant contact times, etc.). This approach was closely followed by our research. Various laboratory reactors designed to simulate pipeline conditions have previously illustrated the effectiveness of glutaraldehyde

control of viable organisms (Costerton and Lashen, 1984; Herbert *et al.*, 1987; Haack *et al.*, 1988).

A rotating biofilm annular reactor was chosen for use in this research for several reasons: high surface area to volume ratio, completely mixed behavior, temperature control, fluid shear stress management, and the ability to potentially corroborate laboratory data with a similar field reactor. Gjaltema *et al.* (1994) described the annular reactor as a valuable tool for qualitative and morphological experiments. Other researchers (Nielsen, 1987; Sanderson, 1996; Okabe *et al.*, 1994) have illustrated the ability to extract kinetic related parameters to model biofilm kinetics. The reactor behavior mimicked a continuously stirred tank reactor (CSTR) as confirmed by the non-reactive tracer analysis.

As previously stated, a series of tracer experiments were conducted for the purpose of evaluating reactor hydrodynamics. The input of the non-reactive tracer produced an effluent curve with a prompt increase to a maximum concentration followed by a decreasing tracer concentration. The effluent concentration followed an exponential decay function, equation (1),

$$\frac{C_t}{C_o} = e^{-t/t_R} \quad (1)$$

with C_o being the initial concentration and t_R the theoretical residence time (ratio of reactor volume to flow rate). This type of profile further validated CSTR behavior.

Two of the non-reactive tracer experiments were processed at a higher inner

cylinder rotational speed of 230 rpm as opposed to the biofilm experiment operating conditions of 150 rpm. The objective of these evaluations was to address mixing behavior as a function of influent port orientation. Subtle differences were observed between the top and bottom configurations. No significant differences were noted in the analysis of mean hydraulic residence time data when compared to the 150 rpm tracer experiments. The mixing behavior of the bottom fed studies operated at 150 rpm exemplified good reproducibility and indicated a well-mixed behavior with minimal stagnant zones. Oil pipeline mixing characteristics are described more accurately as plug-flow and could be simulated by connecting multiple annular reactors in series. Utilization of the rotating biofilm annular reactor provided a reproducible, CSTR working system in which assessments of glutaraldehyde and nitrite efficacy were performed with sulfate-reducing bacterial biofilms under anoxic conditions.

Planktonic – Biofilm Comparison

The metabolic activity of the lactate-utilizing sulfate-reducing bacteria was strongly correlated to sulfide production and sulfate uptake as evidenced by the three enrichment studies. Concentrations in excess of 50 mg/L of glutaraldehyde completely blocked sulfide generation and sulfate consumption by the microorganisms. The observation that biocide efficacy increased with increasing biocide concentration agrees with the work of McIlwaine (1998). Grab and Theis (1993) did not measure a complete kill with a *D.desulfuricans* strain and a similar

7-hour dose of 100 mg/L glutaraldehyde. These discrepancies could possibly be related to a differing target organism or experimental procedure. In any case, it has been shown that increasing the biocide concentration will have a positive effect upon the efficacy of that biocide.

The recalcitrant and resilient nature of biofilms enabled them to resist the detrimental effects of biocidal and bacteriostatic agents. Despite successful biocide penetration of the biofilm, the sulfate-reducing bacterial biofilms of this study were less susceptible to disinfection than their planktonic counterparts. This observation concurs with widely published results of reduced antimicrobial efficacy on sulfate-reducing bacterial biofilms (Cheung and Beech, 1996; Eagar *et al.*, 1988; Grab and Theis, 1993; McIlwaine, 1998; Ruseska *et al.*, 1982; Whitham and Gilbert, 1993). The fact that biocides penetrated the biofilms but were still less susceptible suggests that resistance mechanisms based on the distinct heterogeneous physiological status of biofilm bacteria may play a role in protecting these cells from antimicrobial agents (Vrany, 1996).

Equivalent Biocide Concentration

Planktonic assessment of glutaraldehyde action was tested in batch vials at a fixed concentration of antimicrobial agent. Glutaraldehyde was delivered to the biofilm annular reactor by a pulse injection of 500 mg/L, which then washed out of the reactor creating a changing dose concentration in time. An equivalent concentration was used to compare glutaraldehyde effectiveness between the

planktonic and biofilm experiments. This effective concentration in the annular reactor was mathematically described by equation (2)

$$C_{\text{biofilm_equivalent}} = \frac{1}{t} \int_0^t C dt \quad (2)$$

where the biocide concentration can be written as,

$$C = C_o e^{-Dt} \quad (3)$$

and D is the dilution rate. Substituting (3) into (2) and evaluating the integral results in

$$C_{\text{equivalent}} = \left[\frac{C_o}{t} \left(\frac{-1}{D} e^{-Dt} \right) \right]_0^t \quad (4)$$

Substitution of the experimental variables $C_o = 500 \text{ mg/L}$, $D = 0.45 \text{ hr}^{-1}$, and $t = 6.75 \text{ hr}$ generates an equivalent glutaraldehyde concentration of

$$C_{\text{equivalent}} = 157 \text{ mg/L}$$

This is the concentration, that if applied in a batch test for 6.75 hr would be predicted to have the same effect as the 500 mg/L pulse dose in the continuous flow reactor.

Observations that biofilm bacteria are more resistant to antimicrobial treatment was confirmed by the exposure of planktonic and biofilm populations to glutaraldehyde. Figure 24 summarizes the efficacy of treatments against the

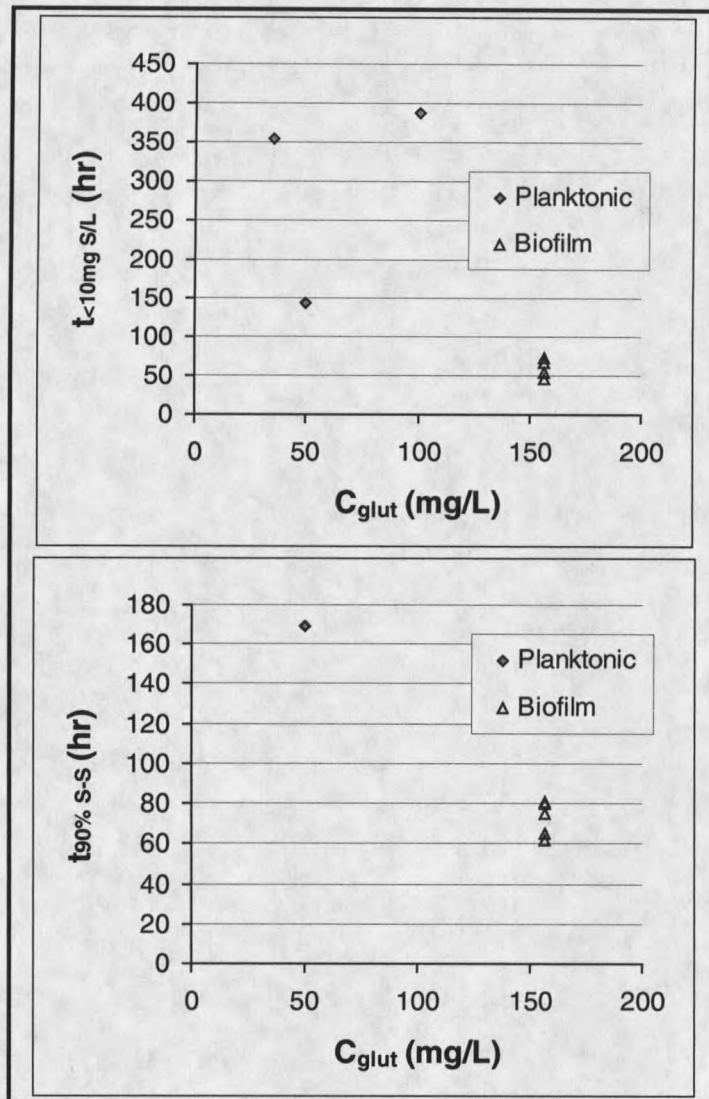


Figure 24. Comparison of microbial sulfide production recovery time as a result of biocide treatment. Recovery indices are expressed in terms of the time sulfide production is suppressed below 10 mg-S/L and the time required to reach 90% of steady-state sulfide levels. Recovery time was measured beginning at the time when the nutrient amendment was reinitiated.

planktonic and sessile SRB populations. The equivalent biocide concentration for the biofilm experiments was used for this comparison. The time required for the SRB biofilm to rebound to 90% of pretreatment sulfide levels was determined to occur more rapidly than for the planktonic organisms. Similarly, the sessile organisms spent less time suppressed below 10 mg-S/L of sulfide as compared to their planktonic counterparts. While these selectively enriched laboratory organisms fail to capture all the physiological characteristics of the field biofilm, the overall efficacy of the glutaraldehyde treatment gives valuable insight to potential treatment strategies. The laboratory results might establish a protocol of delivery that is more efficacious, e.g. high levels of biocide with short contact times as opposed to a low level of continuous feed.

Relative Efficacy of Treatments

To quantify glutaraldehyde and nitrite effectiveness on sulfate-reducing bacteria, changes in sulfide production were used to track microbial activity. Other researchers have shown repeated success with alternative activity interpolations: log-reductions via most probable numbers or CFU/mL, hydrogenase activity (Cheung and Beech, 1996), sulfate uptake rate (Reis *et al.*, 1992), etc. The sulfide profiles mirrored the observed reaction rate profiles for hydrogen sulfide during the course of an experiment. A complimentary behavior was illustrated between sulfide production and sulfate consumption. In the absence of antimicrobial agents, metabolic activity was unimpeded and sulfide levels consistently

approached 50% of the influent sulfate concentration. The presence of yeast extract in the nutrient medium necessitated medium removal prior to glutaraldehyde dosing to preclude biocide reaction with medium components. The flow of the Postgate C nutrient medium was restored immediately following the dosing interval to capture the period of inhibition and resurgence in metabolic activity. The lasting effects of glutaraldehyde produced a 72 to 96 hour period of sulfide inhibition. Two repetitive dosing experiments were performed. Reproducibility between experiments and from dose to dose was observed. A 10 to 13% difference was noted between doses for the time at which sulfide production was suppressed below 10 mg-S/L. In a similar manner, only a 5 to 8% difference was observed in the required to return to 90% of pretreatment sulfide concentration. Therefore, no significant differences were observed between the first and second doses. The consistency between doses argues against microbial adaptation in response to the glutaraldehyde dosing protocol.

As an alternative means of controlling microbial sulfide production, sodium nitrite was added to a SRB biofilm in the rotating annular reactor. Nitrite has long been known to inhibit a variety of microorganisms. For our investigation, a repetitive nitrite dose was administered using the same protocol as the glutaraldehyde studies with one exception. The nutrient flow to the reactor was maintained throughout the experiment to capture changes in sulfide production. Assessment of the overall treatment efficacy was focused on the sulfide monitors of $t_{<10 \text{ mg-S/L}}$ and $t_{90\% \text{ S-S}}$. Comparison of these nitrite inhibition times yielded a

distinct difference when compared to the glutaraldehyde doses. The time to reach 90 percent of pretreatment sulfide levels and the time below 10 mg-S/L of produced sulfide was less than observed with the glutaraldehyde treatments.

This data is incongruent with the works of Reinsel *et al.* (1996). Their findings illustrated an absence of long-term inhibitory effects when they subjected their porous media SRB biofilm to glutaraldehyde. Hydrogen sulfide production quickly recovered after removal of the biocide. The revival of the SRB post nitrite addition was slower than the glutaraldehyde recovery. However, once the remaining inhibitory effects of the nitrite dissipated, the microorganisms proliferated at rate faster than their initial growth curve. These observations supported their classification of glutaraldehyde as cidal to the microorganisms and nitrite appeared to be an inhibitor of sulfide generation.

Comparison of Glutaraldehyde Efficiency – $C \cdot t$ data

A comparison was made with published glutaraldehyde effectiveness data by plotting log reduction versus concentration-time data as described by Wickramanayake (1991). This analysis was performed to determine an apparent log reduction of the Lost Hills sulfate-reducing bacteria in response to the pulse injection of glutaraldehyde. The washout behavior of glutaraldehyde followed an exponential decay function as described by the previous tracer study and is restated here:

$$C = C_0 e^{-Dt} \quad (5)$$

where D is the dilution rate. Integration of the area under the C_{glut} vs. time provided insight into the relative efficacy of glutaraldehyde with our biofilm. Substituting equation (2) into this integral yields,

$$\frac{1}{T} \int_0^T C^* t dt = \frac{C_0}{T} \int_0^T t^* e^{-Dt} dt \quad (6)$$

which when integrated provides a solution of the format,

$$C^* t = \frac{C_0}{T^* D^2} [1 - e^{-Dt} (DT + 1)] \quad (7)$$

Substituting a biocide contact time of $T = 6.75$ hour, $D = 0.45 \text{ hr}^{-1}$, and $C_0 = 500$ mg/L, renders a concentration-time value of

$$C^* t = 295 \text{ mg}^* \text{hr/L}$$

Plotting this value with some $C^* t$ values reported in the literature reveals an estimated 2 to 4 log reduction as illustrated in Figure 25 (See Appendix L). While the concentration of the 500 mg/L pulse injection initially appeared inconsistent with levels commonly experienced in the field, Figure 25 revealed we were actually operating at a lower range of disinfection concentrations.

Changes in cellular carbon were determined by removal of the polycarbonate slides from the reactor. The biomass was scrapped, homogenized and analyzed for total organic carbon. While this event was managed to minimize the changes

to the reactor environment, any process that potentially disrupts the biomass can limit the value of such a measurement {Eagar, 1986}. Nevertheless, the procedures and assessments were performed. Aside from the Control #3 experiment, the plot of areal carbon density (Figure 21) demonstrated a relatively constant level of $50 \mu\text{g-C}/\text{cm}^2$ during the course of an experiment. This value was

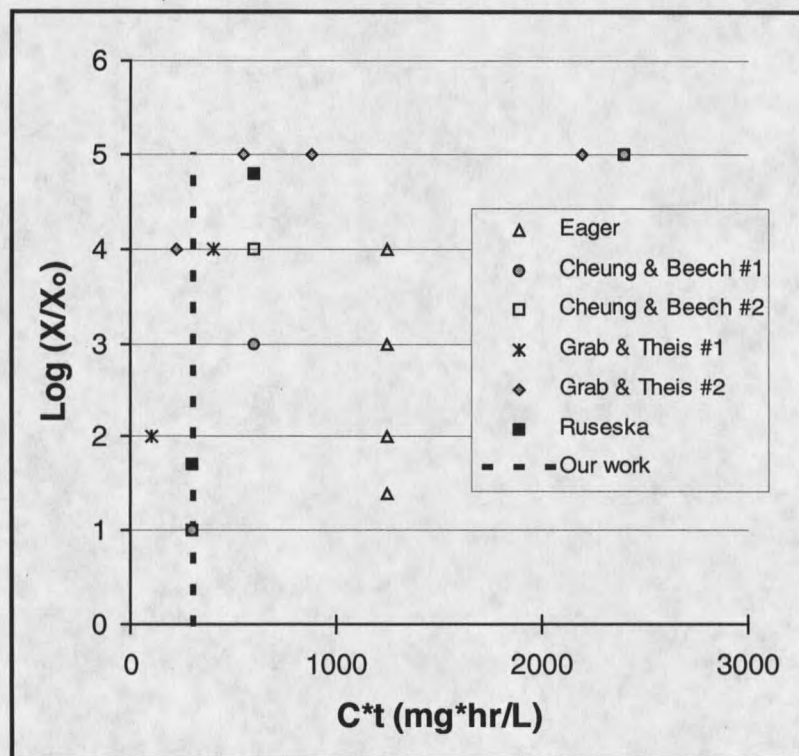


Figure 25. Comparison of inactivation efficiency at different disinfection concentrations. (Eagar: *P.fluorescens*; Cheung & Beech #2: Portsmouth Harbor SRB; Cheung & Beech #2: Indonesian coast SRB; Grab & Theis #1: *D.desulfuricans*; Grab & Theis #2: Prudhoe Bay SRB isolate; Ruseska: undefined SRB population; Our Work: undefined SRB population)

50% lower than the *P.aeruginosa* biofilm mass reported by Eagar *et al.* (1986).

The experimental data from Control #3 were fairly consistent with the other biofilm

experiments in terms of biofilm thickness, sulfate and sulfide levels, but varied considerably as depicted by the plot of areal carbon density. Qualitative observations during the course of Control #3 revealed a higher degree of biomass accumulating on the protruding edge of the polycarbonate slide. It appeared that the slide collected suspended biomass as the inner drum was rotating. The biomass was more mucoid when compared to the remaining biomass on the slide. The distribution of the biomass along the length of the slides was noted to be consistent except for this region. Therefore, the inclusion of this additional biomass accounts for the differences in the Control #3 carbon levels when compared to the remaining biofilm experiments.

Review of the biofilm thickness data and the respective standard deviations revealed a heterogeneous SRB biofilm. Qualitative examination also exposed regions of sparse colonization followed by areas of dense biofilm structures. Extrapolation of biofilm thickness trends could proceed in one of two directions.

A higher degree of variation was observed for the initial steady-state thickness values (Figure 20). Narrowing of this band would bring the data more in-line with the following three intervals (AD1, SS2, AD2). The final steady-state thickness groupings (SS3) are, however, at least 60% higher than the other intervals. At first glance there appears to be a trend in increasing biofilm thickness. A different interpretation could be construed if only the first four experimental intervals were considered. This focus would display a relatively constant biofilm thickness that would be in agreement with the discussion of areal carbon density.

Biocide Penetration Analysis

Analysis of diffusion and reaction employs a parameter termed the Thiele Modulus, Φ . The square of the Thiele Modulus presents a physical interpretation of the first-order reaction rate divided by the diffusion rate (Bailey and Ollis, 1986). In the absence of readily available intrinsic kinetic parameters and concentration profiles within a biofilm, manipulation of the Thiele Modulus yields a dimensionless relationship independent of these items, i.e. the observable modulus, Φ .

Transport limitation in a biofilm likely depends upon which organisms are present, the biofilm architecture, and the chemical properties of the antimicrobial (Vrany, 1996). The extent of glutaraldehyde and nitrite mass transfer resistance in our biofilm experiments was evaluated by calculating the observable modulus, Φ . Conceptually, the modulus reflects the relative rates of reaction and diffusion as described by equation (8).

$$\Phi = \frac{\text{REACTION RATE}}{\text{DIFFUSION RATE}} \quad (8)$$

Values for the observable modulus were calculated for the discrete periods of antimicrobial delivery. Regardless of the inhibiting agent, each individual application resulted in $\Phi \ll 1$. This can be interpreted to mean that the biofilm did not provide a penetration barrier to glutaraldehyde or nitrite. The fact that the observable modulus was less than 1 suggests that mechanisms other than

transport limitation are responsible for the biofilms reduced susceptibility to chemical agents. These results contrast the recent findings performed by Stewart *et al.* (1998) with glutaraldehyde and artificial biofilms. These researchers observed transport limitations as a factor in reduced glutaraldehyde efficacy ($\Phi > 1$).

Reaction of Glutaraldehyde with Biomass – Annular Reactor vs. Pipeline

From the onset, this research embraced the relevance of using field conditions for laboratory experiments. The biofilm annular reactor was operated using field variables and conditions. In a reciprocal manner, analysis of the experimental data was mathematically returned to a field related setting. This was accomplished through a comparative mass balance analysis using the physical characteristics and hydrodynamics of a hypothetical pipeline. The reaction of glutaraldehyde with the annular reactor biofilm was utilized as the common link between the two mass balance relationships. The glutaraldehyde reaction rate coefficient, derived from the annular reactor mass balance and experimental data, was used to predict biocide concentration behavior for distances down a pipeline.

Turbulent flow conditions proceed through a pipeline in an orderly and uniform manner such that each fluid element is transported as a “plug”. Therefore, a mass balance with a differential volume element more accurately captures the reaction rate behavior of pipeline operations (Weber, Jr. and DiGiano, 1996). The mass

balance on glutaraldehyde incorporated accumulation, bulk flow in and out of the reactor, and the surface reaction of the biocide with the biomass. The governing mass balance proceeded as follows.

$$\text{Accumulation} = \text{Flow In} - \text{Flow Out} - \text{Surface Reaction} \quad (9)$$

The accumulation term reduces to zero if a steady-state is assumed. Substituting the parameters that describe the remaining processes results in equation (10),

$$Q_z C_z - Q_{z+\Delta z} = r_B V \quad (10)$$

where Q is the flow rate of the bulk fluid and r_B the reaction rate of biocide with the biomass. They are further defined by the relationships

$$Q = v A_{\text{cross-section}} \quad (11)$$

$$r_B = k A_{\text{circum}} L_f C \quad (12)$$

$$A_{\text{cross-section}} = \pi R^2 \quad (13)$$

$$A_{\text{circum}} = 2\pi R \Delta z \quad (14)$$

where v is the velocity of the bulk fluid (m/sec), $A_{\text{cross-section}}$ the cross sectional surface area (m²), k the first-order reaction rate coefficient (hr⁻¹) as derived in the Results section, A_{circum} the biofilm surface area (m²), L_f biofilm thickness (m), C biocide concentration (mg/m³), R pipeline radius (m), and Δz the width of the differential fluid element (m). Substituting the relationships (11) through (14) into (10) yields,

$$v\pi R^2 [C_z - C_{z+\Delta z}] = k(2\pi R\Delta z)L_f C \quad (15)$$

Dividing by $\pi R\Delta z$, rearranging, and integrating results in equation (16).

$$\ln\left(\frac{C}{C_0}\right) = \frac{-2kL_f z}{vR} \quad (16)$$

Evaluating this relationship for z using an average pipeline diameter and fluid velocity with the glutaraldehyde data from Experiment #4 dose #1 ($C = 37\%C_0$, $L_f = 5.7 \mu\text{m}$, $v = 1.83 \text{ m/s}$ (6 ft/s), $R = 0.15 \text{ m}$ (6 in), and using $k = 20 \text{ hr}^{-1}$) results in a 63% reduction in the initial slug of glutaraldehyde at 4400 km downstream. Glutaraldehyde is known to disappear in actual pipelines more rapidly (within several km) than observed in this analysis. Granted this number does not equate to field results nor does our reactor account for the effects of corrosion by-products or scale inhibitors. The analysis only confirms the difficulty in simulating field conditions. Environmental conditions dictate the development of bacterial biofilms. Selective enrichment of microorganisms in the laboratory could also account for a less reactive and much thinner biofilm. If, for example, another experiment resulted in a $100 \mu\text{m}$ thick biofilm, 37% of the initial biocide concentration would be present 250 km downstream. This is still two orders of magnitude too high and validates the necessity of including the "dirty" components of pipeline operations.

Recommendations for Future Work

It was readily apparent that the treatment of sulfate-reducing bacterial biofilms with glutaraldehyde resulted in a period of suspended sulfide production, reduced accumulation of cells, and a diminished bacterial population. The latter two conclusions were hypothesized based upon qualitative observations of the annular reactor environment as the nutrient amendment was replenished. However, a surviving fraction of microorganisms served as an inoculum for new biofilm development after the addition of nutrients. According to the Observable Modulus, the biocide should have penetrated completely, but the observation of new growth questions this conclusion. This implies the penetration of the biocide was not critical to the reduced susceptibility of the biofilm. In addition, evaluation of the Observable Modulus with an average biofilm thickness did not account for diffusion of the biocide into thicker regions of the biofilm.

Future direction should focus on the diffusion of antimicrobial agents through the biofilm to the underlying substratum. Strategies could include the utilization of microelectrodes to capture the gradient of the diffusing agent. Complimentary to this assessment is the ability to address microorganism viability within a treated biofilm. The use of different physiological staining methods, such as the Molecular Probes Live Dead kit, could provide valuable insight by defining regions of live and dead microbial cells. These techniques would either support or oppose the

inferred hypothesis based upon the Observable Modulus calculation; i.e. the antimicrobial agents fully penetrated the biofilm and contacted all microorganisms. These methods would provide insight into mechanisms of biofilm resistance. Such mechanisms include the physiological status of the cell as well as penetration of the biocide.

The evolution of antimicrobial screening processes has progressed from suspended evaluations and cumbersome pipeloop studies to quick turn around systems such as the annular and disk reactors. While each possess unique advantages and disadvantages, further work is needed to develop biocide screening models that adequately capture naturally occurring biofilms. This was underscored by the attempt to extrapolate annular reactor mass balance information to predict biocide concentrations at discrete points down a pipeline.

Additional advances have been made using artificial biofilms in the assessment of biocide efficacy (McIlwaine, 1998; Stewart *et al.*, 1998; Whitham and Gilbert, 1993). This approach provides the flexibility of adding "dirty" components such as oil, metal scrapings, and scale inhibitors. Emulating field conditions with the alginate matrix may provide a simple, reproducible platform to address the topics of reaction, transport phenomena, and microbial adaptation.

CONCLUSIONS

The purpose of this research project was to evaluate the effectiveness of glutaraldehyde and nitrite treatments in controlling microbial sulfide production. This task was accomplished by developing a reproducible laboratory reactor system and the associated methodologies to effectively establish sulfate-reducing bacterial biofilms under anoxic conditions.

Within the scope of this research, the results have led to several conclusions.

1. Biofilm populations of SRB were less susceptible to glutaraldehyde compared to planktonic populations.
2. Calculations showed that the glutaraldehyde and nitrite fully penetrated the sulfate-reducing bacterial biofilms.
3. With the dose concentration and protocols applied, glutaraldehyde treatment resulted in a more effective suppression of sulfide generation than did the nitrite treatment.
4. An adaptive response by the SRB biofilms was not evident for glutaraldehyde. Preliminary data from a single experiment points to the possibility of biofilm adaptation to repeated doses of nitrite.

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APPENDICES

APPENDIX A: Biofilm Reactor Specifications

BIOFILM REACTOR SPECIFICATIONS AND EQUIPMENT SET

Table 16. BST Model 920 Biofilm Annular Reactor Specifications

Inner Cylinder	Height (mm)	156
	Diameter (mm)	140
	Surface Area (mm ²)*	100,140
Outer Cylinder	Height (mm)	181
	Inner Diameter (mm)	157
	Surface Area (mm ²)	89,424
Top Plate	Inner Diameter (mm)	157
	Outer Diameter (mm)	262
	Surface Area (mm ²)	23,417
Bottom Plate	Inner Diameter (mm)	157
	Outer Diameter (mm)	262
	Surface Area (mm ²)	23,279
Draft Tubes	Number	4
	Length (mm)	151
	Diameter (mm)	12.7
	Total Surface Area (mm ²)	24,038
	Angle of Inclination	75.8°
Coupons	Material	Polycarbonate
	Number	20
	Length (mm)	150
	Width (mm)	14.7
	Thickness (mm)	1.5
	Surface Area/coupon (mm ²)	2,203
Reactor	Volume (mm ³)	10 ⁶
	Total Reactor Surface Area (mm ²)	260,298
	Surface area to Volume ratio (mm ⁻¹)	0.26

◦ Includes coupon area.

Table 17. Additional Apparatus Specifications

Component	Function	Source	Specification
Heat exchanger	Reactor temperature	Neslab RTE-221	T = 36°C
Pumps	Medium delivery	<ul style="list-style-type: none"> Masterflex Digital Console L/S Drive (Cole-Palmer #07523-30) Masterflex L/S size #13 tubing pump head (Cole-Palmer #07013-52) 	Q = 0.79 ml/min
	Dilution water delivery	<ul style="list-style-type: none"> Masterflex Variable-Speed Console L/S Drive (Cole-Palmer #07521-50) L/S size #16 tubing pump head (Cole-Palmer #07016-52) 	Q = 7.13 ml/min
Carboys	Medium	13 L Pyrex®	Continuously sparged and stirred
	Dilution water	20 L Pyrex®	Continuously sparged and stirred
Sparging medium	High purity nitrogen (99.99%)	---	6.1-8.6 mL/min at 15 psi using a Cole-Palmer (#03216-04) flowmeter

SURFACE AREA CALCULATIONS

Rotating Inner Cylinder

The inner cylinder surface area is comprised of four major components: SA_{top} , $SA_{circumference}$, and SA_{bottom} , less $SA_{draft tubes}$. These areas account for recessed vertical areas. The inner cylinder dimensions are listed in Table 18.

Table 18. Inner cylinder surface area calculation parameters.

Parameter	Variable	in	mm
Outer radius	r_1	2.75	69.85
Overall height	h_1	6.13	155.7
Base ring radius	r_2	1.6	40.6
Base ring height	h_2	0.38	9.65
Bolt radius	r_3	0.46	11.68
Bolt height	h_3	0.21	5.33
Draft tube radius	r_4	0.25	6.35

The discrete surface areas are defined as.

$$SA_{top} = \pi r_1^2 \quad (1)$$

$$SA_{circumference} = 2\pi r_1 h_1 \quad (2)$$

$$SA_{bottom} = \pi r_1^2 + SA_{recessed_circumference} + SA_{bolt_circumference} \quad (3)$$

$$SA_{recessed_circumference} = 2\pi(r_1 - r_2)h_2 \quad (4)$$

$$SA_{bolt_circumference} = 2\pi r_3 h_3 \quad (5)$$

$$SA_{top_draft_tube} = 8 * \pi r_4^2 \quad (6)$$

Hence, the overall inner cylinder surface area relationship is described by equation (7). This relationship assumes a smooth outer surface with the inserted polycarbonate coupons.

$$SA_{inner_cylinder} = SA_{top} + SA_{circumference} + SA_{bottom} - SA_{top_draft_tube} \quad (7)$$

Outer Cylinder

The calculation of the outer cylinder area accounts for only the inner circumference with the upper and lower areas determined with the top and bottom plate calculations.

Table 19. Outer cylinder surface area calculation parameters.

Parameter	Variable	in	mm
Inner radius	r_1	3.096	78.64
Outer radius	r_2	3.346	84.99
Overall height	h	7.125	180.98

The corresponding surface area is a straight forward calculation where

$$SA_{outer_cylinder} = 2\pi r_1 h \quad (8)$$

Top Plate

The horizontal surface of the top plate is assumed to be all inclusive of the inner diameter of the outer cylinder. Additional compensations have been made to include the vertical surfaces of the recessed coupon port and the innermost ring of the top plate. The respective parameters are defined in Table 20.

Table 20. Top plate surface area calculation parameters.

Parameter	Variable	in	mm
Innermost ring radius	r_1	3.046	77.37
Innermost ring height	h_1	0.25	6.35
Coupon port radius	r_2	0.575	14.6
Coupon port recessed height	h_2	0.387	9.83
Inner radius of outer cylinder	r_3	3.096	78.64

The overall surface area is assembled with the following relationships and is portrayed by Equation (12).

$$SA_{inner_ring} = 2\pi r_1 h_1 \quad (9)$$

$$SA_{coupon_port} = 2\pi r_2 h_2 \quad (10)$$

$$SA_{top} = \pi r_3^2 \quad (11)$$

$$SA_{top_plate} = SA_{inner_ring} + SA_{coupon_port} + SA_{top} \quad (12)$$

Bottom Plate

This calculation is analogous to the top plate with additional consideration for the vertical surfaces of the bearing housing. Again, the associated dimensions used in this calculation are listed in Table 21.

Table 21. Bottom plate surface area calculation parameters.

Parameter	Variable	in	mm
Bearing inner radius	r_1	1.25	31.75
Bearing inner ring height	h_1	0.41	10.41
Bearing outer radius	r_2	1.75	44.45
Bearing outer ring height	h_2	0.25	6.35
Inner radius of outer cylinder	r_3	3.096	78.64

The bottom plate surface area is represented by Equation (13).

$$SA_{bottom_plate} = 2\pi[r_1h_1 + r_2h_2] + \pi r_3^2 \quad (13)$$

Coupon and Draft Tube Surface Area

As with the other surface area subsets, these calculations are straight forward but are listed here for completeness. The respective dimensions for these entities are listed in Table 22.

Table 22. Draft tube and coupon surface area calculation parameters.

Parameter	Variable	in	mm
Coupon height	h_1	5.9	149.86
Coupon width	w_1	0.58	14.73
Removal hole radius	r_1	0.045	1.14
Draft tube height	h_2	5.93	150.62
Draft tube radius	r_2	0.25	6.35

The surface area equations are listed as follows.

$$SA_{coupon} = h_1 w_1 - \pi r_1^2 \quad (14)$$

$$SA_{draft_tube} = 4 * [2\pi r_2 h_2] \quad (15)$$

APPENDIX B: Analysis of Annular Reactor Mixing Characteristics

BIOFILM REACTOR BEHAVIOR

Stimulus-Response (*C*) and Exit Age Distribution (*E*) Curves

A tracer study was used to evaluate the hydraulic residence time distribution, HRT, in a real reactor by introducing an identifiable dye into the reactor influent and measuring its concentration over time in the effluent. A pulse input of the non-reactive tracer, red food coloring, was introduced into the BioSurface Technologies biofilm annular reactor and measured in the effluent stream.

The stimulus-response or *C* curve depicts the fluid element concentration as it is distributed in time with its subsequent washout from the reactor. The ordinate of this concentration versus time distribution is often expressed as a dimensionless number by normalizing the effluent concentration, C_{out} , by the instantaneously achieved concentration, C_{Δ} , where

$$C_{\Delta} = \frac{M_T}{V_R} \quad (1)$$

and M_T and V_R are the mass of the tracer and reactor volume, respectively. The tracer mass can be expressed as the flowrate through the reactor multiplied by the area under the *C* curve.

$$M_T = Q \int_0^{\infty} C_{OUT}(t) dt \quad (2)$$

The exit age distribution curve (E curve) of a fluid element is a normalized version of the C curve. This curve depicts a time-normalized age distribution where

$$\text{Fraction of fluid elements with age } < t = \int_0^t E(t) dt \quad (3)$$

The ordinate of the E curve, $E(t)$, is represented as the normalized C curve ordinate divided by the area under this curve, A_C . Hence,

$$E(t) = \frac{C_{OUT}(t)/C_{\Delta}}{A_C} \quad (4)$$

where

$$A_C = \frac{1}{C_{\Delta}} \int_0^{\infty} C_{OUT}(t) dt \quad (5)$$

Using equations (4) and (5), the area under the E curve can be defined as

$$\int_0^{\infty} E(t) dt = \int_0^{\infty} \frac{C_{OUT}(t)/C_{\Delta}}{\frac{1}{C_{\Delta}} \int_0^{\infty} C_{OUT}(t) dt} dt \quad (6)$$

and when evaluated yields,

$$\int_0^{\infty} E(t) dt = 1 \quad (7)$$

Annular Reactor Analysis

Equation 7 served as a confirmational statement that the analysis of the biofilm annular reactor was performed correctly. The afore-mentioned equations were

performed to generate the C and E curves. The mean hydraulic residence time was calculated by the *first moment* of the centroid method and expressed as

$$t_m = \frac{\int_0^t t^* E(t) dt}{\int_0^t E(t) dt} \quad (8)$$

Recalling the relationship given in equation 7 and verifying with experimental data, the mean value of the hydraulic residence time simplifies to

$$t_m = \int_0^t t^* E(t) dt \quad (9)$$

Experimental Data Notation

The spectrophotometer data were gathered and imported into an Microsoft Excel worksheet. Analysis was performed by calculating the areas under curves using Simpsons Rule for determination of the integral relationships outlined above and in *Process Dynamics in Environmental Systems* (Weber, Jr. and DiGiano, 1996). The Excel worksheet notation listed in Table 23 cross-references the equations mentioned above. This notation is listed with the experimental data in Appendix C.

Table 23. Hydraulic residence time analysis notation

Parameter	Symbol	Weber	Analysis
Effluent concentration of tracer	C_{OUT}	C_{OUT}	C_{OUT}
Influent flowrate	Q	Q	Q
Height of effluent concentration rectangle using Simpson's Rule	C'	---	$C_1 + (C_2 - C_1)/2$
Time interval of Simpson's Rule rectangle	Δt	---	$t_2 - t_1$
Mass of tracer	M_T	$Q \int_0^{\infty} C_{OUT} dt$	$Q * \Sigma(\Delta t * C')$
Reactor Volume	V_R	V_R	V_R
Instantaneous concentration of tracer	C_{Δ}	M_T / C_{Δ}	M_T / C_{Δ}
Area under normalized C curve	A_C or $A_{C/C_{\Delta}}$	$\frac{1}{C_{\Delta}} \int_0^{\infty} C_{OUT} dt$	$\Sigma(\Delta t * C' / C_{\Delta})$
E curve	$E(t)$	$\frac{C_{OUT}(t) / C_{\Delta}}{A_C}$	$\frac{C_{OUT}(t) / C_{\Delta}}{A_{C/C_{\Delta}}}$
Height of exit age concentration rectangle using Simpson's Rule	E'	---	$E_1 + (E_2 - E_1)/2$
Area under E curve	A_E	$\int_0^{\infty} E(t) dt$	$\Sigma(\Delta t * E')$
Area under $t * E(t)$ curve	$A_{t * E}$	$\int_0^{\infty} t * E(t) dt$	$\Sigma(t * \Delta t * E')$
Mean hydraulic residence time	t_m	$\frac{\int_0^{\infty} t * E(t) dt}{\int_0^{\infty} E(t) dt}$	$\Sigma(\Delta t * t * E') / (\Sigma(\Delta t * E'))$

The hydraulic residence time data analysis for the bottom feed annular reactor tracer studies is listed in Table 24 with the corresponding computation of the raw data listed in Appendix C.

Table 24. Hydraulic residence time analysis for biofilm tracer studies.

Parameter	Units	Tracer #3	Tracer #4
Area under C Curve (A_C) = $\Sigma[\Delta t \cdot C'(t)]$	min*abs	205.89	205.89
Area under $t \cdot A_C$ Curve = $\Sigma[t \cdot \Delta t \cdot C'(t)]$	min ² *abs	25664.54	26830.34
$t_{m-C \text{ curve}} = \Sigma[t \cdot \Delta t \cdot C'(t)] / \Sigma[\Delta t \cdot C'(t)]$	min	124.67	130.33
Q	mL/min	7.9	7.9
$M_T = Q \cdot A_C$	mL*abs	1626.51	1626.37
V_R	mL	1000	1000
$V_{R - \text{calculated}} = t_M \cdot Q$	mL	985	1030
$C_A = M_T / V_R$	abs	1.627	1.626
Area under normalized C Curve (A_{C/C_A}) = $\Sigma[\Delta t \cdot C'(t) / C_A]$	min	126.58	126.58
Area under E Curve (A_E) = $\Sigma[\Delta t \cdot E'(t)]$		1	1
Area under $t \cdot A_E$ Curve = $\Sigma[t \cdot \Delta t \cdot E'(t)]$	min	124.67	130.33
$t_{m-C \text{ curve}} = \Sigma[t \cdot \Delta t \cdot E'(t)] / \Sigma[\Delta t \cdot E'(t)]$	min	124.67	130.33

APPENDIX C: Experimental Data for Hydraulic Mixing Study

Table 25. Computational analysis for a bottom fed non-reactive tracer (Tracer #1, $\omega = 230$ rpm).

$\text{Area under } C \text{ curve } (A_C) = \Sigma [\Delta t^* C'(t)]$	135.45	min*abs
$\text{Area under } t^* A_C \text{ curve} = \Sigma [t^* \Delta t^* C'(t)]$	18640.23	min ² *abs
$t_{m-C \text{ curve}} = \Sigma [\Delta t^* t^* C'(t)] / (\Sigma [\Delta t^* C'(t)])$	137.61	min
Q	6.8	mL/min
$M_T = Q * A_C$	921.08	mL*abs
V_R	950	mL
$V_{R-\text{calculated}} = t_m * Q$	936	mL
$C_\Delta = M_T / V_R$	0.97	abs
$\text{Area under normalized } C \text{ curve } (A_{C/C_\Delta}) = \Sigma [\Delta t^* C'(t) / C_\Delta]$	139.71	min
$\text{Area under } E \text{ curve } (A_E) = \Sigma [\Delta t^* E'(t)]$	1.00	
$\text{Area under } t^* E \text{ curve } (A_{t^* E}) = \Sigma [\Delta t^* t^* E'(t)]$	137.61	min
$t_{m-E \text{ curve}} = \Sigma [\Delta t^* t^* E'(t)] / (\Sigma [\Delta t^* E'(t)])$	137.61	min

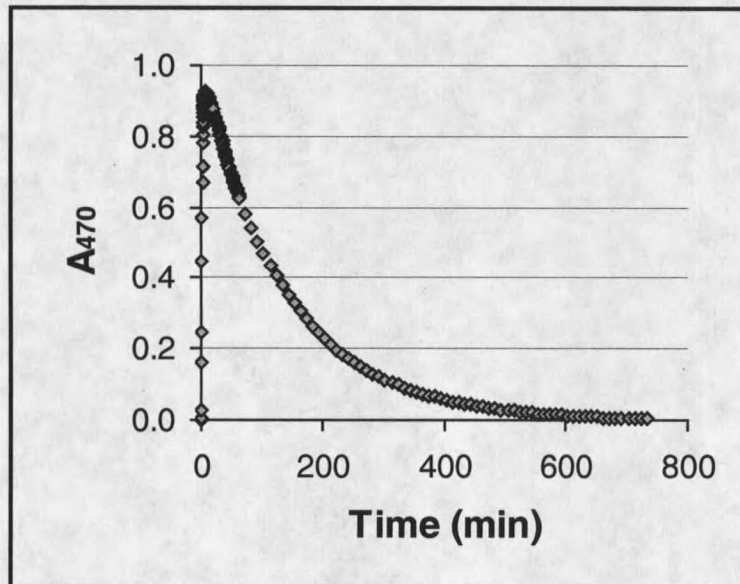


Figure 26. Effluent tracer concentration profile for Tracer #1 ($\omega = 230$ rpm). A 1-mL pulse injection of red food coloring with absorbance levels recorded at $\lambda = 470$ nm.

Table 26. Computational analysis for a bottom fed non-reactive tracer (Tracer #2, $\omega = 230$ rpm).

$\text{Area under } C \text{ curve } (A_C) = \Sigma [\Delta t^* C'(t)]$	218.35	min*abs
$\text{Area under } t^* A_C \text{ curve} = \Sigma [t^* \Delta t^* C'(t)]$	38226.40	min ² *abs
$t_{m-C \text{ curve}} = \Sigma [\Delta t^* t^* C'(t)] / (\Sigma [\Delta t^* C'(t)])$	175.07	min
Q	6.8	mL/min
$M_T = Q * A_C$	1484.81	mL*abs
V_R	1000	mL
$V_{R-\text{calculated}} = t_m * Q$	1190	mL
$C_A = M_T / V_R$	1.48	abs
$\text{Area under normalized } C \text{ curve } (A_{C/C_A}) = \Sigma [\Delta t^* C'(t) / C_A]$	147.06	min
$\text{Area under } E \text{ curve } (A_E) = \Sigma [\Delta t^* E'(t)]$	1.00	
$\text{Area under } t^* E \text{ curve } (A_{t^* E}) = \Sigma [\Delta t^* t^* E'(t)]$	175.07	min
$t_{m-E \text{ curve}} = \Sigma [\Delta t^* t^* E'(t)] / (\Sigma [\Delta t^* E'(t)])$	175.07	min

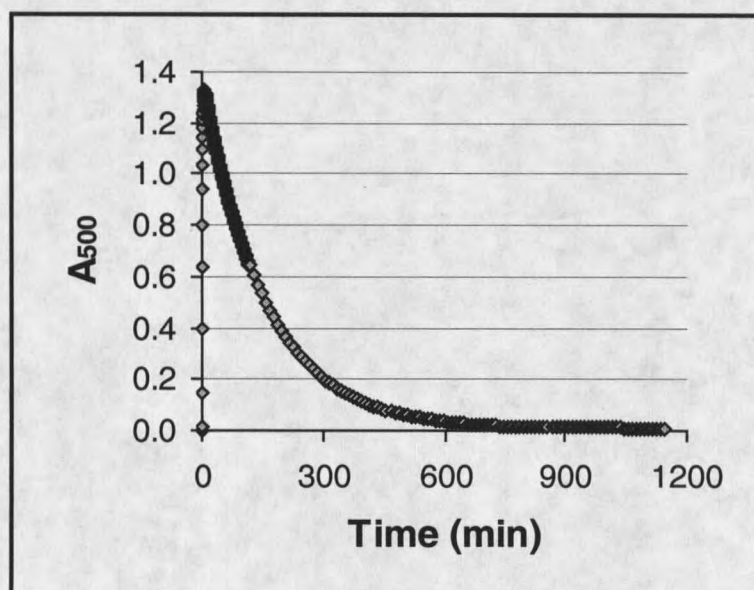


Figure 27. Effluent tracer concentration profile for Tracer #2 ($\omega = 230$ rpm). A 1-mL pulse injection of red food coloring with absorbance levels recorded at $\lambda = 500$ nm.

Table 27. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #3, $\omega = 150$ rpm).

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/CA}(t)=$		$E(t)=$		$A_{RE}(t)=$	
	Time (min)	$\Delta t=t_2-t_1$ (min)	Time $t=t_1+(t_2-t_1)/2$ (min)	$C_{out}(t)$ (abs)	$C_1+(C_2-C_1)/2$ (abs)	$\Delta t^*C'(t)$ (abs*min)	$\Delta t^*t^*C'(t)$ (abs*min ²)	$C_{out}(t)/C_A$	$\Delta t^*C'(t)/C_A$ (min)	$A_{C/CA}$ (min ⁻¹)	$E_1+(E_2-E_1)/2$ (min ⁻¹)	$A_E(t)=$ $\Delta t^*E'(t)$	$t^*E'(t)$ (min)	$\Delta t^*t^*E'(t)$ (min)
13:00:08	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
13:00:18	0.17	0.17	0.08	0.000	0.000	0.000	0.000	0.000	0.000	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
13:00:28	0.33	0.17	0.25	0.000	0.000	0.000	0.000	0.000	0.000	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
13:00:39	0.52	0.18	0.42	0.000	0.000	0.000	0.000	0.000	0.000	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
13:00:48	0.67	0.15	0.59	0.042	0.021	0.003	0.002	0.026	0.002	2.04E-04	1.02E-04	1.53E-05	6.03E-05	9.05E-06
13:00:59	0.85	0.18	0.76	0.222	0.132	0.024	0.018	0.136	0.015	1.08E-03	6.41E-04	1.18E-04	4.86E-04	8.91E-05
13:01:09	1.02	0.17	0.93	0.417	0.320	0.053	0.050	0.256	0.033	2.03E-03	1.55E-03	2.59E-04	1.45E-03	2.41E-04
13:01:19	1.18	0.17	1.10	0.593	0.505	0.084	0.093	0.365	0.052	2.88E-03	2.45E-03	4.09E-04	2.70E-03	4.50E-04
13:01:29	1.35	0.17	1.27	0.749	0.671	0.112	0.142	0.460	0.069	3.64E-03	3.26E-03	5.43E-04	4.13E-03	6.88E-04
13:01:39	1.52	0.17	1.43	0.870	0.810	0.135	0.193	0.535	0.083	4.23E-03	3.93E-03	6.55E-04	5.64E-03	9.39E-04
13:01:49	1.68	0.17	1.60	0.941	0.906	0.151	0.241	0.579	0.093	4.57E-03	4.40E-03	7.33E-04	7.04E-03	1.17E-03
13:01:59	1.85	0.17	1.77	1.030	0.986	0.164	0.290	0.633	0.101	5.00E-03	4.79E-03	7.98E-04	8.46E-03	1.41E-03
13:02:09	2.02	0.17	1.93	1.106	1.068	0.178	0.344	0.680	0.109	5.37E-03	5.19E-03	8.65E-04	1.00E-02	1.67E-03
13:02:19	2.18	0.17	2.10	1.154	1.130	0.188	0.395	0.709	0.116	5.61E-03	5.49E-03	9.15E-04	1.15E-02	1.92E-03
13:02:29	2.35	0.17	2.27	1.205	1.180	0.197	0.446	0.741	0.121	5.85E-03	5.73E-03	9.55E-04	1.30E-02	2.16E-03
13:02:40	2.53	0.18	2.44	1.240	1.223	0.224	0.547	0.762	0.138	6.02E-03	5.94E-03	1.09E-03	1.45E-02	2.66E-03
13:02:49	2.68	0.15	2.61	1.273	1.257	0.188	0.492	0.783	0.116	6.18E-03	6.10E-03	9.15E-04	1.59E-02	2.39E-03
13:03:00	2.87	0.18	2.77	1.293	1.283	0.235	0.653	0.795	0.145	6.28E-03	6.23E-03	1.14E-03	1.73E-02	3.17E-03
13:03:10	3.03	0.17	2.95	1.318	1.306	0.218	0.642	0.810	0.134	6.40E-03	6.34E-03	1.06E-03	1.87E-02	3.12E-03
13:03:20	3.20	0.17	3.12	1.334	1.326	0.221	0.689	0.820	0.136	6.48E-03	6.44E-03	1.07E-03	2.01E-02	3.35E-03
13:03:30	3.37	0.17	3.28	1.355	1.345	0.224	0.736	0.833	0.138	6.58E-03	6.53E-03	1.09E-03	2.14E-02	3.57E-03
13:03:40	3.53	0.17	3.45	1.366	1.361	0.227	0.782	0.840	0.139	6.63E-03	6.61E-03	1.10E-03	2.28E-02	3.80E-03
13:03:50	3.70	0.17	3.62	1.380	1.373	0.229	0.828	0.848	0.141	6.70E-03	6.67E-03	1.11E-03	2.41E-02	4.02E-03
13:04:00	3.87	0.17	3.78	1.393	1.387	0.231	0.874	0.856	0.142	6.77E-03	6.73E-03	1.12E-03	2.55E-02	4.25E-03
13:04:10	4.03	0.17	3.95	1.404	1.399	0.233	0.921	0.863	0.143	6.82E-03	6.79E-03	1.13E-03	2.68E-02	4.47E-03
13:04:20	4.20	0.17	4.12	1.411	1.408	0.235	0.966	0.868	0.144	6.85E-03	6.84E-03	1.14E-03	2.81E-02	4.69E-03
13:04:30	4.37	0.17	4.28	1.420	1.416	0.236	1.011	0.873	0.145	6.90E-03	6.88E-03	1.15E-03	2.94E-02	4.91E-03
13:04:40	4.53	0.17	4.45	1.428	1.424	0.237	1.056	0.878	0.146	6.94E-03	6.92E-03	1.15E-03	3.08E-02	5.13E-03
13:04:50	4.70	0.17	4.62	1.436	1.432	0.239	1.102	0.883	0.147	6.97E-03	6.96E-03	1.16E-03	3.21E-02	5.35E-03
13:05:00	4.87	0.17	4.78	1.442	1.439	0.240	1.147	0.887	0.147	7.00E-03	6.99E-03	1.16E-03	3.34E-02	5.57E-03
13:05:10	5.03	0.17	4.95	1.448	1.445	0.241	1.192	0.890	0.148	7.03E-03	7.02E-03	1.17E-03	3.47E-02	5.79E-03

Table 27. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #3, $\omega = 150$ rpm). *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/CA}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time	$\Delta t=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$\Delta t^*C'(t)$	$\Delta t^*t^*C'(t)$	$C_{out}(t)/C_A$	$\Delta t^*C'(t)/C_A$	$(C_{out}(t)/C_A)/$	$E'(t)=$	$A_E(t)=$	$t^*E(t)=$	$\Delta t^*t^*E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)		(min)	(min ⁻¹)	(min ⁻¹)	$\Delta t^*E'(t)$	$t^*E'(t)$	(min)
13:05:20	5.20	0.17	5.12	1.453	1.451	0.242	1.237	0.893	0.149	7.06E-03	7.05E-03	1.17E-03	3.60E-02	6.01E-03
13:05:30	5.37	0.17	5.28	1.456	1.455	0.242	1.281	0.895	0.149	7.07E-03	7.06E-03	1.18E-03	3.73E-02	6.22E-03
13:05:40	5.53	0.17	5.45	1.461	1.459	0.243	1.325	0.898	0.149	7.10E-03	7.08E-03	1.18E-03	3.86E-02	6.43E-03
13:05:50	5.70	0.17	5.62	1.465	1.463	0.244	1.370	0.901	0.150	7.12E-03	7.11E-03	1.18E-03	3.99E-02	6.65E-03
13:06:00	5.87	0.17	5.78	1.469	1.467	0.244	1.414	0.903	0.150	7.13E-03	7.13E-03	1.19E-03	4.12E-02	6.87E-03
13:06:10	6.03	0.17	5.95	1.472	1.471	0.245	1.458	0.905	0.151	7.15E-03	7.14E-03	1.19E-03	4.25E-02	7.08E-03
13:06:20	6.20	0.17	6.12	1.475	1.474	0.246	1.502	0.907	0.151	7.16E-03	7.16E-03	1.19E-03	4.38E-02	7.30E-03
13:06:30	6.37	0.17	6.28	1.477	1.476	0.246	1.546	0.908	0.151	7.17E-03	7.17E-03	1.19E-03	4.50E-02	7.51E-03
13:06:40	6.53	0.17	6.45	1.480	1.479	0.246	1.589	0.910	0.152	7.19E-03	7.18E-03	1.20E-03	4.63E-02	7.72E-03
13:06:51	6.72	0.18	6.62	1.481	1.481	0.271	1.798	0.911	0.167	7.19E-03	7.19E-03	1.32E-03	4.76E-02	8.73E-03
13:07:00	6.87	0.15	6.79	1.484	1.483	0.222	1.510	0.912	0.137	7.21E-03	7.20E-03	1.08E-03	4.89E-02	7.34E-03
13:07:11	7.05	0.18	6.96	1.485	1.485	0.272	1.894	0.913	0.167	7.21E-03	7.21E-03	1.32E-03	5.02E-02	9.20E-03
13:07:21	7.22	0.17	7.13	1.487	1.486	0.248	1.767	0.914	0.152	7.22E-03	7.22E-03	1.20E-03	5.15E-02	8.58E-03
13:07:30	7.37	0.15	7.29	1.488	1.488	0.223	1.627	0.915	0.137	7.23E-03	7.22E-03	1.08E-03	5.27E-02	7.90E-03
13:07:41	7.55	0.18	7.46	1.489	1.489	0.273	2.035	0.915	0.168	7.23E-03	7.23E-03	1.33E-03	5.39E-02	9.89E-03
13:07:51	7.72	0.17	7.63	1.490	1.490	0.248	1.895	0.916	0.153	7.24E-03	7.23E-03	1.21E-03	5.52E-02	9.20E-03
13:08:01	7.88	0.17	7.80	1.491	1.491	0.248	1.938	0.917	0.153	7.24E-03	7.24E-03	1.21E-03	5.65E-02	9.41E-03
13:08:11	8.05	0.17	7.97	1.492	1.492	0.249	1.980	0.917	0.153	7.25E-03	7.24E-03	1.21E-03	5.77E-02	9.62E-03
13:08:21	8.22	0.17	8.13	1.492	1.492	0.249	2.022	0.917	0.153	7.25E-03	7.25E-03	1.21E-03	5.89E-02	9.82E-03
13:08:31	8.38	0.17	8.30	1.492	1.492	0.249	2.064	0.917	0.153	7.25E-03	7.25E-03	1.21E-03	6.01E-02	1.00E-02
13:08:41	8.55	0.17	8.47	1.493	1.493	0.249	2.106	0.918	0.153	7.25E-03	7.25E-03	1.21E-03	6.14E-02	1.02E-02
13:08:51	8.72	0.17	8.63	1.493	1.493	0.249	2.148	0.918	0.153	7.25E-03	7.25E-03	1.21E-03	6.26E-02	1.04E-02
13:09:01	8.88	0.17	8.80	1.493	1.493	0.249	2.190	0.918	0.153	7.25E-03	7.25E-03	1.21E-03	6.38E-02	1.06E-02
13:09:11	9.05	0.17	8.97	1.493	1.493	0.249	2.231	0.918	0.153	7.25E-03	7.25E-03	1.21E-03	6.50E-02	1.08E-02
13:09:21	9.22	0.17	9.13	1.493	1.493	0.249	2.273	0.918	0.153	7.25E-03	7.25E-03	1.21E-03	6.62E-02	1.10E-02
13:09:31	9.38	0.17	9.30	1.493	1.493	0.249	2.314	0.918	0.153	7.25E-03	7.25E-03	1.21E-03	6.74E-02	1.12E-02
13:09:41	9.55	0.17	9.47	1.493	1.493	0.249	2.356	0.918	0.153	7.25E-03	7.25E-03	1.21E-03	6.86E-02	1.14E-02
13:09:51	9.72	0.17	9.63	1.493	1.493	0.249	2.397	0.918	0.153	7.25E-03	7.25E-03	1.21E-03	6.99E-02	1.16E-02
13:10:01	9.88	0.17	9.80	1.492	1.493	0.249	2.438	0.917	0.153	7.25E-03	7.25E-03	1.21E-03	7.10E-02	1.18E-02
13:10:11	10.05	0.17	9.97	1.492	1.492	0.249	2.478	0.917	0.153	7.25E-03	7.25E-03	1.21E-03	7.22E-02	1.20E-02
13:10:21	10.22	0.17	10.13	1.492	1.492	0.249	2.520	0.917	0.153	7.25E-03	7.25E-03	1.21E-03	7.34E-02	1.22E-02

Table 27. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #3, $\omega = 150$ rpm). *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/\Delta}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time	$\Delta t=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$\Delta t^*C'(t)$	$\Delta t^*t^*C'(t)$	$C_{out}(t)/C_{\Delta}$	$\Delta t^*C'(t)/C_{\Delta}$	$C_{C/\Delta}$	$E_1+(E_2-E_1)/2$	$A_E(t)=$	$t^*E(t)=$	$\Delta t^*t^*E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)		(min)	(min ⁻¹)	(min ⁻¹)	$\Delta t^*E'(t)$	$t^*E'(t)$	(min)
13:10:31	10.38	0.17	10.30	1.492	1.492	0.249	2.561	0.917	0.153	7.25E-03	7.25E-03	1.21E-03	7.46E-02	1.24E-02
13:10:42	10.57	0.18	10.48	1.491	1.492	0.273	2.864	0.917	0.168	7.24E-03	7.24E-03	1.33E-03	7.59E-02	1.39E-02
13:10:52	10.73	0.17	10.65	1.490	1.491	0.248	2.646	0.916	0.153	7.24E-03	7.24E-03	1.21E-03	7.71E-02	1.28E-02
13:11:02	10.90	0.17	10.82	1.490	1.490	0.248	2.686	0.916	0.153	7.24E-03	7.24E-03	1.21E-03	7.83E-02	1.30E-02
13:11:12	11.07	0.17	10.98	1.489	1.490	0.248	2.727	0.915	0.153	7.23E-03	7.23E-03	1.21E-03	7.95E-02	1.32E-02
13:11:21	11.22	0.15	11.14	1.489	1.489	0.223	2.488	0.915	0.137	7.23E-03	7.23E-03	1.08E-03	8.06E-02	1.21E-02
13:11:32	11.40	0.18	11.31	1.488	1.489	0.273	3.086	0.915	0.168	7.23E-03	7.23E-03	1.33E-03	8.18E-02	1.50E-02
13:11:42	11.57	0.17	11.48	1.487	1.488	0.248	2.847	0.914	0.152	7.22E-03	7.22E-03	1.20E-03	8.30E-02	1.38E-02
13:11:52	11.73	0.17	11.65	1.486	1.487	0.248	2.886	0.914	0.152	7.22E-03	7.22E-03	1.20E-03	8.41E-02	1.40E-02
13:12:02	11.90	0.17	11.82	1.485	1.486	0.248	2.926	0.913	0.152	7.21E-03	7.22E-03	1.20E-03	8.53E-02	1.42E-02
13:12:12	12.07	0.17	11.98	1.485	1.485	0.247	2.966	0.913	0.152	7.21E-03	7.21E-03	1.20E-03	8.64E-02	1.44E-02
13:12:23	12.25	0.18	12.16	1.484	1.485	0.272	3.309	0.912	0.167	7.21E-03	7.21E-03	1.32E-03	8.77E-02	1.61E-02
13:12:32	12.40	0.15	12.33	1.483	1.484	0.223	2.743	0.912	0.137	7.20E-03	7.21E-03	1.08E-03	8.88E-02	1.33E-02
13:12:42	12.57	0.17	12.48	1.482	1.483	0.247	3.084	0.911	0.152	7.20E-03	7.20E-03	1.20E-03	8.99E-02	1.50E-02
13:12:52	12.73	0.17	12.65	1.481	1.482	0.247	3.123	0.911	0.152	7.19E-03	7.20E-03	1.20E-03	9.10E-02	1.52E-02
13:13:02	12.90	0.17	12.82	1.480	1.481	0.247	3.163	0.910	0.152	7.19E-03	7.19E-03	1.20E-03	9.22E-02	1.54E-02
13:13:12	13.07	0.17	12.98	1.479	1.480	0.247	3.201	0.909	0.152	7.18E-03	7.19E-03	1.20E-03	9.33E-02	1.55E-02
13:13:22	13.23	0.17	13.15	1.478	1.479	0.246	3.240	0.909	0.152	7.18E-03	7.18E-03	1.20E-03	9.44E-02	1.57E-02
13:13:32	13.40	0.17	13.32	1.477	1.478	0.246	3.279	0.908	0.151	7.17E-03	7.18E-03	1.20E-03	9.56E-02	1.59E-02
13:13:42	13.57	0.17	13.48	1.476	1.477	0.246	3.318	0.907	0.151	7.17E-03	7.17E-03	1.20E-03	9.67E-02	1.61E-02
13:13:54	13.77	0.20	13.67	1.475	1.476	0.295	4.033	0.907	0.181	7.16E-03	7.17E-03	1.43E-03	9.79E-02	1.96E-02
13:14:03	13.92	0.15	13.84	1.475	1.475	0.221	3.062	0.907	0.136	7.16E-03	7.16E-03	1.07E-03	9.92E-02	1.49E-02
13:14:12	14.07	0.15	13.99	1.474	1.475	0.221	3.095	0.906	0.136	7.16E-03	7.16E-03	1.07E-03	1.00E-01	1.50E-02
13:14:22	14.23	0.17	14.15	1.473	1.474	0.246	3.475	0.906	0.151	7.15E-03	7.16E-03	1.19E-03	1.01E-01	1.69E-02
13:14:32	14.40	0.17	14.32	1.472	1.473	0.245	3.514	0.905	0.151	7.15E-03	7.15E-03	1.19E-03	1.02E-01	1.71E-02
13:14:42	14.57	0.17	14.48	1.471	1.472	0.245	3.552	0.904	0.151	7.14E-03	7.15E-03	1.19E-03	1.04E-01	1.73E-02
13:14:52	14.73	0.17	14.65	1.470	1.471	0.245	3.590	0.904	0.151	7.14E-03	7.14E-03	1.19E-03	1.05E-01	1.74E-02
13:15:03	14.92	0.18	14.82	1.469	1.470	0.269	3.994	0.903	0.166	7.13E-03	7.14E-03	1.31E-03	1.06E-01	1.94E-02
13:15:13	15.08	0.17	15.00	1.467	1.468	0.245	3.670	0.902	0.150	7.13E-03	7.13E-03	1.19E-03	1.07E-01	1.78E-02
13:15:23	15.25	0.17	15.17	1.466	1.467	0.244	3.707	0.901	0.150	7.12E-03	7.12E-03	1.19E-03	1.08E-01	1.80E-02
13:15:33	15.42	0.17	15.33	1.465	1.466	0.244	3.745	0.901	0.150	7.12E-03	7.12E-03	1.19E-03	1.09E-01	1.82E-02

Table 27. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #3, $\omega = 150$ rpm). *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/\Delta}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time	$\Delta t=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$\Delta t^*C'(t)$	$\Delta t^*t^*C'(t)$	$C_{out}(t)/C_\Delta$	$\Delta t^*C'(t)/C_\Delta$	$A_{C/\Delta}$	$E_1+(E_2-E_1)/2$	$A_E(t)=$	$t^*E(t)=$	$\Delta t^*t^*E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)		(min)	(min ⁻¹)	(min ⁻¹)	$\Delta t^*E'(t)$	$t^*E'(t)$	(min)
13:15:42	15.57	0.15	15.49	1.464	1.465	0.220	3.403	0.900	0.135	7.11E-03	7.11E-03	1.07E-03	1.10E-01	1.65E-02
13:15:53	15.75	0.18	15.66	1.462	1.463	0.268	4.200	0.899	0.165	7.10E-03	7.11E-03	1.30E-03	1.11E-01	2.04E-02
13:16:03	15.92	0.17	15.83	1.462	1.462	0.244	3.858	0.899	0.150	7.10E-03	7.10E-03	1.18E-03	1.12E-01	1.87E-02
13:16:13	16.08	0.17	16.00	1.461	1.462	0.244	3.897	0.898	0.150	7.10E-03	7.10E-03	1.18E-03	1.14E-01	1.89E-02
13:16:23	16.25	0.17	16.17	1.459	1.460	0.243	3.934	0.897	0.150	7.09E-03	7.09E-03	1.18E-03	1.15E-01	1.91E-02
13:16:33	16.42	0.17	16.33	1.458	1.459	0.243	3.970	0.896	0.149	7.08E-03	7.08E-03	1.18E-03	1.16E-01	1.93E-02
13:16:43	16.58	0.17	16.50	1.457	1.458	0.243	4.008	0.896	0.149	7.08E-03	7.08E-03	1.18E-03	1.17E-01	1.95E-02
13:16:53	16.75	0.17	16.67	1.456	1.457	0.243	4.046	0.895	0.149	7.07E-03	7.07E-03	1.18E-03	1.18E-01	1.97E-02
13:17:03	16.92	0.17	16.83	1.454	1.455	0.242	4.082	0.894	0.149	7.06E-03	7.07E-03	1.18E-03	1.19E-01	1.98E-02
13:17:13	17.08	0.17	17.00	1.453	1.454	0.242	4.118	0.893	0.149	7.06E-03	7.06E-03	1.18E-03	1.20E-01	2.00E-02
13:17:23	17.25	0.17	17.17	1.453	1.453	0.242	4.157	0.893	0.149	7.06E-03	7.06E-03	1.18E-03	1.21E-01	2.02E-02
13:17:33	17.42	0.17	17.33	1.451	1.452	0.242	4.195	0.892	0.149	7.05E-03	7.05E-03	1.18E-03	1.22E-01	2.04E-02
13:17:43	17.58	0.17	17.50	1.450	1.451	0.242	4.231	0.891	0.149	7.04E-03	7.05E-03	1.17E-03	1.23E-01	2.05E-02
13:17:53	17.75	0.17	17.67	1.448	1.449	0.241	4.266	0.890	0.148	7.03E-03	7.04E-03	1.17E-03	1.24E-01	2.07E-02
13:18:03	17.92	0.17	17.83	1.447	1.448	0.241	4.302	0.890	0.148	7.03E-03	7.03E-03	1.17E-03	1.25E-01	2.09E-02
13:18:13	18.08	0.17	18.00	1.445	1.446	0.241	4.338	0.888	0.148	7.02E-03	7.02E-03	1.17E-03	1.26E-01	2.11E-02
13:18:23	18.25	0.17	18.17	1.445	1.445	0.241	4.375	0.888	0.148	7.02E-03	7.02E-03	1.17E-03	1.28E-01	2.13E-02
13:18:33	18.42	0.17	18.33	1.444	1.445	0.241	4.414	0.888	0.148	7.01E-03	7.02E-03	1.17E-03	1.29E-01	2.14E-02
13:18:43	18.58	0.17	18.50	1.442	1.443	0.240	4.449	0.887	0.148	7.00E-03	7.01E-03	1.17E-03	1.30E-01	2.16E-02
13:18:53	18.75	0.17	18.67	1.441	1.442	0.240	4.485	0.886	0.148	7.00E-03	7.00E-03	1.17E-03	1.31E-01	2.18E-02
13:19:04	18.93	0.18	18.84	1.439	1.440	0.264	4.974	0.885	0.162	6.99E-03	6.99E-03	1.28E-03	1.32E-01	2.42E-02
13:19:14	19.10	0.17	19.02	1.438	1.439	0.240	4.559	0.884	0.147	6.98E-03	6.99E-03	1.16E-03	1.33E-01	2.21E-02
13:19:24	19.27	0.17	19.18	1.437	1.438	0.240	4.596	0.883	0.147	6.98E-03	6.98E-03	1.16E-03	1.34E-01	2.23E-02
13:19:34	19.43	0.17	19.35	1.436	1.437	0.239	4.633	0.883	0.147	6.97E-03	6.98E-03	1.16E-03	1.35E-01	2.25E-02
13:19:44	19.60	0.17	19.52	1.434	1.435	0.239	4.668	0.882	0.147	6.96E-03	6.97E-03	1.16E-03	1.36E-01	2.27E-02
13:19:54	19.77	0.17	19.68	1.432	1.433	0.239	4.701	0.880	0.147	6.96E-03	6.96E-03	1.16E-03	1.37E-01	2.28E-02
13:20:04	19.93	0.17	19.85	1.432	1.432	0.239	4.738	0.880	0.147	6.96E-03	6.96E-03	1.16E-03	1.38E-01	2.30E-02
13:20:14	20.10	0.17	20.02	1.431	1.432	0.239	4.776	0.880	0.147	6.95E-03	6.95E-03	1.16E-03	1.39E-01	2.32E-02
13:22:49	22.68	2.58	21.39	1.409	1.420	3.668	78.472	0.866	2.255	6.84E-03	6.90E-03	1.78E-02	1.48E-01	3.81E-01
13:25:54	25.77	3.08	24.23	1.381	1.395	4.301	104.198	0.849	2.644	6.71E-03	6.78E-03	2.09E-02	1.64E-01	5.06E-01
13:26:54	26.77	1.00	26.27	1.373	1.377	1.377	36.169	0.844	0.847	6.67E-03	6.69E-03	6.69E-03	1.76E-01	1.76E-01

Table 27. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #3, $\omega = 150$ rpm). *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/C_A}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time (min)	$\Delta t=t_2-t_1$ (min)	Time $t=t_1+(t_2-t_1)/2$	$C_{out}(t)$ (abs)	$C_1+(C_2-C_1)/2$ (abs)	$\Delta t^*C'(t)$ (abs*min)	$\Delta t^*t^*C'(t)$ (abs*min ²)	$C_{out}(t)/C_A$	$\Delta t^*C'(t)/C_A$ (min)	$(C_{out}(t)/C_A)/$ A_{C/C_A} (min ⁻¹)	$E_1+(E_2-E_1)/2$ (min ⁻¹)	$A_E(t)=$ $\Delta t^*E'(t)$	$t^*E'(t)$	$\Delta t^*t^*E'(t)$ (min)
13:27:52	27.73	0.97	27.25	1.365	1.369	1.323	36.062	0.839	0.814	6.63E-03	6.65E-03	6.43E-03	1.81E-01	1.75E-01
13:28:52	28.73	1.00	28.23	1.355	1.360	1.360	38.397	0.833	0.836	6.58E-03	6.61E-03	6.61E-03	1.86E-01	1.86E-01
13:29:52	29.73	1.00	29.23	1.346	1.351	1.350	39.480	0.828	0.830	6.54E-03	6.56E-03	6.56E-03	1.92E-01	1.92E-01
13:30:52	30.73	1.00	30.23	1.336	1.341	1.341	40.543	0.821	0.824	6.49E-03	6.51E-03	6.51E-03	1.97E-01	1.97E-01
13:31:52	31.73	1.00	31.23	1.326	1.331	1.331	41.572	0.815	0.818	6.44E-03	6.46E-03	6.46E-03	2.02E-01	2.02E-01
13:32:52	32.73	1.00	32.23	1.315	1.321	1.321	42.564	0.808	0.812	6.39E-03	6.41E-03	6.41E-03	2.07E-01	2.07E-01
13:33:52	33.73	1.00	33.23	1.304	1.310	1.309	43.519	0.802	0.805	6.33E-03	6.36E-03	6.36E-03	2.11E-01	2.11E-01
13:34:52	34.73	1.00	34.23	1.294	1.299	1.299	44.469	0.796	0.799	6.28E-03	6.31E-03	6.31E-03	2.16E-01	2.16E-01
13:35:52	35.73	1.00	35.23	1.284	1.289	1.289	45.416	0.789	0.792	6.24E-03	6.26E-03	6.26E-03	2.21E-01	2.21E-01
13:36:52	36.73	1.00	36.23	1.274	1.279	1.279	46.342	0.783	0.786	6.19E-03	6.21E-03	6.21E-03	2.25E-01	2.25E-01
13:37:52	37.73	1.00	37.23	1.264	1.269	1.269	47.249	0.777	0.780	6.14E-03	6.16E-03	6.16E-03	2.29E-01	2.29E-01
13:38:52	38.73	1.00	38.23	1.253	1.259	1.259	48.117	0.770	0.774	6.09E-03	6.11E-03	6.11E-03	2.34E-01	2.34E-01
13:39:54	39.77	1.03	39.25	1.243	1.248	1.290	50.617	0.764	0.793	6.04E-03	6.06E-03	6.26E-03	2.38E-01	2.46E-01
13:40:53	40.75	0.98	40.26	1.233	1.238	1.217	49.009	0.758	0.748	5.99E-03	6.01E-03	5.91E-03	2.42E-01	2.38E-01
13:41:54	41.77	1.02	41.26	1.223	1.228	1.248	51.510	0.752	0.768	5.94E-03	5.96E-03	6.06E-03	2.46E-01	2.50E-01
13:42:56	42.80	1.03	42.28	1.213	1.218	1.259	53.218	0.746	0.774	5.89E-03	5.92E-03	6.11E-03	2.50E-01	2.58E-01
13:43:53	43.75	0.95	43.28	1.204	1.209	1.148	49.683	0.740	0.706	5.85E-03	5.87E-03	5.58E-03	2.54E-01	2.41E-01
13:44:54	44.77	1.02	44.26	1.194	1.199	1.219	53.950	0.734	0.749	5.80E-03	5.82E-03	5.92E-03	2.58E-01	2.62E-01
13:45:53	45.75	0.98	45.26	1.184	1.189	1.169	52.915	0.728	0.719	5.75E-03	5.77E-03	5.68E-03	2.61E-01	2.57E-01
13:46:53	46.75	1.00	46.25	1.175	1.180	1.180	54.552	0.722	0.725	5.71E-03	5.73E-03	5.73E-03	2.65E-01	2.65E-01
13:47:53	47.75	1.00	47.25	1.166	1.171	1.171	55.306	0.717	0.720	5.66E-03	5.69E-03	5.69E-03	2.69E-01	2.69E-01
13:48:53	48.75	1.00	48.25	1.156	1.161	1.161	56.018	0.711	0.714	5.61E-03	5.64E-03	5.64E-03	2.72E-01	2.72E-01
13:49:53	49.75	1.00	49.25	1.147	1.152	1.152	56.711	0.705	0.708	5.57E-03	5.59E-03	5.59E-03	2.75E-01	2.75E-01
13:50:53	50.75	1.00	50.25	1.137	1.142	1.142	57.385	0.699	0.702	5.52E-03	5.55E-03	5.55E-03	2.79E-01	2.79E-01
13:51:54	51.77	1.02	51.26	1.128	1.133	1.151	59.018	0.694	0.708	5.48E-03	5.50E-03	5.59E-03	2.82E-01	2.87E-01
13:52:53	52.75	0.98	52.26	1.118	1.123	1.104	57.708	0.687	0.679	5.43E-03	5.45E-03	5.36E-03	2.85E-01	2.80E-01
13:53:53	53.75	1.00	53.25	1.109	1.114	1.114	59.294	0.682	0.685	5.39E-03	5.41E-03	5.41E-03	2.88E-01	2.88E-01
13:54:53	54.75	1.00	54.25	1.100	1.105	1.105	59.919	0.676	0.679	5.34E-03	5.36E-03	5.36E-03	2.91E-01	2.91E-01
13:55:54	55.77	1.02	55.26	1.091	1.096	1.114	61.544	0.671	0.685	5.30E-03	5.32E-03	5.41E-03	2.94E-01	2.99E-01
13:56:54	56.77	1.00	56.27	1.082	1.087	1.087	61.134	0.665	0.668	5.26E-03	5.28E-03	5.28E-03	2.97E-01	2.97E-01
13:57:54	57.77	1.00	57.27	1.074	1.078	1.078	61.733	0.660	0.663	5.22E-03	5.24E-03	5.24E-03	3.00E-01	3.00E-01

Table 27. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #3, $\omega = 150$ rpm). *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/\Delta}(t)=$		$E(t)=$		$A_{RE}(t)=$	
	Time	$\Delta t=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$\Delta t^*C'(t)$	$\Delta t^*t^*C'(t)$	$C_{out}(t)/C_\Delta$	$\Delta t^*C'(t)/C_\Delta$	$A_{C/\Delta}$	$E_1+(E_2-E_1)/2$	$A_E(t)=$	$t^*E(t)=$	$\Delta t^*t^*E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)		(min)	(min ⁻¹)	(min ⁻¹)	$\Delta t^*E'(t)$	$t^*E'(t)$	(min)
13:58:54	58.77	1.00	58.27	1.065	1.070	1.069	62.316	0.655	0.658	5.17E-03	5.19E-03	5.19E-03	3.03E-01	3.03E-01
13:59:54	59.77	1.00	59.27	1.056	1.061	1.060	62.852	0.649	0.652	5.13E-03	5.15E-03	5.15E-03	3.05E-01	3.05E-01
14:00:54	60.77	1.00	60.27	1.048	1.052	1.052	63.401	0.644	0.647	5.09E-03	5.11E-03	5.11E-03	3.08E-01	3.08E-01
14:01:54	61.77	1.00	61.27	1.039	1.044	1.044	63.932	0.639	0.642	5.05E-03	5.07E-03	5.07E-03	3.11E-01	3.11E-01
14:02:54	62.77	1.00	62.27	1.031	1.035	1.035	64.446	0.634	0.636	5.01E-03	5.03E-03	5.03E-03	3.13E-01	3.13E-01
14:03:54	63.77	1.00	63.27	1.022	1.027	1.026	64.943	0.628	0.631	4.96E-03	4.99E-03	4.99E-03	3.15E-01	3.15E-01
14:04:54	64.77	1.00	64.27	1.014	1.018	1.018	65.423	0.623	0.626	4.93E-03	4.94E-03	4.94E-03	3.18E-01	3.18E-01
14:05:56	65.80	1.03	65.28	1.006	1.010	1.044	68.134	0.619	0.642	4.89E-03	4.91E-03	5.07E-03	3.20E-01	3.31E-01
14:06:55	66.78	0.98	66.29	0.998	1.002	0.985	65.317	0.614	0.606	4.85E-03	4.87E-03	4.79E-03	3.23E-01	3.17E-01
14:07:55	67.78	1.00	67.28	0.990	0.994	0.994	66.880	0.609	0.611	4.81E-03	4.83E-03	4.83E-03	3.25E-01	3.25E-01
14:08:55	68.78	1.00	68.28	0.982	0.986	0.986	67.327	0.604	0.606	4.77E-03	4.79E-03	4.79E-03	3.27E-01	3.27E-01
14:09:56	69.80	1.02	69.29	0.973	0.978	0.994	68.861	0.598	0.611	4.73E-03	4.75E-03	4.83E-03	3.29E-01	3.34E-01
14:10:55	70.78	0.98	70.29	0.965	0.969	0.953	66.977	0.593	0.586	4.69E-03	4.71E-03	4.63E-03	3.31E-01	3.25E-01
14:11:55	71.78	1.00	71.28	0.958	0.962	0.962	68.539	0.589	0.591	4.65E-03	4.67E-03	4.67E-03	3.33E-01	3.33E-01
14:12:55	72.78	1.00	72.28	0.950	0.954	0.954	68.958	0.584	0.587	4.61E-03	4.63E-03	4.63E-03	3.35E-01	3.35E-01
14:13:55	73.78	1.00	73.28	0.942	0.946	0.946	69.326	0.579	0.582	4.58E-03	4.59E-03	4.59E-03	3.37E-01	3.37E-01
14:14:55	74.78	1.00	74.28	0.934	0.938	0.938	69.678	0.574	0.577	4.54E-03	4.56E-03	4.56E-03	3.38E-01	3.38E-01
14:15:55	75.78	1.00	75.28	0.926	0.930	0.930	70.013	0.569	0.572	4.50E-03	4.52E-03	4.52E-03	3.40E-01	3.40E-01
14:16:55	76.78	1.00	76.28	0.919	0.923	0.923	70.371	0.565	0.567	4.46E-03	4.48E-03	4.48E-03	3.42E-01	3.42E-01
14:17:55	77.78	1.00	77.28	0.911	0.915	0.915	70.714	0.560	0.563	4.42E-03	4.44E-03	4.44E-03	3.43E-01	3.43E-01
14:18:55	78.78	1.00	78.28	0.904	0.908	0.908	71.042	0.556	0.558	4.39E-03	4.41E-03	4.41E-03	3.45E-01	3.45E-01
14:19:55	79.78	1.00	79.28	0.896	0.900	0.900	71.355	0.551	0.553	4.35E-03	4.37E-03	4.37E-03	3.47E-01	3.47E-01
14:20:55	80.78	1.00	80.28	0.889	0.893	0.893	71.653	0.547	0.549	4.32E-03	4.33E-03	4.33E-03	3.48E-01	3.48E-01
14:21:55	81.78	1.00	81.28	0.882	0.886	0.885	71.976	0.542	0.544	4.28E-03	4.30E-03	4.30E-03	3.50E-01	3.50E-01
14:22:55	82.78	1.00	82.28	0.875	0.879	0.879	72.286	0.538	0.540	4.25E-03	4.27E-03	4.27E-03	3.51E-01	3.51E-01
14:23:55	83.78	1.00	83.28	0.868	0.872	0.871	72.581	0.534	0.536	4.22E-03	4.23E-03	4.23E-03	3.53E-01	3.53E-01
14:24:55	84.78	1.00	84.28	0.861	0.865	0.865	72.863	0.529	0.532	4.18E-03	4.20E-03	4.20E-03	3.54E-01	3.54E-01
14:25:55	85.78	1.00	85.28	0.853	0.857	0.857	73.088	0.524	0.527	4.14E-03	4.16E-03	4.16E-03	3.55E-01	3.55E-01
14:26:55	86.78	1.00	86.28	0.846	0.850	0.850	73.298	0.520	0.522	4.11E-03	4.13E-03	4.13E-03	3.56E-01	3.56E-01
14:27:55	87.78	1.00	87.28	0.840	0.843	0.843	73.580	0.516	0.518	4.08E-03	4.09E-03	4.09E-03	3.57E-01	3.57E-01
14:28:55	88.78	1.00	88.28	0.833	0.837	0.837	73.849	0.512	0.514	4.05E-03	4.06E-03	4.06E-03	3.59E-01	3.59E-01

Table 27. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #3, $\omega = 150$ rpm). *continued*

Time	Elapsed	$\Delta t = t_2 - t_1$ (min)	1st Moment	$C_{out}(t)$ (abs)	$C'(t) =$	$A_C(t) =$	$\Delta t^* C'(t)$ (abs*min ²)	$C_{out}(t)/C_\Delta$ (min)	$E(t) =$		$E'(t) =$ $E_1 + (E_2 - E_1)/2$ (min ⁻¹)	$A_E(t) =$ $\Delta t^* E'(t)$ (min)	$t^* E(t) =$ $t^* E'(t)$ (min)	$A_{rE}(t) =$ $\Delta t^* t^* E'(t)$ (min)
	Time (min)		Time $t = t_1 + (t_2 - t_1)/2$ (min)		$C_1 + (C_2 - C_1)/2$ (abs)	$\Delta t^* C'(t)$ (abs*min)			$A_{C/C_\Delta}(t) =$ $\Delta t^* C'(t)/C_\Delta$ (min)	$(C_{out}(t)/C_\Delta)/$ A_{C/C_Δ} (min ⁻¹)				
14:29:55	89.78	1.00	89.28	0.826	0.830	0.830	74.061	0.508	0.510	4.01E-03	4.03E-03	4.03E-03	3.60E-01	3.60E-01
14:30:55	90.78	1.00	90.28	0.819	0.823	0.822	74.258	0.504	0.506	3.98E-03	3.99E-03	3.99E-03	3.61E-01	3.61E-01
14:31:55	91.78	1.00	91.28	0.812	0.816	0.816	74.442	0.499	0.501	3.94E-03	3.96E-03	3.96E-03	3.62E-01	3.62E-01
14:32:55	92.78	1.00	92.28	0.806	0.809	0.809	74.657	0.496	0.497	3.91E-03	3.93E-03	3.93E-03	3.63E-01	3.63E-01
14:33:55	93.78	1.00	93.28	0.799	0.803	0.802	74.860	0.491	0.493	3.88E-03	3.90E-03	3.90E-03	3.64E-01	3.64E-01
14:34:55	94.78	1.00	94.28	0.792	0.796	0.796	75.002	0.487	0.489	3.85E-03	3.86E-03	3.86E-03	3.64E-01	3.64E-01
14:35:56	95.80	1.02	95.29	0.786	0.789	0.802	76.438	0.483	0.493	3.82E-03	3.83E-03	3.90E-03	3.65E-01	3.71E-01
14:36:56	96.80	1.00	96.30	0.779	0.783	0.782	75.355	0.479	0.481	3.78E-03	3.80E-03	3.80E-03	3.66E-01	3.66E-01
14:37:56	97.80	1.00	97.30	0.773	0.776	0.776	75.505	0.475	0.477	3.75E-03	3.77E-03	3.77E-03	3.67E-01	3.67E-01
14:38:56	98.80	1.00	98.30	0.767	0.770	0.770	75.691	0.472	0.473	3.73E-03	3.74E-03	3.74E-03	3.68E-01	3.68E-01
14:39:56	99.80	1.00	99.30	0.761	0.764	0.764	75.865	0.468	0.470	3.70E-03	3.71E-03	3.71E-03	3.68E-01	3.68E-01
14:40:56	100.80	1.00	100.30	0.754	0.758	0.758	75.977	0.464	0.466	3.66E-03	3.68E-03	3.68E-03	3.69E-01	3.69E-01
14:41:56	101.80	1.00	101.30	0.748	0.751	0.751	76.076	0.460	0.462	3.63E-03	3.65E-03	3.65E-03	3.70E-01	3.70E-01
14:43:17	103.15	1.35	102.48	0.740	0.744	1.004	102.926	0.455	0.618	3.59E-03	3.61E-03	4.88E-03	3.70E-01	5.00E-01
14:53:14	113.10	9.95	108.13	0.682	0.711	7.074	764.925	0.419	4.349	3.31E-03	3.45E-03	3.44E-02	3.73E-01	3.72E+00
15:03:16	123.13	10.03	118.12	0.628	0.655	6.572	776.243	0.386	4.040	3.05E-03	3.18E-03	3.19E-02	3.76E-01	3.77E+00
15:13:14	133.10	9.97	128.12	0.579	0.604	6.015	770.607	0.356	3.698	2.81E-03	2.93E-03	2.92E-02	3.76E-01	3.74E+00
15:23:14	143.10	10.00	138.10	0.533	0.556	5.560	767.836	0.328	3.418	2.59E-03	2.70E-03	2.70E-02	3.73E-01	3.73E+00
15:33:14	153.10	10.00	148.10	0.491	0.512	5.120	758.272	0.302	3.148	2.38E-03	2.49E-03	2.49E-02	3.68E-01	3.68E+00
15:43:15	163.12	10.02	158.11	0.453	0.472	4.728	747.515	0.279	2.907	2.20E-03	2.29E-03	2.30E-02	3.62E-01	3.63E+00
15:53:14	173.10	9.98	168.11	0.417	0.435	4.343	730.052	0.256	2.670	2.03E-03	2.11E-03	2.11E-02	3.55E-01	3.55E+00
16:03:15	183.12	10.02	178.11	0.385	0.401	4.017	715.405	0.237	2.470	1.87E-03	1.95E-03	1.95E-02	3.47E-01	3.47E+00
16:13:15	193.12	10.00	188.12	0.354	0.370	3.695	695.091	0.218	2.272	1.72E-03	1.79E-03	1.79E-02	3.38E-01	3.38E+00
16:23:16	203.13	10.02	198.13	0.327	0.341	3.411	675.740	0.201	2.097	1.59E-03	1.65E-03	1.66E-02	3.28E-01	3.28E+00
16:33:16	213.13	10.00	208.13	0.301	0.314	3.140	653.539	0.185	1.931	1.46E-03	1.53E-03	1.53E-02	3.17E-01	3.17E+00
16:43:15	223.12	9.98	218.13	0.278	0.290	2.890	630.419	0.171	1.777	1.35E-03	1.41E-03	1.40E-02	3.07E-01	3.06E+00
16:53:15	233.12	10.00	228.12	0.256	0.267	2.670	609.071	0.157	1.642	1.24E-03	1.30E-03	1.30E-02	2.96E-01	2.96E+00
17:03:17	243.15	10.03	238.13	0.236	0.246	2.468	587.761	0.145	1.517	1.15E-03	1.19E-03	1.20E-02	2.85E-01	2.85E+00
17:13:15	253.12	9.97	248.13	0.218	0.227	2.262	561.385	0.134	1.391	1.06E-03	1.10E-03	1.10E-02	2.74E-01	2.73E+00
17:23:18	263.17	10.05	258.14	0.201	0.210	2.105	543.511	0.124	1.294	9.76E-04	1.02E-03	1.02E-02	2.63E-01	2.64E+00
17:33:17	273.15	9.98	268.16	0.185	0.193	1.927	516.683	0.114	1.185	8.99E-04	9.37E-04	9.36E-03	2.51E-01	2.51E+00

Table 27. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #3, $\omega = 150$ rpm). *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/C_A}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time	$\Delta t=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$\Delta t^*C'(t)$	$\Delta t^{*2}C'(t)$	$C_{out}(t)/C_A$	$\Delta t^*C'(t)/C_A$	A_{C/C_A}	$E_1+(E_2-E_1)/2$	$A_E(t)=$	$t^*E(t)=$	$\Delta t^{*2}E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)	(min)	(min)	(min ⁻¹)	(min ⁻¹)	$\Delta t^*E'(t)$	$t^*E'(t)$	(min)
17:43:16	283.13	9.98	278.14	0.171	0.178	1.777	494.267	0.105	1.093	8.31E-04	8.65E-04	8.63E-03	2.40E-01	2.40E+00
17:53:17	293.15	10.02	288.14	0.158	0.165	1.648	474.783	0.097	1.013	7.67E-04	7.99E-04	8.00E-03	2.30E-01	2.31E+00
18:03:17	303.15	10.00	298.15	0.145	0.152	1.515	451.697	0.089	0.931	7.04E-04	7.36E-04	7.36E-03	2.19E-01	2.19E+00
18:13:17	313.15	10.00	308.15	0.134	0.140	1.395	429.869	0.082	0.858	6.51E-04	6.78E-04	6.78E-03	2.09E-01	2.09E+00
18:23:19	323.18	10.03	318.17	0.124	0.129	1.294	411.803	0.076	0.796	6.02E-04	6.27E-04	6.29E-03	1.99E-01	2.00E+00
18:33:18	333.17	9.98	328.18	0.114	0.119	1.188	389.877	0.070	0.730	5.54E-04	5.78E-04	5.77E-03	1.90E-01	1.89E+00
18:43:17	343.15	9.98	338.16	0.106	0.110	1.098	371.354	0.065	0.675	5.15E-04	5.34E-04	5.33E-03	1.81E-01	1.80E+00
18:53:18	353.17	10.02	348.16	0.098	0.102	1.022	355.713	0.060	0.628	4.76E-04	4.95E-04	4.96E-03	1.72E-01	1.73E+00
18:56:36	356.47	3.30	354.82	0.095	0.097	0.318	112.991	0.058	0.196	4.61E-04	4.69E-04	1.55E-03	1.66E-01	5.49E-01
19:16:38	376.50	20.03	366.48	0.081	0.088	1.763	646.086	0.050	1.084	3.93E-04	4.27E-04	8.56E-03	1.57E-01	3.14E+00
19:36:36	396.47	19.97	386.48	0.070	0.076	1.507	582.617	0.043	0.927	3.40E-04	3.67E-04	7.32E-03	1.42E-01	2.83E+00
19:56:36	416.47	20.00	406.47	0.060	0.065	1.300	528.407	0.037	0.799	2.91E-04	3.16E-04	6.31E-03	1.28E-01	2.57E+00
20:16:36	436.47	20.00	426.47	0.051	0.056	1.110	473.378	0.031	0.682	2.48E-04	2.70E-04	5.39E-03	1.15E-01	2.30E+00
20:36:37	456.48	20.02	446.48	0.044	0.048	0.951	424.505	0.027	0.585	2.14E-04	2.31E-04	4.62E-03	1.03E-01	2.06E+00
20:56:37	476.48	20.00	466.48	0.038	0.041	0.820	382.516	0.023	0.504	1.85E-04	1.99E-04	3.98E-03	9.29E-02	1.86E+00
21:16:37	496.48	20.00	486.48	0.033	0.036	0.710	345.403	0.020	0.437	1.60E-04	1.72E-04	3.45E-03	8.39E-02	1.68E+00
21:36:39	516.52	20.03	506.50	0.029	0.031	0.621	314.553	0.018	0.382	1.41E-04	1.51E-04	3.02E-03	7.63E-02	1.53E+00
21:56:40	536.53	20.02	526.53	0.025	0.027	0.540	284.560	0.015	0.332	1.21E-04	1.31E-04	2.62E-03	6.90E-02	1.38E+00
22:16:38	556.50	19.97	546.52	0.022	0.024	0.469	256.435	0.014	0.288	1.07E-04	1.14E-04	2.28E-03	6.24E-02	1.25E+00
22:36:40	576.53	20.03	566.52	0.019	0.021	0.411	232.659	0.012	0.252	9.23E-05	9.96E-05	1.99E-03	5.64E-02	1.13E+00
22:56:40	596.53	20.00	586.53	0.017	0.018	0.360	211.152	0.010	0.221	8.26E-05	8.74E-05	1.75E-03	5.13E-02	1.03E+00
23:16:40	616.53	20.00	606.53	0.015	0.016	0.320	194.091	0.009	0.197	7.29E-05	7.77E-05	1.55E-03	4.71E-02	9.43E-01
23:36:40	636.53	20.00	626.53	0.013	0.014	0.280	175.429	0.008	0.172	6.31E-05	6.80E-05	1.36E-03	4.26E-02	8.52E-01
23:56:40	656.53	20.00	646.53	0.012	0.013	0.250	161.633	0.007	0.154	5.83E-05	6.07E-05	1.21E-03	3.93E-02	7.85E-01
0:16:40	676.53	20.00	666.53	0.011	0.012	0.230	153.303	0.007	0.141	5.34E-05	5.59E-05	1.12E-03	3.72E-02	7.45E-01

Table 28. Computational analysis for a bottom fed non-reactive tracer (Tracer #3, $\omega = 150$ rpm).

$\text{Area under } C \text{ curve } (A_C) = \Sigma [\Delta t^* C'(t)]$	205.89	min*abs
$\text{Area under } t^* A_C \text{ curve} = \Sigma [t^* \Delta t^* C'(t)]$	25667.54	min ² *abs
$t_{m-C \text{ curve}} = \Sigma [\Delta t^* t^* C'(t)] / (\Sigma [\Delta t^* C'(t)])$	124.67	min
Q	7.9	mL/min
$M_T = Q * A_C$	1626.51	mL*abs
V_R	1000	mL
$V_{R-\text{calculated}} = t_m * Q$	985	mL
$C_\Delta = M_T / V_R$	1.627	abs
$\text{Area under normalized } C \text{ curve } (A_{C/C_\Delta}) = \Sigma [\Delta t^* C'(t) / C_\Delta]$	126.58	min
$\text{Area under } E \text{ curve } (A_E) = \Sigma [\Delta t^* E'(t)]$	1.00	
$\text{Area under } t^* E \text{ curve } (A_{t^* E}) = \Sigma [\Delta t^* t^* E'(t)]$	124.67	min
$t_{m-E \text{ curve}} = \Sigma [\Delta t^* t^* E'(t)] / (\Sigma [\Delta t^* E'(t)])$	124.67	min

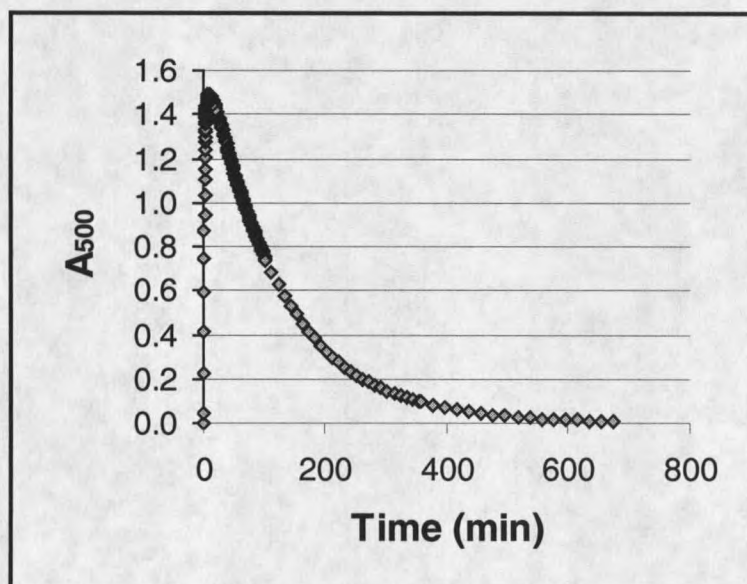


Figure 28. Effluent tracer concentration profile for Tracer #3 ($\omega = 150$ rpm). A 1-mL pulse injection of red food coloring with absorbance levels recorded at $\lambda = 500$ nm.

Table 29. Linear regression summary for the natural logarithm transformation of the concentration versus time data (Tracer #3, $\omega = 150$ rpm).

Regression Statistics	
Multiple R	0.99948
R Square	0.99896
Adjusted R Square	0.99895
Standard Error	0.03852
Observations	184

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	258.640	258.640	174296.069	0.000
Residual	182	0.27007	0.00148		
Total	183	258.910			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.50252	0.00352	142.576	0.000	0.49557	0.50948	0.49557	0.50948
X Variable 1	-0.46743	0.00112	-417.488	0.000	-0.46964	-0.46522	-0.46964	-0.46522

Table 30. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm)

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$E(t)=$		$E'(t)=$		$A_{rE}(t)=$	
	Time	$dt=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$dt^*C'(t)$	$dt^*t^*C'(t)$	$C_{out}(t)/C_A$	$A_{C/C_A}(t)=$	$(C_{out}(t)/C_A)/$	$E_1+(E_2-E_1)/2$	$A_E(t)=$	$t^*E(t)=$	$dt^*t^*E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)	(min)	(min ⁻¹)	(min ⁻¹)	(min ⁻¹)	$dt^*E'(t)$	$t^*E'(t)$	(min)
0:50:12	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
0:50:21	0.15	0.15	0.08	0.000	0.000	0.000	0.000	0.000	0.000	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
0:50:31	0.32	0.17	0.23	0.000	0.000	0.000	0.000	0.000	0.000	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
0:50:41	0.48	0.17	0.40	0.004	0.002	0.000	0.000	0.002	0.000	1.94E-05	9.71E-06	1.62E-06	3.89E-06	6.48E-07
0:50:51	0.65	0.17	0.57	0.070	0.037	0.006	0.003	0.043	0.004	3.40E-04	1.80E-04	3.00E-05	1.02E-04	1.70E-05
0:51:01	0.82	0.17	0.73	0.199	0.135	0.022	0.016	0.122	0.014	9.67E-04	6.53E-04	1.09E-04	4.79E-04	7.99E-05
0:51:11	0.98	0.17	0.90	0.366	0.283	0.047	0.042	0.225	0.029	1.78E-03	1.37E-03	2.29E-04	1.24E-03	2.06E-04
0:51:21	1.15	0.17	1.07	0.523	0.445	0.074	0.079	0.322	0.046	2.54E-03	2.16E-03	3.60E-04	2.30E-03	3.84E-04
0:51:31	1.32	0.17	1.23	0.635	0.579	0.096	0.119	0.390	0.059	3.08E-03	2.81E-03	4.69E-04	3.47E-03	5.78E-04
0:51:41	1.48	0.17	1.40	0.773	0.704	0.117	0.164	0.475	0.072	3.75E-03	3.42E-03	5.70E-04	4.79E-03	7.98E-04
0:51:52	1.67	0.18	1.58	0.867	0.820	0.150	0.237	0.533	0.092	4.21E-03	3.98E-03	7.30E-04	6.27E-03	1.15E-03
0:52:01	1.82	0.15	1.74	0.959	0.913	0.137	0.239	0.590	0.084	4.66E-03	4.43E-03	6.65E-04	7.72E-03	1.16E-03
0:52:11	1.98	0.17	1.90	1.045	1.002	0.167	0.317	0.643	0.103	5.08E-03	4.87E-03	8.11E-04	9.25E-03	1.54E-03
0:52:22	2.17	0.18	2.07	1.115	1.080	0.198	0.411	0.686	0.122	5.42E-03	5.25E-03	9.62E-04	1.09E-02	2.00E-03
0:52:31	2.32	0.15	2.24	1.173	1.144	0.172	0.385	0.721	0.106	5.70E-03	5.56E-03	8.34E-04	1.25E-02	1.87E-03
0:52:42	2.50	0.18	2.41	1.202	1.188	0.218	0.524	0.739	0.134	5.84E-03	5.77E-03	1.06E-03	1.39E-02	2.55E-03
0:52:51	2.65	0.15	2.58	1.235	1.219	0.183	0.471	0.759	0.112	6.00E-03	5.92E-03	8.88E-04	1.52E-02	2.29E-03
0:53:02	2.83	0.18	2.74	1.265	1.250	0.229	0.628	0.778	0.141	6.14E-03	6.07E-03	1.11E-03	1.66E-02	3.05E-03
0:53:12	3.00	0.17	2.92	1.289	1.277	0.213	0.621	0.793	0.131	6.26E-03	6.20E-03	1.03E-03	1.81E-02	3.02E-03
0:53:22	3.17	0.17	3.08	1.318	1.304	0.217	0.670	0.810	0.134	6.40E-03	6.33E-03	1.06E-03	1.95E-02	3.25E-03
0:53:32	3.33	0.17	3.25	1.338	1.328	0.221	0.719	0.823	0.136	6.50E-03	6.45E-03	1.08E-03	2.10E-02	3.49E-03
0:53:42	3.50	0.17	3.42	1.351	1.345	0.224	0.766	0.831	0.138	6.56E-03	6.53E-03	1.09E-03	2.23E-02	3.72E-03
0:53:52	3.67	0.17	3.58	1.366	1.359	0.226	0.811	0.840	0.139	6.64E-03	6.60E-03	1.10E-03	2.36E-02	3.94E-03
0:54:02	3.83	0.17	3.75	1.377	1.372	0.229	0.857	0.847	0.141	6.69E-03	6.66E-03	1.11E-03	2.50E-02	4.16E-03
0:54:12	4.00	0.17	3.92	1.386	1.382	0.230	0.902	0.852	0.142	6.73E-03	6.71E-03	1.12E-03	2.63E-02	4.38E-03
0:54:22	4.17	0.17	4.08	1.398	1.392	0.232	0.947	0.860	0.143	6.79E-03	6.76E-03	1.13E-03	2.76E-02	4.60E-03
0:54:32	4.33	0.17	4.25	1.407	1.403	0.234	0.993	0.865	0.144	6.83E-03	6.81E-03	1.14E-03	2.90E-02	4.83E-03
0:54:42	4.50	0.17	4.42	1.416	1.412	0.235	1.039	0.871	0.145	6.88E-03	6.86E-03	1.14E-03	3.03E-02	5.05E-03
0:54:52	4.67	0.17	4.58	1.423	1.420	0.237	1.084	0.875	0.145	6.91E-03	6.90E-03	1.15E-03	3.16E-02	5.27E-03
0:55:02	4.83	0.17	4.75	1.428	1.426	0.238	1.129	0.878	0.146	6.94E-03	6.92E-03	1.15E-03	3.29E-02	5.48E-03
0:55:12	5.00	0.17	4.92	1.435	1.432	0.239	1.173	0.882	0.147	6.97E-03	6.95E-03	1.16E-03	3.42E-02	5.70E-03

Table 30. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm) *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/CA}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time (min)	$dt=t_2-t_1$ (min)	Time $t=t_1+(t_2-t_1)/2$	$C_{out}(t)$ (abs)	$C_1+(C_2-C_1)/2$ (abs)	$dt^*C'(t)$ (abs*min)	$dt^{**}C'(t)$ (abs*min ²)	$C_{out}(t)/C_A$	$dt^*C'(t)/C_A$ (min)	$A_{C/CA}$ (min ⁻¹)	$E_1+(E_2-E_1)/2$ (min ⁻¹)	$A_E(t)=$ $dt^*E'(t)$	$t^*E(t)=$ $t^*E'(t)$	$dt^{**}E'(t)$ (min)
0:55:22	5.17	0.17	5.08	1.439	1.437	0.239	1.217	0.885	0.147	6.99E-03	6.98E-03	1.16E-03	3.55E-02	5.91E-03
0:55:32	5.33	0.17	5.25	1.443	1.441	0.240	1.261	0.887	0.148	7.01E-03	7.00E-03	1.17E-03	3.67E-02	6.12E-03
0:55:42	5.50	0.17	5.42	1.449	1.446	0.241	1.305	0.891	0.148	7.04E-03	7.02E-03	1.17E-03	3.80E-02	6.34E-03
0:55:52	5.67	0.17	5.58	1.452	1.451	0.242	1.350	0.893	0.149	7.05E-03	7.05E-03	1.17E-03	3.93E-02	6.56E-03
0:56:02	5.83	0.17	5.75	1.455	1.454	0.242	1.393	0.895	0.149	7.07E-03	7.06E-03	1.18E-03	4.06E-02	6.77E-03
0:56:13	6.02	0.18	5.93	1.459	1.457	0.267	1.583	0.897	0.164	7.09E-03	7.08E-03	1.30E-03	4.19E-02	7.69E-03
0:56:22	6.17	0.15	6.09	1.461	1.460	0.219	1.334	0.898	0.135	7.10E-03	7.09E-03	1.06E-03	4.32E-02	6.48E-03
0:56:32	6.33	0.17	6.25	1.463	1.462	0.244	1.523	0.900	0.150	7.11E-03	7.10E-03	1.18E-03	4.44E-02	7.40E-03
0:56:42	6.50	0.17	6.42	1.465	1.464	0.244	1.566	0.901	0.150	7.12E-03	7.11E-03	1.19E-03	4.56E-02	7.61E-03
0:56:53	6.68	0.18	6.59	1.468	1.467	0.269	1.772	0.903	0.165	7.13E-03	7.12E-03	1.31E-03	4.70E-02	8.61E-03
0:57:03	6.85	0.17	6.77	1.468	1.468	0.245	1.656	0.903	0.150	7.13E-03	7.13E-03	1.19E-03	4.83E-02	8.04E-03
0:57:12	7.00	0.15	6.92	1.469	1.469	0.220	1.525	0.903	0.135	7.14E-03	7.13E-03	1.07E-03	4.94E-02	7.41E-03
0:57:23	7.18	0.18	7.09	1.470	1.470	0.269	1.911	0.904	0.166	7.14E-03	7.14E-03	1.31E-03	5.06E-02	9.28E-03
0:57:33	7.35	0.17	7.27	1.471	1.471	0.245	1.781	0.904	0.151	7.15E-03	7.14E-03	1.19E-03	5.19E-02	8.65E-03
0:57:43	7.52	0.17	7.43	1.472	1.472	0.245	1.823	0.905	0.151	7.15E-03	7.15E-03	1.19E-03	5.31E-02	8.86E-03
0:57:53	7.68	0.17	7.60	1.474	1.473	0.245	1.866	0.906	0.151	7.16E-03	7.15E-03	1.19E-03	5.44E-02	9.06E-03
0:58:03	7.85	0.17	7.77	1.474	1.474	0.246	1.908	0.906	0.151	7.16E-03	7.16E-03	1.19E-03	5.56E-02	9.27E-03
0:58:13	8.02	0.17	7.93	1.474	1.474	0.246	1.949	0.906	0.151	7.16E-03	7.16E-03	1.19E-03	5.68E-02	9.47E-03
0:58:23	8.18	0.17	8.10	1.474	1.474	0.246	1.990	0.906	0.151	7.16E-03	7.16E-03	1.19E-03	5.80E-02	9.67E-03
0:58:33	8.35	0.17	8.27	1.475	1.475	0.246	2.032	0.907	0.151	7.16E-03	7.16E-03	1.19E-03	5.92E-02	9.87E-03
0:58:43	8.52	0.17	8.43	1.475	1.475	0.246	2.073	0.907	0.151	7.16E-03	7.16E-03	1.19E-03	6.04E-02	1.01E-02
0:58:53	8.68	0.17	8.60	1.475	1.475	0.246	2.114	0.907	0.151	7.16E-03	7.16E-03	1.19E-03	6.16E-02	1.03E-02
0:59:03	8.85	0.17	8.77	1.475	1.475	0.246	2.155	0.907	0.151	7.16E-03	7.16E-03	1.19E-03	6.28E-02	1.05E-02
0:59:13	9.02	0.17	8.93	1.475	1.475	0.246	2.196	0.907	0.151	7.16E-03	7.16E-03	1.19E-03	6.40E-02	1.07E-02
0:59:23	9.18	0.17	9.10	1.475	1.475	0.246	2.237	0.907	0.151	7.16E-03	7.16E-03	1.19E-03	6.52E-02	1.09E-02
0:59:33	9.35	0.17	9.27	1.474	1.475	0.246	2.277	0.906	0.151	7.16E-03	7.16E-03	1.19E-03	6.64E-02	1.11E-02
0:59:43	9.52	0.17	9.43	1.474	1.474	0.246	2.317	0.906	0.151	7.16E-03	7.16E-03	1.19E-03	6.75E-02	1.13E-02
0:59:53	9.68	0.17	9.60	1.473	1.474	0.246	2.358	0.906	0.151	7.15E-03	7.16E-03	1.19E-03	6.87E-02	1.15E-02
1:00:03	9.85	0.17	9.77	1.473	1.473	0.245	2.398	0.906	0.151	7.15E-03	7.15E-03	1.19E-03	6.99E-02	1.16E-02
1:00:13	10.02	0.17	9.93	1.473	1.473	0.246	2.439	0.906	0.151	7.15E-03	7.15E-03	1.19E-03	7.11E-02	1.18E-02
1:00:23	10.18	0.17	10.10	1.472	1.473	0.245	2.479	0.905	0.151	7.15E-03	7.15E-03	1.19E-03	7.22E-02	1.20E-02

Table 30. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm) *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/\Delta}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time	$dt=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$dt^*C'(t)$	$dt^*t^*C'(t)$	$C_{out}(t)/C_\Delta$	$dt^*C'(t)/C_\Delta$	$(C_{out}(t)/C_\Delta)/$	$E_1+(E_2-E_1)/2$	$A_E(t)=$	$t^*E(t)=$	$dt^*t^*E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)		(min)	(min ⁻¹)	(min ⁻¹)	$dt^*E'(t)$	$t^*E'(t)$	(min)
1:00:32	10.33	0.15	10.26	1.471	1.472	0.221	2.264	0.904	0.136	7.15E-03	7.15E-03	1.07E-03	7.33E-02	1.10E-02
1:00:43	10.52	0.18	10.43	1.471	1.471	0.270	2.811	0.904	0.166	7.15E-03	7.15E-03	1.31E-03	7.45E-02	1.37E-02
1:00:54	10.70	0.18	10.61	1.470	1.471	0.270	2.860	0.904	0.166	7.14E-03	7.14E-03	1.31E-03	7.58E-02	1.39E-02
1:01:04	10.87	0.17	10.78	1.469	1.470	0.245	2.641	0.903	0.151	7.14E-03	7.14E-03	1.19E-03	7.70E-02	1.28E-02
1:01:14	11.03	0.17	10.95	1.469	1.469	0.245	2.681	0.903	0.151	7.14E-03	7.14E-03	1.19E-03	7.81E-02	1.30E-02
1:01:24	11.20	0.17	11.12	1.468	1.469	0.245	2.721	0.903	0.150	7.13E-03	7.13E-03	1.19E-03	7.93E-02	1.32E-02
1:01:34	11.37	0.17	11.28	1.467	1.468	0.245	2.760	0.902	0.150	7.13E-03	7.13E-03	1.19E-03	8.04E-02	1.34E-02
1:01:44	11.53	0.17	11.45	1.466	1.467	0.244	2.799	0.901	0.150	7.12E-03	7.12E-03	1.19E-03	8.16E-02	1.36E-02
1:01:54	11.70	0.17	11.62	1.465	1.466	0.244	2.837	0.901	0.150	7.12E-03	7.12E-03	1.19E-03	8.27E-02	1.38E-02
1:02:04	11.87	0.17	11.78	1.465	1.465	0.244	2.877	0.901	0.150	7.12E-03	7.12E-03	1.19E-03	8.39E-02	1.40E-02
1:02:14	12.03	0.17	11.95	1.465	1.465	0.244	2.918	0.901	0.150	7.12E-03	7.12E-03	1.19E-03	8.50E-02	1.42E-02
1:02:24	12.20	0.17	12.12	1.463	1.464	0.244	2.956	0.900	0.150	7.11E-03	7.11E-03	1.19E-03	8.62E-02	1.44E-02
1:02:34	12.37	0.17	12.28	1.463	1.463	0.244	2.995	0.900	0.150	7.11E-03	7.11E-03	1.18E-03	8.73E-02	1.45E-02
1:02:44	12.53	0.17	12.45	1.461	1.462	0.244	3.034	0.898	0.150	7.10E-03	7.10E-03	1.18E-03	8.84E-02	1.47E-02
1:02:54	12.70	0.17	12.62	1.461	1.461	0.244	3.072	0.898	0.150	7.10E-03	7.10E-03	1.18E-03	8.95E-02	1.49E-02
1:03:04	12.87	0.17	12.78	1.460	1.461	0.243	3.112	0.898	0.150	7.09E-03	7.09E-03	1.18E-03	9.07E-02	1.51E-02
1:03:14	13.03	0.17	12.95	1.459	1.460	0.243	3.150	0.897	0.150	7.09E-03	7.09E-03	1.18E-03	9.18E-02	1.53E-02
1:03:24	13.20	0.17	13.12	1.458	1.459	0.243	3.188	0.896	0.149	7.08E-03	7.08E-03	1.18E-03	9.29E-02	1.55E-02
1:03:34	13.37	0.17	13.28	1.456	1.457	0.243	3.226	0.895	0.149	7.07E-03	7.08E-03	1.18E-03	9.40E-02	1.57E-02
1:03:44	13.53	0.17	13.45	1.455	1.456	0.243	3.263	0.895	0.149	7.07E-03	7.07E-03	1.18E-03	9.51E-02	1.58E-02
1:03:54	13.70	0.17	13.62	1.454	1.455	0.242	3.301	0.894	0.149	7.06E-03	7.07E-03	1.18E-03	9.62E-02	1.60E-02
1:04:04	13.87	0.17	13.78	1.453	1.454	0.242	3.339	0.893	0.149	7.06E-03	7.06E-03	1.18E-03	9.73E-02	1.62E-02
1:04:14	14.03	0.17	13.95	1.452	1.453	0.242	3.377	0.893	0.149	7.05E-03	7.06E-03	1.18E-03	9.84E-02	1.64E-02
1:04:24	14.20	0.17	14.12	1.452	1.452	0.242	3.416	0.893	0.149	7.05E-03	7.05E-03	1.18E-03	9.96E-02	1.66E-02
1:04:34	14.37	0.17	14.28	1.450	1.451	0.242	3.454	0.892	0.149	7.04E-03	7.05E-03	1.17E-03	1.01E-01	1.68E-02
1:04:45	14.55	0.18	14.46	1.449	1.450	0.266	3.842	0.891	0.163	7.04E-03	7.04E-03	1.29E-03	1.02E-01	1.87E-02
1:04:55	14.72	0.17	14.63	1.448	1.449	0.241	3.533	0.890	0.148	7.03E-03	7.04E-03	1.17E-03	1.03E-01	1.72E-02
1:05:04	14.87	0.15	14.79	1.446	1.447	0.217	3.211	0.889	0.133	7.02E-03	7.03E-03	1.05E-03	1.04E-01	1.56E-02
1:05:14	15.03	0.17	14.95	1.445	1.446	0.241	3.602	0.888	0.148	7.02E-03	7.02E-03	1.17E-03	1.05E-01	1.75E-02
1:05:25	15.22	0.18	15.13	1.444	1.445	0.265	4.005	0.888	0.163	7.01E-03	7.02E-03	1.29E-03	1.06E-01	1.95E-02
1:05:35	15.38	0.17	15.30	1.443	1.444	0.241	3.681	0.887	0.148	7.01E-03	7.01E-03	1.17E-03	1.07E-01	1.79E-02

Table 30. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm) *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/C_A}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time (min)	$dt=t_2-t_1$ (min)	Time $t=t_1+(t_2-t_1)/2$	$C_{out}(t)$ (abs)	$C_1+(C_2-C_1)/2$ (abs)	$dt^*C'(t)$ (abs*min)	$dt^*t^*C'(t)$ (abs*min ²)	$C_{out}(t)/C_A$	$dt^*C'(t)/C_A$ (min)	$(C_{out}(t)/C_A)/$ A_{C/C_A} (min ⁻¹)	$E_1+(E_2-E_1)/2$ (min ⁻¹)	$A_E(t)=$ $dt^*E'(t)$	$t^*E(t)=$ $t^*E'(t)$	$dt^*t^*E'(t)$ (min)
1:05:46	15.57	0.18	15.48	1.442	1.443	0.264	4.092	0.887	0.163	7.00E-03	7.01E-03	1.28E-03	1.08E-01	1.99E-02
1:05:55	15.72	0.15	15.64	1.440	1.441	0.216	3.381	0.885	0.133	6.99E-03	7.00E-03	1.05E-03	1.09E-01	1.64E-02
1:06:05	15.88	0.17	15.80	1.439	1.440	0.240	3.791	0.885	0.148	6.99E-03	6.99E-03	1.17E-03	1.10E-01	1.84E-02
1:06:15	16.05	0.17	15.97	1.438	1.439	0.240	3.828	0.884	0.147	6.98E-03	6.99E-03	1.16E-03	1.12E-01	1.86E-02
1:06:25	16.22	0.17	16.13	1.437	1.438	0.240	3.865	0.884	0.147	6.98E-03	6.98E-03	1.16E-03	1.13E-01	1.88E-02
1:06:35	16.38	0.17	16.30	1.435	1.436	0.239	3.901	0.882	0.147	6.97E-03	6.98E-03	1.16E-03	1.14E-01	1.89E-02
1:06:45	16.55	0.17	16.47	1.434	1.435	0.239	3.937	0.882	0.147	6.97E-03	6.97E-03	1.16E-03	1.15E-01	1.91E-02
1:06:55	16.72	0.17	16.63	1.433	1.434	0.239	3.974	0.881	0.147	6.96E-03	6.96E-03	1.16E-03	1.16E-01	1.93E-02
1:07:05	16.88	0.17	16.80	1.431	1.432	0.239	4.010	0.880	0.147	6.95E-03	6.96E-03	1.16E-03	1.17E-01	1.95E-02
1:07:15	17.05	0.17	16.97	1.430	1.431	0.238	4.045	0.879	0.147	6.95E-03	6.95E-03	1.16E-03	1.18E-01	1.96E-02
1:07:25	17.22	0.17	17.13	1.429	1.430	0.238	4.082	0.879	0.146	6.94E-03	6.94E-03	1.16E-03	1.19E-01	1.98E-02
1:07:35	17.38	0.17	17.30	1.428	1.429	0.238	4.119	0.878	0.146	6.94E-03	6.94E-03	1.16E-03	1.20E-01	2.00E-02
1:07:45	17.55	0.17	17.47	1.427	1.428	0.238	4.156	0.877	0.146	6.93E-03	6.93E-03	1.16E-03	1.21E-01	2.02E-02
1:07:55	17.72	0.17	17.63	1.425	1.426	0.238	4.191	0.876	0.146	6.92E-03	6.93E-03	1.15E-03	1.22E-01	2.04E-02
1:08:05	17.88	0.17	17.80	1.423	1.424	0.237	4.225	0.875	0.146	6.91E-03	6.92E-03	1.15E-03	1.23E-01	2.05E-02
1:08:15	18.05	0.17	17.97	1.422	1.423	0.237	4.260	0.874	0.146	6.91E-03	6.91E-03	1.15E-03	1.24E-01	2.07E-02
1:08:25	18.22	0.17	18.13	1.421	1.422	0.237	4.296	0.874	0.146	6.90E-03	6.90E-03	1.15E-03	1.25E-01	2.09E-02
1:08:35	18.38	0.17	18.30	1.420	1.421	0.237	4.333	0.873	0.146	6.90E-03	6.90E-03	1.15E-03	1.26E-01	2.10E-02
1:08:45	18.55	0.17	18.47	1.418	1.419	0.237	4.367	0.872	0.145	6.89E-03	6.89E-03	1.15E-03	1.27E-01	2.12E-02
1:08:55	18.72	0.17	18.63	1.417	1.418	0.236	4.402	0.871	0.145	6.88E-03	6.89E-03	1.15E-03	1.28E-01	2.14E-02
1:09:06	18.90	0.18	18.81	1.415	1.416	0.260	4.883	0.870	0.160	6.87E-03	6.88E-03	1.26E-03	1.29E-01	2.37E-02
1:09:15	19.05	0.15	18.98	1.414	1.415	0.212	4.026	0.869	0.130	6.87E-03	6.87E-03	1.03E-03	1.30E-01	1.96E-02
1:09:26	19.23	0.18	19.14	1.412	1.413	0.259	4.959	0.868	0.159	6.86E-03	6.86E-03	1.26E-03	1.31E-01	2.41E-02
1:09:35	19.38	0.15	19.31	1.411	1.412	0.212	4.088	0.868	0.130	6.85E-03	6.86E-03	1.03E-03	1.32E-01	1.99E-02
1:09:46	19.57	0.18	19.48	1.410	1.411	0.259	5.036	0.867	0.159	6.85E-03	6.85E-03	1.26E-03	1.33E-01	2.45E-02
1:09:56	19.73	0.17	19.65	1.408	1.409	0.235	4.614	0.866	0.144	6.84E-03	6.84E-03	1.14E-03	1.34E-01	2.24E-02
1:10:06	19.90	0.17	19.82	1.407	1.408	0.235	4.649	0.865	0.144	6.83E-03	6.84E-03	1.14E-03	1.35E-01	2.26E-02
1:10:16	20.07	0.17	19.98	1.406	1.407	0.234	4.684	0.864	0.144	6.83E-03	6.83E-03	1.14E-03	1.37E-01	2.28E-02
1:10:26	20.23	0.17	20.15	1.404	1.405	0.234	4.718	0.863	0.144	6.82E-03	6.82E-03	1.14E-03	1.38E-01	2.29E-02
1:10:36	20.40	0.17	20.32	1.402	1.403	0.234	4.751	0.862	0.144	6.81E-03	6.81E-03	1.14E-03	1.38E-01	2.31E-02
1:10:46	20.57	0.17	20.48	1.402	1.402	0.234	4.786	0.862	0.144	6.81E-03	6.81E-03	1.14E-03	1.39E-01	2.32E-02

Table 30. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm) *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/C_A}(t)=$		$E(t)=$		$A_{E(t)}=$	
	Time	$dt=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$dt^*C'(t)$	$dt^*C'(t)$	$C_{out}(t)/C_A$	$dt^*C'(t)/C_A$	$(C_{out}(t)/C_A)/$	$E'(t)=$	$E_1+(E_2-E_1)/2$	$A_E(t)=$	$t^*E(t)=$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)		(min)	A_{C/C_A}	(min ⁻¹)	(min ⁻¹)	$dt^*E'(t)$	$t^*E'(t)$
1:10:56	20.73	0.17	20.65	1.400	1.401	0.233	4.822	0.861	0.144	6.80E-03	6.81E-03	1.13E-03	1.41E-01	2.34E-02
1:11:06	20.90	0.17	20.82	1.398	1.399	0.233	4.854	0.860	0.143	6.79E-03	6.80E-03	1.13E-03	1.41E-01	2.36E-02
1:11:16	21.07	0.17	20.98	1.397	1.398	0.233	4.887	0.859	0.143	6.79E-03	6.79E-03	1.13E-03	1.42E-01	2.37E-02
1:11:26	21.23	0.17	21.15	1.396	1.397	0.233	4.923	0.858	0.143	6.78E-03	6.78E-03	1.13E-03	1.43E-01	2.39E-02
1:11:36	21.40	0.17	21.32	1.394	1.395	0.233	4.956	0.857	0.143	6.77E-03	6.78E-03	1.13E-03	1.44E-01	2.41E-02
1:11:46	21.57	0.17	21.48	1.393	1.394	0.232	4.990	0.857	0.143	6.77E-03	6.77E-03	1.13E-03	1.45E-01	2.42E-02
1:11:56	21.73	0.17	21.65	1.392	1.393	0.232	5.025	0.856	0.143	6.76E-03	6.76E-03	1.13E-03	1.46E-01	2.44E-02
1:12:06	21.90	0.17	21.82	1.390	1.391	0.232	5.058	0.855	0.143	6.75E-03	6.76E-03	1.13E-03	1.47E-01	2.46E-02
1:12:16	22.07	0.17	21.98	1.389	1.390	0.232	5.091	0.854	0.142	6.75E-03	6.75E-03	1.12E-03	1.48E-01	2.47E-02
1:12:31	22.32	0.25	22.19	1.386	1.388	0.347	7.698	0.852	0.213	6.73E-03	6.74E-03	1.68E-03	1.50E-01	3.74E-02
1:22:31	32.32	10.00	27.32	1.294	1.340	13.400	366.043	0.796	8.239	6.29E-03	6.51E-03	6.51E-02	1.78E-01	1.78E+00
1:32:31	42.32	10.00	37.32	1.195	1.245	12.445	464.406	0.735	7.652	5.80E-03	6.05E-03	6.05E-02	2.26E-01	2.26E+00
1:42:32	52.33	10.02	47.33	1.101	1.148	11.499	544.196	0.677	7.070	5.35E-03	5.58E-03	5.59E-02	2.64E-01	2.64E+00
1:52:31	62.32	9.98	57.33	1.016	1.059	10.567	605.774	0.625	6.497	4.94E-03	5.14E-03	5.13E-02	2.95E-01	2.94E+00
2:02:31	72.32	10.00	67.32	0.937	0.977	9.765	657.347	0.576	6.004	4.55E-03	4.74E-03	4.74E-02	3.19E-01	3.19E+00
2:05:49	75.62	3.30	73.97	0.912	0.925	3.051	225.661	0.561	1.876	4.43E-03	4.49E-03	1.48E-02	3.32E-01	1.10E+00
2:06:50	76.63	1.02	76.13	0.904	0.908	0.923	70.274	0.556	0.568	4.39E-03	4.41E-03	4.48E-03	3.36E-01	3.41E-01
2:07:50	77.63	1.00	77.13	0.897	0.901	0.901	69.459	0.552	0.554	4.36E-03	4.37E-03	4.37E-03	3.37E-01	3.37E-01
2:08:50	78.63	1.00	78.13	0.890	0.894	0.893	69.812	0.547	0.549	4.32E-03	4.34E-03	4.34E-03	3.39E-01	3.39E-01
2:09:50	79.63	1.00	79.13	0.883	0.887	0.887	70.152	0.543	0.545	4.29E-03	4.31E-03	4.31E-03	3.41E-01	3.41E-01
2:10:50	80.63	1.00	80.13	0.876	0.880	0.880	70.477	0.539	0.541	4.26E-03	4.27E-03	4.27E-03	3.42E-01	3.42E-01
2:11:50	81.63	1.00	81.13	0.869	0.873	0.873	70.789	0.534	0.536	4.22E-03	4.24E-03	4.24E-03	3.44E-01	3.44E-01
2:12:50	82.63	1.00	82.13	0.862	0.866	0.866	71.086	0.530	0.532	4.19E-03	4.20E-03	4.20E-03	3.45E-01	3.45E-01
2:13:50	83.63	1.00	83.13	0.855	0.859	0.859	71.370	0.526	0.528	4.15E-03	4.17E-03	4.17E-03	3.47E-01	3.47E-01
2:14:50	84.63	1.00	84.13	0.848	0.852	0.852	71.640	0.521	0.524	4.12E-03	4.14E-03	4.14E-03	3.48E-01	3.48E-01
2:15:50	85.63	1.00	85.13	0.841	0.845	0.845	71.895	0.517	0.519	4.09E-03	4.10E-03	4.10E-03	3.49E-01	3.49E-01
2:16:50	86.63	1.00	86.13	0.834	0.838	0.838	72.137	0.513	0.515	4.05E-03	4.07E-03	4.07E-03	3.50E-01	3.50E-01
2:17:50	87.63	1.00	87.13	0.827	0.831	0.831	72.364	0.508	0.511	4.02E-03	4.03E-03	4.03E-03	3.52E-01	3.52E-01
2:18:50	88.63	1.00	88.13	0.821	0.824	0.824	72.622	0.505	0.507	3.99E-03	4.00E-03	4.00E-03	3.53E-01	3.53E-01
2:19:51	89.65	1.02	89.14	0.814	0.818	0.831	74.088	0.500	0.511	3.95E-03	3.97E-03	4.04E-03	3.54E-01	3.60E-01
2:20:50	90.63	0.98	90.14	0.807	0.811	0.797	71.842	0.496	0.490	3.92E-03	3.94E-03	3.87E-03	3.55E-01	3.49E-01

Table 30. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm) *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_c(t)=$		$A_{C/C_A}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time (min)	$dt=t_2-t_1$ (min)	Time $t=t_1+(t_2-t_1)/2$	$C_{out}(t)$ (abs)	$C_1+(C_2-C_1)/2$ (abs)	$dt^*C'(t)$ (abs*min)	$dt^*t^*C'(t)$ (abs*min ²)	$C_{out}(t)/C_A$	$dt^*C'(t)/C_A$ (min)	$(C_{out}(t)/C_A)/$ A_{C/C_A} (min ⁻¹)	$E_1+(E_2-E_1)/2$ (min ⁻¹)	$A_E(t)=$ $dt^*E'(t)$	$t^*E(t)=$ $t^*E'(t)$	$dt^*t^*E'(t)$ (min)
2:21:51	91.65	1.02	91.14	0.801	0.804	0.817	74.499	0.493	0.503	3.89E-03	3.91E-03	3.97E-03	3.56E-01	3.62E-01
2:22:51	92.65	1.00	92.15	0.794	0.798	0.798	73.490	0.488	0.490	3.86E-03	3.87E-03	3.87E-03	3.57E-01	3.57E-01
2:23:50	93.63	0.98	93.14	0.788	0.791	0.778	72.447	0.485	0.478	3.83E-03	3.84E-03	3.78E-03	3.58E-01	3.52E-01
2:24:50	94.63	1.00	94.13	0.782	0.785	0.785	73.895	0.481	0.483	3.80E-03	3.81E-03	3.81E-03	3.59E-01	3.59E-01
2:25:51	95.65	1.02	95.14	0.775	0.779	0.791	75.302	0.477	0.487	3.76E-03	3.78E-03	3.84E-03	3.60E-01	3.66E-01
2:26:51	96.65	1.00	96.15	0.769	0.772	0.772	74.228	0.473	0.475	3.74E-03	3.75E-03	3.75E-03	3.61E-01	3.61E-01
2:27:51	97.65	1.00	97.15	0.763	0.766	0.766	74.417	0.469	0.471	3.71E-03	3.72E-03	3.72E-03	3.61E-01	3.61E-01
2:28:50	98.63	0.98	98.14	0.757	0.760	0.747	73.345	0.465	0.460	3.68E-03	3.69E-03	3.63E-03	3.62E-01	3.56E-01
2:29:51	99.65	1.02	99.14	0.751	0.754	0.767	75.999	0.462	0.471	3.65E-03	3.66E-03	3.72E-03	3.63E-01	3.69E-01
2:30:50	100.63	0.98	100.14	0.745	0.748	0.736	73.658	0.458	0.452	3.62E-03	3.63E-03	3.57E-03	3.64E-01	3.58E-01
2:31:51	101.65	1.02	101.14	0.739	0.742	0.754	76.298	0.454	0.464	3.59E-03	3.60E-03	3.66E-03	3.65E-01	3.71E-01
2:32:51	102.65	1.00	102.15	0.733	0.736	0.736	75.182	0.451	0.453	3.56E-03	3.58E-03	3.58E-03	3.65E-01	3.65E-01
2:33:50	103.63	0.98	103.14	0.727	0.730	0.718	74.039	0.447	0.441	3.53E-03	3.55E-03	3.49E-03	3.66E-01	3.60E-01
2:34:54	104.70	1.07	104.17	0.721	0.724	0.772	80.444	0.443	0.475	3.50E-03	3.52E-03	3.75E-03	3.66E-01	3.91E-01
2:35:52	105.67	0.97	105.18	0.715	0.718	0.694	73.004	0.440	0.427	3.47E-03	3.49E-03	3.37E-03	3.67E-01	3.55E-01
2:36:53	106.68	1.02	106.18	0.709	0.712	0.724	76.857	0.436	0.445	3.44E-03	3.46E-03	3.52E-03	3.67E-01	3.73E-01
2:37:51	107.65	0.97	107.17	0.703	0.706	0.682	73.138	0.432	0.420	3.41E-03	3.43E-03	3.32E-03	3.68E-01	3.55E-01
2:38:40	108.47	0.82	108.06	0.699	0.701	0.572	61.862	0.430	0.352	3.40E-03	3.41E-03	2.78E-03	3.68E-01	3.00E-01
2:48:42	118.50	10.03	113.48	0.644	0.672	6.737	764.581	0.396	4.143	3.13E-03	3.26E-03	3.27E-02	3.70E-01	3.71E+00
2:58:40	128.47	9.97	123.48	0.595	0.620	6.174	762.429	0.366	3.796	2.89E-03	3.01E-03	3.00E-02	3.72E-01	3.70E+00
3:08:42	138.50	10.03	133.48	0.549	0.572	5.739	766.070	0.338	3.529	2.67E-03	2.78E-03	2.79E-02	3.71E-01	3.72E+00
3:18:41	148.48	9.98	143.49	0.506	0.528	5.266	755.657	0.311	3.238	2.46E-03	2.56E-03	2.56E-02	3.68E-01	3.67E+00
3:28:43	158.52	10.03	153.50	0.467	0.487	4.881	749.267	0.287	3.001	2.27E-03	2.36E-03	2.37E-02	3.63E-01	3.64E+00
3:38:41	168.48	9.97	163.50	0.431	0.449	4.475	731.668	0.265	2.752	2.09E-03	2.18E-03	2.17E-02	3.57E-01	3.55E+00
3:48:41	178.48	10.00	173.48	0.398	0.415	4.145	719.088	0.245	2.549	1.93E-03	2.01E-03	2.01E-02	3.49E-01	3.49E+00
3:58:41	188.48	10.00	183.48	0.367	0.383	3.825	701.824	0.226	2.352	1.78E-03	1.86E-03	1.86E-02	3.41E-01	3.41E+00
4:08:42	198.50	10.02	193.49	0.339	0.353	3.536	684.164	0.208	2.174	1.65E-03	1.71E-03	1.72E-02	3.32E-01	3.32E+00
4:18:43	208.52	10.02	203.51	0.313	0.326	3.265	664.543	0.192	2.008	1.52E-03	1.58E-03	1.59E-02	3.22E-01	3.23E+00
4:28:44	218.53	10.02	213.53	0.289	0.301	3.015	643.781	0.178	1.854	1.40E-03	1.46E-03	1.46E-02	3.12E-01	3.13E+00
4:38:42	228.50	9.97	223.52	0.267	0.278	2.771	619.305	0.164	1.704	1.30E-03	1.35E-03	1.35E-02	3.02E-01	3.01E+00
4:48:42	238.50	10.00	233.50	0.247	0.257	2.570	600.095	0.152	1.580	1.20E-03	1.25E-03	1.25E-02	2.91E-01	2.91E+00

Table 30. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm) *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_c(t)=$		$A_{C/C_A}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time	$dt=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$dt^*C'(t)$	$dt^*t^*C'(t)$	$C_{out}(t)/C_A$	$dt^*C'(t)/C_A$	$(C_{out}(t)/C_A)/$	$E_1+(E_2-E_1)/2$	$A_E(t)=$	$t^*E(t)=$	$dt^*t^*E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)		(min)	(min ⁻¹)	(min ⁻¹)	$dt^*E'(t)$	$t^*E'(t)$	(min)
4:58:42	248.50	10.00	243.50	0.228	0.238	2.375	578.313	0.140	1.460	1.11E-03	1.15E-03	1.15E-02	2.81E-01	2.81E+00
5:08:43	258.52	10.02	253.51	0.211	0.220	2.199	557.378	0.130	1.352	1.02E-03	1.07E-03	1.07E-02	2.70E-01	2.71E+00
5:18:44	268.53	10.02	263.53	0.195	0.203	2.033	535.847	0.120	1.250	9.47E-04	9.86E-04	9.88E-03	2.60E-01	2.60E+00
5:28:44	278.53	10.00	273.53	0.180	0.188	1.875	512.875	0.111	1.153	8.74E-04	9.11E-04	9.11E-03	2.49E-01	2.49E+00
5:38:45	288.55	10.02	283.54	0.167	0.174	1.738	492.765	0.103	1.069	8.11E-04	8.43E-04	8.44E-03	2.39E-01	2.39E+00
5:48:45	298.55	10.00	293.55	0.154	0.161	1.605	471.148	0.095	0.987	7.48E-04	7.80E-04	7.80E-03	2.29E-01	2.29E+00
5:58:43	308.52	9.97	303.53	0.143	0.149	1.480	449.245	0.088	0.910	6.95E-04	7.21E-04	7.19E-03	2.19E-01	2.18E+00
6:08:44	318.53	10.02	313.53	0.132	0.138	1.377	431.815	0.081	0.847	6.41E-04	6.68E-04	6.69E-03	2.09E-01	2.10E+00
6:18:44	328.53	10.00	323.53	0.122	0.127	1.270	410.887	0.075	0.781	5.93E-04	6.17E-04	6.17E-03	2.00E-01	2.00E+00
6:28:43	338.52	9.98	333.53	0.113	0.118	1.173	391.239	0.069	0.721	5.49E-04	5.71E-04	5.70E-03	1.90E-01	1.90E+00
6:38:44	348.53	10.02	343.53	0.105	0.109	1.092	375.066	0.065	0.671	5.10E-04	5.29E-04	5.30E-03	1.82E-01	1.82E+00
6:48:44	358.53	10.00	353.53	0.097	0.101	1.010	357.069	0.060	0.621	4.71E-04	4.91E-04	4.91E-03	1.73E-01	1.73E+00
6:58:44	368.53	10.00	363.53	0.090	0.094	0.935	339.904	0.055	0.575	4.37E-04	4.54E-04	4.54E-03	1.65E-01	1.65E+00
7:08:44	378.53	10.00	373.53	0.084	0.087	0.870	324.974	0.052	0.535	4.08E-04	4.23E-04	4.23E-03	1.58E-01	1.58E+00
7:18:45	388.55	10.02	383.54	0.078	0.081	0.811	311.187	0.048	0.499	3.79E-04	3.93E-04	3.94E-03	1.51E-01	1.51E+00
7:28:45	398.55	10.00	393.55	0.072	0.075	0.750	295.163	0.044	0.461	3.50E-04	3.64E-04	3.64E-03	1.43E-01	1.43E+00
7:38:45	408.55	10.00	403.55	0.067	0.070	0.695	280.467	0.041	0.427	3.25E-04	3.38E-04	3.38E-03	1.36E-01	1.36E+00
7:48:46	418.57	10.02	413.56	0.062	0.065	0.646	267.190	0.038	0.397	3.01E-04	3.13E-04	3.14E-03	1.30E-01	1.30E+00
7:58:46	428.57	10.00	423.57	0.058	0.060	0.600	254.140	0.036	0.369	2.82E-04	2.91E-04	2.91E-03	1.23E-01	1.23E+00
8:08:47	438.58	10.02	433.58	0.054	0.056	0.561	243.207	0.033	0.345	2.62E-04	2.72E-04	2.72E-03	1.18E-01	1.18E+00
8:18:48	448.60	10.02	443.59	0.050	0.052	0.521	231.052	0.031	0.320	2.43E-04	2.53E-04	2.53E-03	1.12E-01	1.12E+00
8:28:45	458.55	9.95	453.58	0.047	0.049	0.483	218.884	0.029	0.297	2.28E-04	2.36E-04	2.34E-03	1.07E-01	1.06E+00
8:38:45	468.55	10.00	463.55	0.044	0.046	0.455	210.915	0.027	0.280	2.14E-04	2.21E-04	2.21E-03	1.02E-01	1.02E+00
8:48:47	478.58	10.03	473.57	0.041	0.043	0.426	201.937	0.025	0.262	1.99E-04	2.06E-04	2.07E-03	9.78E-02	9.81E-01
8:58:47	488.58	10.00	483.58	0.038	0.040	0.395	191.015	0.023	0.243	1.85E-04	1.92E-04	1.92E-03	9.28E-02	9.28E-01
9:08:48	498.60	10.02	493.59	0.036	0.037	0.371	182.933	0.022	0.228	1.75E-04	1.80E-04	1.80E-03	8.87E-02	8.89E-01
9:18:46	508.57	9.97	503.58	0.034	0.035	0.349	175.667	0.021	0.214	1.65E-04	1.70E-04	1.69E-03	8.56E-02	8.53E-01
9:28:46	518.57	10.00	513.57	0.032	0.033	0.330	169.477	0.020	0.203	1.55E-04	1.60E-04	1.60E-03	8.23E-02	8.23E-01
9:38:47	528.58	10.02	523.58	0.030	0.031	0.311	162.579	0.018	0.191	1.46E-04	1.51E-04	1.51E-03	7.88E-02	7.90E-01
9:48:48	538.60	10.02	533.59	0.028	0.029	0.290	154.999	0.017	0.179	1.36E-04	1.41E-04	1.41E-03	7.52E-02	7.53E-01
9:58:46	548.57	9.97	543.58	0.026	0.027	0.269	146.278	0.016	0.165	1.26E-04	1.31E-04	1.31E-03	7.13E-02	7.11E-01

Table 30. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm) *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/C_A}(t)=$		$E(t)=$		$A_E(t)=$	
	Time	$dt=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$dt^*C'(t)$	$dt^*t^*C'(t)$	$C_{out}(t)/C_A$	$dt^*C'(t)/C_A$	$(C_{out}(t)/C_A)/$	$E_1+(E_2-E_1)/2$	$A_E(t)=$	$t^*E(t)=$	$dt^*t^*E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)		(min)	(min ⁻¹)	(min ⁻¹)	$dt^*E'(t)$	$t^*E'(t)$	(min)
4:58:42	248.50	10.00	243.50	0.228	0.238	2.375	578.313	0.140	1.460	1.11E-03	1.15E-03	1.15E-02	2.81E-01	2.81E+00
5:08:43	258.52	10.02	253.51	0.211	0.220	2.199	557.378	0.130	1.352	1.02E-03	1.07E-03	1.07E-02	2.70E-01	2.71E+00
5:18:44	268.53	10.02	263.53	0.195	0.203	2.033	535.847	0.120	1.250	9.47E-04	9.86E-04	9.88E-03	2.60E-01	2.60E+00
5:28:44	278.53	10.00	273.53	0.180	0.188	1.875	512.875	0.111	1.153	8.74E-04	9.11E-04	9.11E-03	2.49E-01	2.49E+00
5:38:45	288.55	10.02	283.54	0.167	0.174	1.738	492.765	0.103	1.069	8.11E-04	8.43E-04	8.44E-03	2.39E-01	2.39E+00
5:48:45	298.55	10.00	293.55	0.154	0.161	1.605	471.148	0.095	0.987	7.48E-04	7.80E-04	7.80E-03	2.29E-01	2.29E+00
5:58:43	308.52	9.97	303.53	0.143	0.149	1.480	449.245	0.088	0.910	6.95E-04	7.21E-04	7.19E-03	2.19E-01	2.18E+00
6:08:44	318.53	10.02	313.53	0.132	0.138	1.377	431.815	0.081	0.847	6.41E-04	6.68E-04	6.69E-03	2.09E-01	2.10E+00
6:18:44	328.53	10.00	323.53	0.122	0.127	1.270	410.887	0.075	0.781	5.93E-04	6.17E-04	6.17E-03	2.00E-01	2.00E+00
6:28:43	338.52	9.98	333.53	0.113	0.118	1.173	391.239	0.069	0.721	5.49E-04	5.71E-04	5.70E-03	1.90E-01	1.90E+00
6:38:44	348.53	10.02	343.53	0.105	0.109	1.092	375.066	0.065	0.671	5.10E-04	5.29E-04	5.30E-03	1.82E-01	1.82E+00
6:48:44	358.53	10.00	353.53	0.097	0.101	1.010	357.069	0.060	0.621	4.71E-04	4.91E-04	4.91E-03	1.73E-01	1.73E+00
6:58:44	368.53	10.00	363.53	0.090	0.094	0.935	339.904	0.055	0.575	4.37E-04	4.54E-04	4.54E-03	1.65E-01	1.65E+00
7:08:44	378.53	10.00	373.53	0.084	0.087	0.870	324.974	0.052	0.535	4.08E-04	4.23E-04	4.23E-03	1.58E-01	1.58E+00
7:18:45	388.55	10.02	383.54	0.078	0.081	0.811	311.187	0.048	0.499	3.79E-04	3.93E-04	3.94E-03	1.51E-01	1.51E+00
7:28:45	398.55	10.00	393.55	0.072	0.075	0.750	295.163	0.044	0.461	3.50E-04	3.64E-04	3.64E-03	1.43E-01	1.43E+00
7:38:45	408.55	10.00	403.55	0.067	0.070	0.695	280.467	0.041	0.427	3.25E-04	3.38E-04	3.38E-03	1.36E-01	1.36E+00
7:48:46	418.57	10.02	413.56	0.062	0.065	0.646	267.190	0.038	0.397	3.01E-04	3.13E-04	3.14E-03	1.30E-01	1.30E+00
7:58:46	428.57	10.00	423.57	0.058	0.060	0.600	254.140	0.036	0.369	2.82E-04	2.91E-04	2.91E-03	1.23E-01	1.23E+00
8:08:47	438.58	10.02	433.58	0.054	0.056	0.561	243.207	0.033	0.345	2.62E-04	2.72E-04	2.72E-03	1.18E-01	1.18E+00
8:18:48	448.60	10.02	443.59	0.050	0.052	0.521	231.052	0.031	0.320	2.43E-04	2.53E-04	2.53E-03	1.12E-01	1.12E+00
8:28:45	458.55	9.95	453.58	0.047	0.049	0.483	218.884	0.029	0.297	2.28E-04	2.36E-04	2.34E-03	1.07E-01	1.06E+00
8:38:45	468.55	10.00	463.55	0.044	0.046	0.455	210.915	0.027	0.280	2.14E-04	2.21E-04	2.21E-03	1.02E-01	1.02E+00
8:48:47	478.58	10.03	473.57	0.041	0.043	0.426	201.937	0.025	0.262	1.99E-04	2.06E-04	2.07E-03	9.78E-02	9.81E-01
8:58:47	488.58	10.00	483.58	0.038	0.040	0.395	191.015	0.023	0.243	1.85E-04	1.92E-04	1.92E-03	9.28E-02	9.28E-01
9:08:48	498.60	10.02	493.59	0.036	0.037	0.371	182.933	0.022	0.228	1.75E-04	1.80E-04	1.80E-03	8.87E-02	8.89E-01
9:18:46	508.57	9.97	503.58	0.034	0.035	0.349	175.667	0.021	0.214	1.65E-04	1.70E-04	1.69E-03	8.56E-02	8.53E-01
9:28:46	518.57	10.00	513.57	0.032	0.033	0.330	169.477	0.020	0.203	1.55E-04	1.60E-04	1.60E-03	8.23E-02	8.23E-01
9:38:47	528.58	10.02	523.58	0.030	0.031	0.311	162.579	0.018	0.191	1.46E-04	1.51E-04	1.51E-03	7.88E-02	7.90E-01
9:48:48	538.60	10.02	533.59	0.028	0.029	0.290	154.999	0.017	0.179	1.36E-04	1.41E-04	1.41E-03	7.52E-02	7.53E-01
9:58:46	548.57	9.97	543.58	0.026	0.027	0.269	146.278	0.016	0.165	1.26E-04	1.31E-04	1.31E-03	7.13E-02	7.11E-01

Table 30. Experimental data and computation for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm) *continued*

Time	Elapsed		1st Moment		$C'(t)=$		$A_C(t)=$		$A_{C/C_A}(t)=$		$E(t)=$		$A_{rE}(t)=$	
	Time	$dt=t_2-t_1$	Time	$C_{out}(t)$	$C_1+(C_2-C_1)/2$	$dt^*C'(t)$	$dt^*t^*C'(t)$	$C_{out}(t)/C_A$	$dt^*C'(t)/C_A$	$(C_{out}(t)/C_A)/$	$E'(t)=$	$A_E(t)=$	$t^*E(t)=$	$dt^*t^*E'(t)$
	(min)	(min)	$t=t_1+(t_2-t_1)/2$	(abs)	(abs)	(abs*min)	(abs*min ²)		(min)	(min ⁻¹)	$E_1+(E_2-E_1)/2$	$dt^*E'(t)$	$t^*E'(t)$	(min)
10:08:46	558.57	10.00	553.57	0.025	0.026	0.255	141.160	0.015	0.157	1.21E-04	1.24E-04	1.24E-03	6.86E-02	6.86E-01
10:18:47	568.58	10.02	563.58	0.024	0.025	0.245	138.306	0.015	0.151	1.17E-04	1.19E-04	1.19E-03	6.71E-02	6.72E-01
10:28:48	578.60	10.02	573.59	0.022	0.023	0.230	132.146	0.014	0.142	1.07E-04	1.12E-04	1.12E-03	6.41E-02	6.42E-01
10:38:48	588.60	10.00	583.60	0.021	0.022	0.215	125.474	0.013	0.132	1.02E-04	1.04E-04	1.04E-03	6.09E-02	6.09E-01
10:48:49	598.62	10.02	593.61	0.020	0.021	0.205	121.893	0.012	0.126	9.71E-05	9.96E-05	9.97E-04	5.91E-02	5.92E-01
10:58:47	608.58	9.97	603.60	0.019	0.020	0.194	117.310	0.012	0.119	9.23E-05	9.47E-05	9.44E-04	5.72E-02	5.70E-01
11:08:48	618.60	10.02	613.59	0.018	0.019	0.185	113.704	0.011	0.114	8.74E-05	8.99E-05	9.00E-04	5.51E-02	5.52E-01
11:18:49	628.62	10.02	623.61	0.017	0.018	0.175	109.313	0.010	0.108	8.26E-05	8.50E-05	8.51E-04	5.30E-02	5.31E-01
11:28:50	638.63	10.02	633.63	0.016	0.017	0.165	104.722	0.010	0.102	7.77E-05	8.01E-05	8.03E-04	5.08E-02	5.09E-01
11:38:48	648.60	9.97	643.62	0.016	0.016	0.159	102.635	0.010	0.098	7.77E-05	7.77E-05	7.75E-04	5.00E-02	4.99E-01
11:48:48	658.60	10.00	653.60	0.015	0.016	0.155	101.308	0.009	0.095	7.29E-05	7.53E-05	7.53E-04	4.92E-02	4.92E-01
11:58:49	668.62	10.02	663.61	0.015	0.015	0.150	99.707	0.009	0.092	7.29E-05	7.29E-05	7.30E-04	4.84E-02	4.84E-01
12:08:50	678.63	10.02	673.63	0.014	0.015	0.145	97.838	0.009	0.089	6.80E-05	7.04E-05	7.06E-04	4.74E-02	4.75E-01
12:18:48	688.60	9.97	683.62	0.013	0.014	0.135	91.981	0.008	0.083	6.31E-05	6.56E-05	6.54E-04	4.48E-02	4.47E-01
12:28:49	698.62	10.02	693.61	0.013	0.013	0.130	90.319	0.008	0.080	6.31E-05	6.31E-05	6.33E-04	4.38E-02	4.39E-01
12:38:49	708.62	10.00	703.62	0.013	0.013	0.130	91.470	0.008	0.080	6.31E-05	6.31E-05	6.31E-04	4.44E-02	4.44E-01
12:48:50	718.63	10.02	713.63	0.012	0.013	0.125	89.352	0.007	0.077	5.83E-05	6.07E-05	6.08E-04	4.33E-02	4.34E-01
12:58:49	728.62	9.98	723.63	0.012	0.012	0.120	86.690	0.007	0.074	5.83E-05	5.83E-05	5.82E-04	4.22E-02	4.21E-01
13:08:50	738.63	10.02	733.63	0.011	0.012	0.115	84.507	0.007	0.071	5.34E-05	5.59E-05	5.60E-04	4.10E-02	4.10E-01
13:18:52	748.67	10.03	743.65	0.011	0.011	0.110	82.074	0.007	0.068	5.34E-05	5.34E-05	5.36E-04	3.97E-02	3.99E-01

Table 31. Computational analysis for a bottom fed non-reactive tracer (Tracer #4, $\omega = 150$ rpm).

$\text{Area under } C \text{ curve } (A_C) = \Sigma [\Delta t^*C'(t)]$	205.87	min*abs
$\text{Area under } t^*A_C \text{ curve} = \Sigma [t^*\Delta t^*C'(t)]$	26830.34	min ² *abs
$t_{m-C \text{ curve}} = \Sigma [\Delta t^*t^*C'(t)] / (\Sigma [\Delta t^*C'(t)])$	130.33	min
Q	8	mL/min
$M_T = Q * A_C$	1626.37	mL*abs
V_R	1000	mL
$V_{R-\text{calculated}} = t_m * Q$	1030	mL
$C_A = M_T / V_R$	1.63	abs
$\text{Area under normalized } C \text{ curve } (A_{C/C_A}) = \Sigma [\Delta t^*C'(t)/C_A]$	126.58	min
$\text{Area under } E \text{ curve } (A_E) = \Sigma [\Delta t^*E'(t)]$	1.00	
$\text{Area under } t^*E \text{ curve } (A_{t^*E}) = \Sigma [\Delta t^*t^*E'(t)]$	130.33	min
$t_{m-E \text{ curve}} = \Sigma [\Delta t^*t^*E'(t)] / (\Sigma [\Delta t^*E'(t)])$	130.33	min

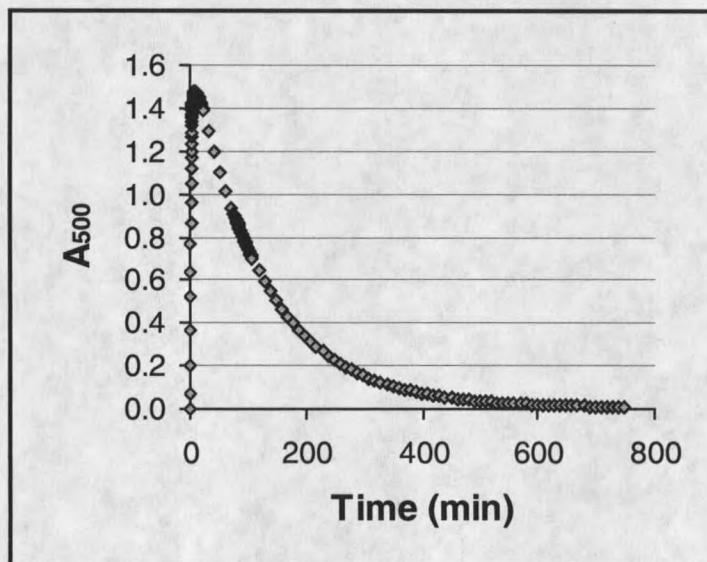


Figure 29. Effluent tracer concentration profile for Tracer #4 ($\omega = 150$ rpm). A 1-mL pulse injection of red food coloring with absorbance levels recorded at $\lambda = 500$ nm.

Table 32. Linear regression summary for the natural logarithm transformation of the concentration versus time data (Tracer #4, $\omega = 150$ rpm).

Regression Statistics	
Multiple R	0.99781
R Square	0.99562
Adjusted R Square	0.99559
Standard Error	0.10607
Observations	182

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	460.174	460.174	40900.755	0.000
Residual	180	2.02518	0.01125		
Total	181	462.199			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.44115	0.01012	43.599	0.000	0.42119	0.46112	0.42119	0.46112
X Variable 1	-0.43436	0.00215	-202.239	0.000	-0.43860	-0.43012	-0.43860	-0.43012

APPENDIX D: Standard Curves

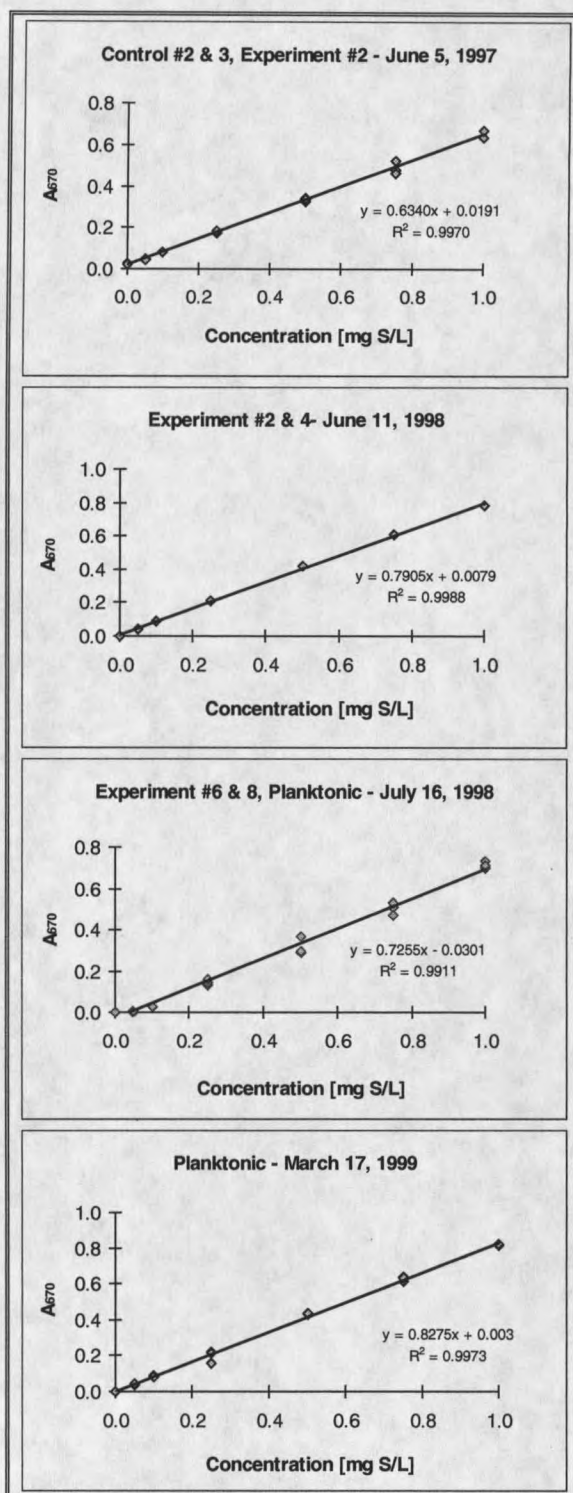


Figure 30. Hydrogen sulfide standard curves used in calculating sulfide levels during the planktonic and biofilm experiments.

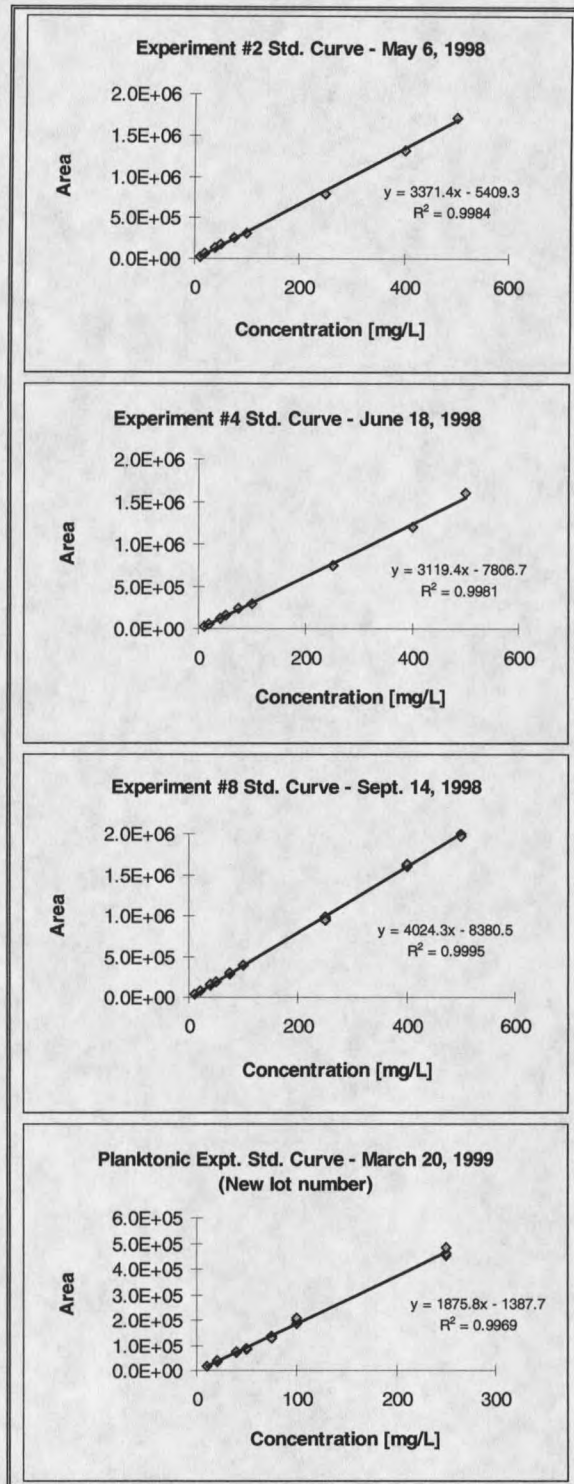


Figure 31. Glutaraldehyde standard curves used in the analysis of biocide levels during the planktonic and biofilm experiments.

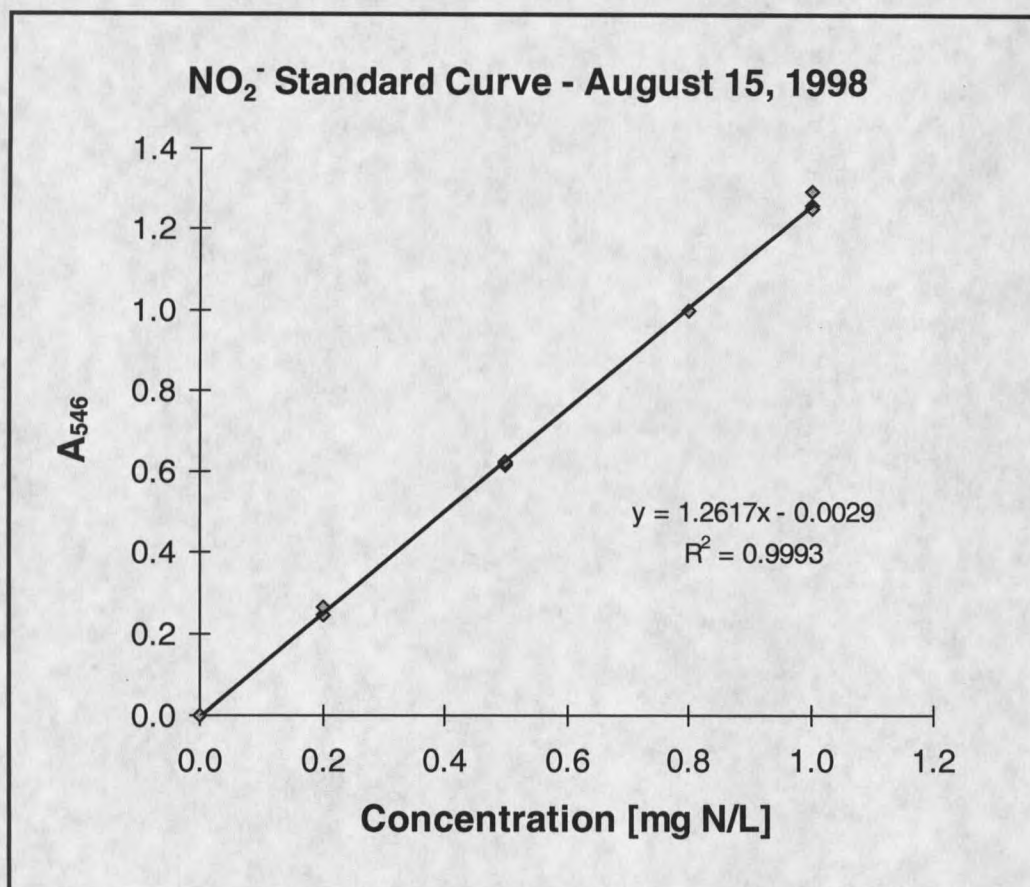


Figure 32. Nitrite standard curve. This linear relationship was utilized to determine nitrite concentration levels during Experiment #6.

APPENDIX E: Planktonic Experimental Data

Table 33. January 7, 1998 batch vial data.

Time (hr)	Sample	Dilution	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	$\Delta\text{H}_2\text{S}$ (mg S/L)	ΔSO_4^{2-} (mg S/L)
			A670	(mg S/L)				
0.17	Vial #1	50	0.150	10.32	1003.25	1013.58	0.00	-0.23
2.00	Vial #1	50	0.177	12.45	984.41	996.86	2.13	18.62
4.00	Vial #1	50	0.207	14.82	1005.34	1020.15	4.50	-2.31
6.00	Vial #1	50	0.288	21.21	1019.10	1040.30	10.88	-16.07
8.00	Vial #2	50	0.191	13.56	993.77	1007.33	3.23	9.25
10.00	Vial #2	50	0.620	47.39	944.98	992.37	37.07	58.04
12.00	Vial #2	100	0.536	81.53	924.21	1005.74	71.21	78.81
14.00	Vial #2	100	0.898	138.63	862.88	1001.51	128.30	140.14
16.00	Vial #3	500	0.364	272.00	700.97	972.98	261.68	302.05
18.00	Vial #3	500	0.553	421.06	546.08	967.14	410.73	456.94
20.00	Vial #3	500	0.552	420.27	553.01	973.28	409.94	450.02
22.00	Vial #3	500	0.570	434.46	560.38	994.85	424.14	442.64
24.00	Vial #4	500	0.584	445.50	518.41	963.91	435.18	484.62
26.00	Vial #4	500	0.559	425.79	556.61	982.40	415.47	446.42
28.00	Vial #4	500	0.556	423.42	510.27	933.70	413.10	492.75
30.00	Vial #4	500	0.506	383.99	534.94	918.93	373.67	468.08
32.00	Vial #5	500	0.565	430.52	524.01	954.53	420.20	479.01
34.00	Vial #5	500	0.568	432.89	556.20	989.09	422.56	446.82
36.00	Vial #5.1	500	0.549	417.90	519.20	937.10	407.58	483.83
36.00	Vial #5.2	500	0.554	421.85	526.57	948.41	411.52	476.46
36.00	Vial #5.3	500	0.566	431.31	523.63	954.94	420.99	479.39

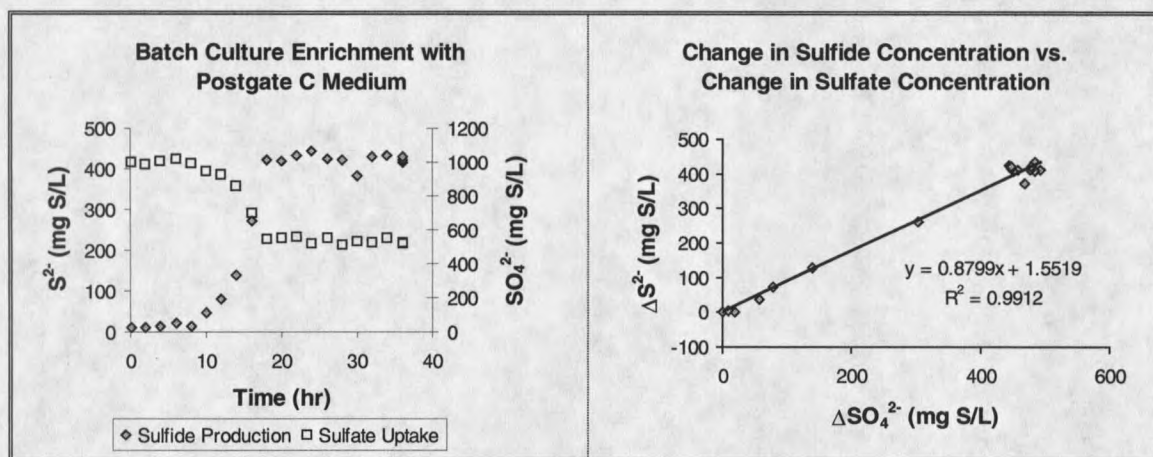


Figure 33. January 7, 1998 batch vial enrichment. Sulfate and sulfide profiles during a 36-hour growth cycle with change in sulfide concentration versus change in sulfate concentration.

Table 34. November 18, 1998 batch vial data.

Time (hr)	Sample	Dilution	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	$\Delta\text{H}_2\text{S}$ (mg S/L)	ΔSO_4^{2-} (mg S/L)
			A_{670}	(mg S/L)				
0.17	Vial #1	50	0.262	20.13	1165.90	1186.04	0.00	-0.78
3.17	Vial #1	50	0.284	21.65	1103.52	1125.17	1.52	61.60
5.00	Vial #1	50	0.389	28.88	1134.13	1163.01	8.75	30.99
7.25	Vial #1	50	0.670	48.25	1085.69	1133.94	28.12	79.43
9.00	Vial #2	100	0.699	100.50	1059.42	1159.91	80.37	105.70
11.00	Vial #2	500	0.218	170.99	927.81	1098.80	150.85	237.31
12.00	Vial #2	500	0.288	219.23	933.33	1152.56	199.10	231.79
13.00	Vial #2	500	0.396	293.66	786.51	1080.17	273.53	378.61
14.00	Vial #3	500	0.608	439.77	658.56	1098.33	419.63	506.56
15.00	Vial #3	500	0.696	500.41	520.13	1020.54	480.28	644.99
16.00	Vial #3	500	0.727	521.78	535.77	1057.55	501.65	629.35
17.00	Vial #3	1250	0.281	536.01	527.14	1063.15	515.88	637.98
19.00	Vial #4	500	0.749	536.94	509.61	1046.55	516.81	655.51
22.08	Vial #4	500	0.751	538.32	509.61	1047.93	518.19	655.51
24.00	Vial #4	1250	0.272	520.50	518.25	1038.76	500.37	646.87
27.00	Vial #4	1250	0.271	518.78	535.71	1054.49	498.65	629.41
30.00	Vial #5	500	0.645	465.27	538.38	1003.64	445.13	626.74
34.00	Vial #5	500	0.751	538.32	562.88	1101.19	518.19	602.24
36.00	Vial #5.3	500	0.700	503.17	548.26	1051.43	483.04	616.86

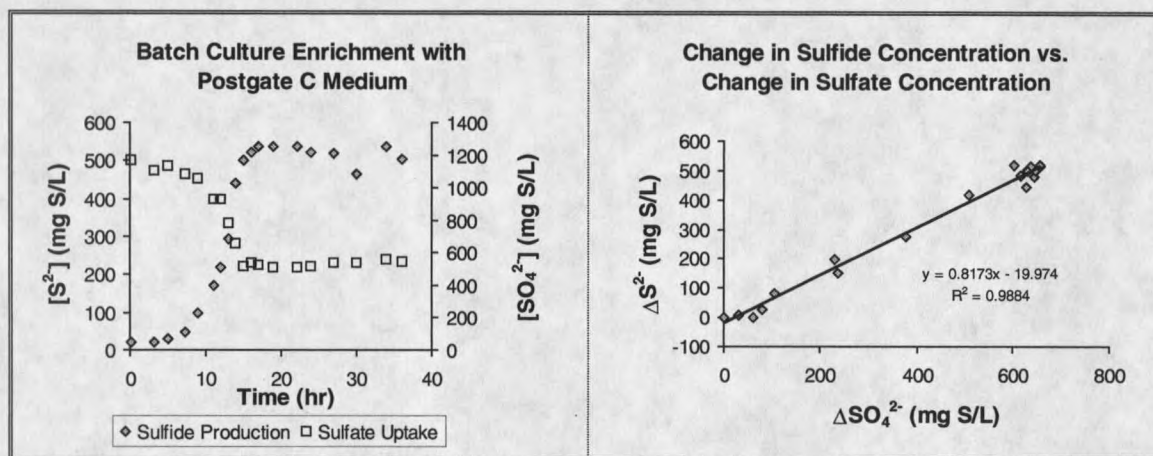


Figure 34. November 18, 1998 batch vial enrichment. Sulfate and sulfide profiles during a 36-hour growth cycle with change in sulfide concentration versus change in sulfate concentration.

Table 35. March 18, 1999 batch vial data.

Time (hr)	Sample	Dilution	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	$\Delta\text{H}_2\text{S}$ (mg S/L)	ΔSO_4^{2-} (mg S/L)
			A_{670}	(mg S/L)				
0.25	Vial #1	50	0.219	13.05	1056.37	1069.42	0.00	0.00
2.50	Vial #1	50	0.255	15.23	1030.37	1045.60	2.18	26.00
6.00	Vial #1	50	0.470	28.22	1042.82	1071.04	15.17	13.55
7.00	Vial #1	50	0.556	33.41	1008.01	1041.42	20.36	48.36
8.50	Vial #1	50	0.835	50.27	1010.73	1061.00	37.22	45.64
10.00	Vial #1	100	0.621	74.68	960.47	1035.15	61.63	95.90
11.00	Vial #2	500	0.227	135.35	888.96	1024.31	122.30	167.41
12.00	Vial #2	500	0.274	163.75	827.63	991.38	150.69	228.74
13.00	Vial #2	500	0.392	235.05	754.97	990.02	221.99	301.40
14.00	Vial #2	500	0.437	262.24	701.34	963.58	249.18	355.03
15.00	Vial #2	500	0.524	314.80	640.30	955.10	301.75	416.07
16.00	Vial #3	500	0.640	384.89	549.65	934.54	371.84	506.72
17.00	Vial #3	1250	0.347	519.64	527.43	1047.07	506.59	528.94
18.00	Vial #3	1250	0.308	460.73	524.96	985.69	447.67	531.41
19.00	Vial #3	1250	0.325	486.40	514.57	1000.97	473.35	541.80
20.00	Vial #3	1250	0.315	471.30	508.93	980.23	458.25	547.44
21.00	Vial #3	1250	0.315	471.30	512.32	983.62	458.25	544.05
25.00	Vial #4	1250	0.329	492.45	507.71	1000.16	479.40	548.66
28.50	Vial #4	1250	0.327	489.43	519.22	1008.65	476.37	537.15
33.00	Vial #4	1250	0.304	454.68	520.66	975.34	441.63	535.71
36.50	Vial #4	1250	0.331	495.47	519.31	1014.78	482.42	537.06

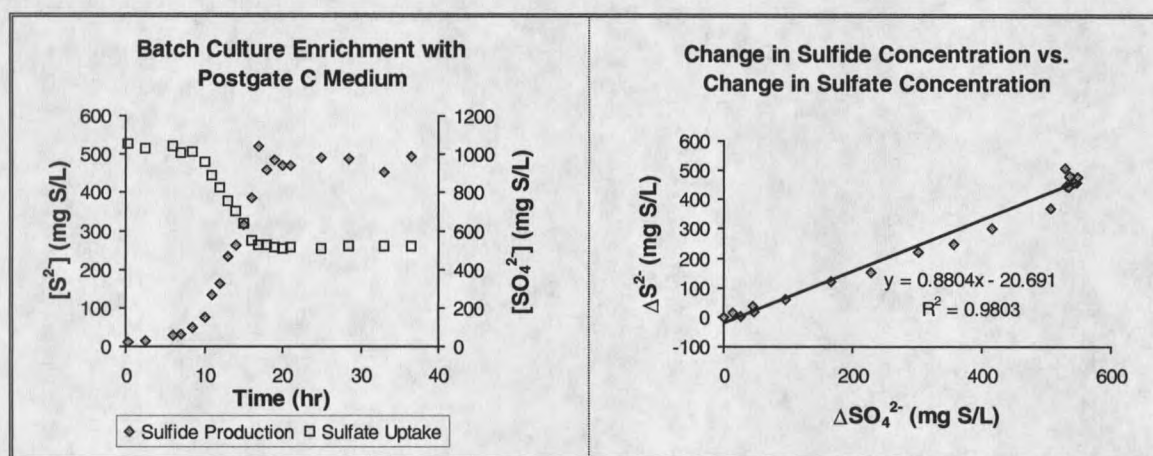


Figure 35. March 18, 1999 batch vial enrichment. Sulfate and sulfide profiles during a 36-hour growth cycle with change in sulfide concentration versus change in sulfate concentration.

Table 36. Planktonic Experiment #1 – Control

Time (hr)	Sulfide			Sulfate (mg S/L)	Total S (mg S/L)
	Dilution	A ₆₇₀	(mg S/L)		
0.00			0.00		
6.17	25	0.002	0.00		
12.17	25	0.002	0.00		
25.17	25	0.004	1.18		
47.17	25	0.151	6.24		
52.00	25	0.426	15.72	1139.48	1155.20
55.50	50	0.492	35.98	1133.07	1169.05
58.00	100	0.470	68.93	1108.66	1177.59
59.00	100	0.633	91.40	1021.77	1113.17
60.00	500	0.138	115.85	1065.93	1181.78
62.00	500	0.212	166.85	1021.78	1188.63
66.00	500	0.391	290.21	822.80	1113.02
69.00	500	0.564	409.44	664.18	1073.62
72.17	500	0.633	457.00	565.17	1022.17
77.08	500	0.659	474.91	566.84	1041.75
79.50	500	0.672	483.87	571.48	1055.35
93.67	500	0.598	432.87	560.95	993.83
101.75	500	0.624	450.79	543.34	994.13
118.50	500	0.636	459.06	548.59	1007.65

Table 37. Planktonic Experiment #1 – 50 mg/L glutaraldehyde

Time (hr)	Sulfide			Sulfate (mg S/L)	Total S (mg S/L)
	Dilution	A ₆₇₀	(mg S/L)		
0.00			0.00		
6.17	25	-0.001	0.00		
12.17	25	0.001	0.00		
25.17	25	0.002	0.00		
47.17	25	0.007	0.00		
52.00	25	0.001	0.00		
55.50	25	-0.002	0.00		
59.00	25	0.004	0.00		
67.00	25	0.006	1.24		
75.00	25	0.006	1.24		
79.50	25	0.007	1.28		
93.67	25	0.016	1.59		
101.75	25	0.006	1.24		
118.50	25	0.007	1.28		
142.67	25	0.013	1.49		
168.08	500	0.527	383.94	769.46	1153.40
170.00	500	0.610	441.14	623.85	1065.00
171.00	500	0.632	456.31	604.45	1060.76
172.50	500	0.650	468.71	634.70	1103.41
174.17	500	0.637	459.75	692.68	1152.43
180.00	500	0.659	474.91	673.00	1147.91
190.33	500	0.638	460.44	663.44	1123.88
198.58	500	0.641	462.51	641.09	1103.60
216.00	500	0.632	456.31	641.15	1097.46

Table 38. Planktonic Experiment #1 – 100 mg/L glutaraldehyde

Time (hr)	Sulfide		
	Dilution	A ₆₇₀	(mg S/L)
0.00			0.00
52.00	25	-0.003	0.00
93.67	25	0.001	0.00
142.67	25	0.003	0.00
168.00	25	0.003	0.00
190.33	25	0.003	0.00
198.58	25	0.003	0.00
216.00	25	0.004	1.18
220.83	25	0.005	1.21
225.42	25	0.005	1.21
239.33	25	0.007	1.28
246.17	25	0.006	1.24
264.92	25	0.009	1.35
272.17	25	0.009	1.35
275.75	25	0.009	1.35
287.33	25	0.008	1.31
293.42	25	0.008	1.31
312.17	25	0.009	1.35
318.42	25	0.006	1.24
336.17	25	0.005	1.21
361.50	25	0.005	1.21
388.42	25	0.002	1.11

Table 39. Planktonic Experiment #1 – 350 mg/L glutaraldehyde

Time (hr)	Sulfide		
	Dilution	A ₆₇₀	(mg S/L)
0.00			0.00
52.00	25	-0.002	0.00
93.67	25	0.005	1.21
142.67	25	0.002	1.11
168.00	25	0.003	1.14
190.33	25	0.004	1.18
198.58	25	0.005	1.21
239.33	25	0.007	1.28
264.92	25	0.009	1.35
272.17	25	0.010	1.38
275.75	25	0.009	1.35
287.33	25	0.009	1.35
312.17	25	0.010	1.38
336.17	25	0.008	1.31
361.50	25	0.009	1.35
388.42	25	0.028	2.00
396.67	25	0.008	1.31
407.42	25	0.008	1.31
483.33	25	0.007	1.28

Table 40. Planktonic Experiment #1 – 500 mg/L glutaraldehyde

Time (hr)	Sulfide		
	Dilution	A ₆₇₀	(mg S/L)
0.00			0.00
52.00	25	0.000	0.00
93.67	25	0.004	1.18
142.67	25	0.005	1.21
168.00	25	0.005	1.21
190.33	25	0.006	1.24
239.33	25	0.005	1.21
336.17	25	0.009	1.35
390.42	25	0.011	1.42
396.67	25	0.010	1.38
407.42	25	0.010	1.38
483.33	25	0.009	1.35

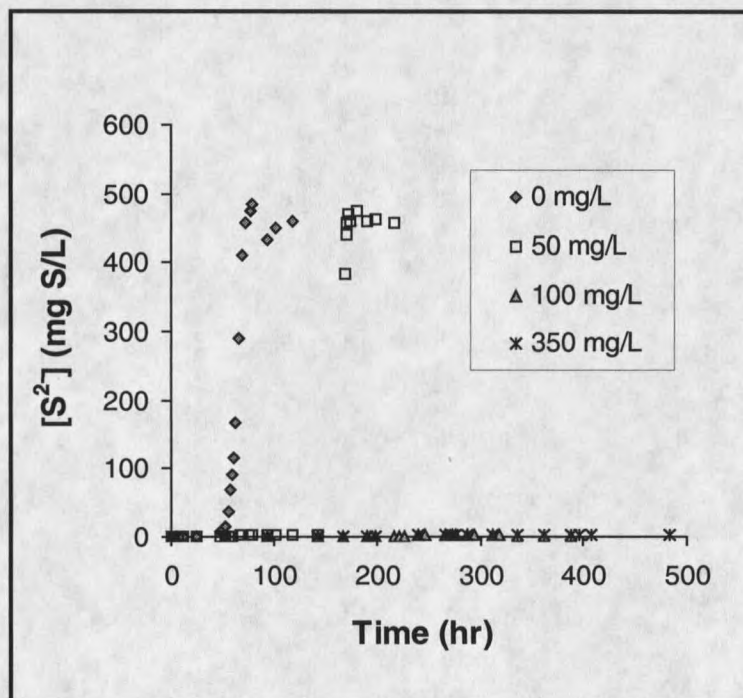


Figure 36. Planktonic Experiment #1. Sulfide recovery profiles in response to varying concentrations of glutaraldehyde.

Table 41. Planktonic Experiment #2 – Control

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)
	Dilution	A ₆₇₀ (mg S/L)		
0.00			0.00	
14.00	25	0.000	0.00	
29.33	25	0.000	0.00	
41.08	25	0.018	0.45	
49.00	25	0.318	9.52	
51.00	25	0.497	14.92	
52.50	25	0.733	22.05	
54.00	50	0.585	35.17	1009.57
55.50	100	0.369	44.23	1044.74
57.50	100	0.660	79.40	999.75
59.00	500	0.194	115.41	
60.50	500	0.300	179.46	
62.50	500	0.392	235.05	750.73
65.58	500	0.608	365.56	985.78
68.50	500	0.650	390.94	
72.50	500	0.600	360.73	533.19
85.25	500	0.614	369.18	893.92
96.25	500	0.593	356.50	928.37

Table 42. Planktonic Experiment #2 – 35 mg/L glutaraldehyde

Time (hr)	Sulfide	
	Dilution	A ₆₇₀ (mg S/L)
0.00		0.00
49.00	25	0.003
55.50	25	0.001
68.50	25	0.003
85.25	25	0.007
96.25	25	0.003
112.50	25	0.002
133.00	25	0.002
149.92	25	0.002
162.50	25	0.002
174.25	25	0.002
186.17	25	0.002
197.58	25	0.001
212.50	25	0.004
233.25	25	0.007
243.17	25	0.007
258.92	25	0.008
268.92	25	0.016
277.75	25	0.008
288.00	25	0.010
304.00	25	0.008
332.33	25	0.010
355.58	25	0.009

Table 43. Planktonic Experiment #2 – 65 mg/L glutaraldehyde

Time (hr)	Dilution	Sulfide	
		A_{670}	(mg S/L)
0.00			0.00
268.92	25	-0.004	0.00
288.00	25	0.004	0.03
304.00	25	0.005	0.06
332.33	25	0.004	0.03
355.58	25	0.007	0.12

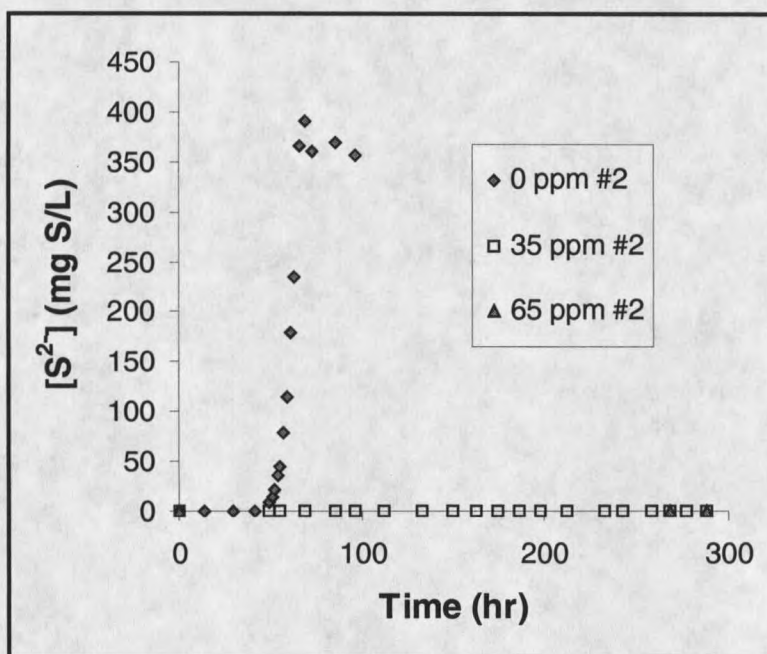


Figure 37. Planktonic Experiment #2. Sulfide recovery profiles in response to varying concentrations of glutaraldehyde.

Table 44. Planktonic Experiment #3 – Control

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)
	Dilution	A ₆₇₀ (mg S/L)		
0.00			0.00	
12.00	25	0.000	0.00	
27.33	25	0.000	0.00	
39.08	25	0.031	0.85	
47.00	25	0.793	23.87	
48.00	50	0.491	29.49	
49.00	50	0.660	39.70	
50.50	50	0.977	58.85	
52.00	100	0.702	84.47	930.88
53.50	500	0.211	125.68	
55.50	500	0.369	221.15	792.18
57.00	500	0.495	297.28	
58.50	500	0.536	322.05	
60.50	500	0.581	349.24	562.29
63.58	500	0.646	388.52	
66.50	500	0.644	387.31	
70.50	500	0.521	312.99	
83.25	500	0.553	332.33	548.36
94.25	500	0.598	359.52	880.69

Table 45. Planktonic Experiment #3 – 35 mg/L glutaraldehyde

Time (hr)	Sulfide	
	Dilution	A ₆₇₀ (mg S/L)
0.00		0.00
47.00	25	0.005
53.50	25	0.002
66.50	25	0.006
83.25	25	0.002
94.25	25	0.007
110.50	25	0.005
131.00	25	0.004
147.92	25	0.003
160.50	25	0.006
172.25	25	0.004
184.17	25	0.007
195.58	25	0.007
210.50	25	0.010
231.25	25	0.011
241.17	25	0.010
256.92	25	0.011
266.92	25	0.021
275.75	25	0.013
286.00	25	0.013
302.00	25	0.011
330.33	25	0.013
353.58	25	0.011

Table 46. Planktonic Experiment #3 – 65 mg/L glutaraldehyde

Time (hr)	Dilution	Sulfide	
		A_{670}	(mg S/L)
0.00			0.00
241.25	25	0.008	0.15
266.92	25	0.003	0.00
286.00	25	0.009	0.18
302.00	25	0.009	0.18
330.33	25	0.007	0.12
353.58	25	0.007	0.12

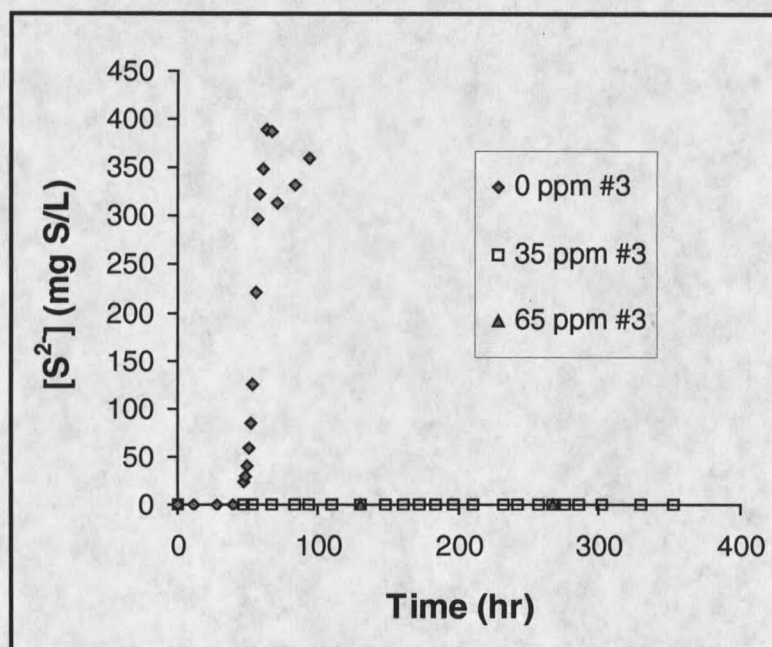


Figure 38. Planktonic Experiment #3. Sulfide recovery profiles in response to varying concentrations of glutaraldehyde.

APPENDIX F: Biofilm Experiment Data

Table 47. Influent data for Control experiment #2 (Single dose of ultrapure water)

Time (hr)	Dilution	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
		A ₆₇₀	(mg S/L)			
81.30	25	0.001	0.00	208.77	208.77	<i>Post ultrapure water treatment</i>
104.80	25	0.004	0.00	100.77	100.77	
112.75	25	0.002	0.00	108.97	108.97	
125.25	25	0.000	0.00	71.36	71.36	
134.00	25	0.000	0.00	0.00	0.00	
140.25	25	0.000	0.00	94.14	94.14	
153.67	25	0.005	0.00	93.35	93.35	
157.25	25	0.000	0.00	92.24	92.24	
164.75	25	0.000	0.00	82.91	82.91	
176.75	25	0.003	0.00	100.25	100.25	
199.67	25	0.000	0.00	81.92	81.92	
230.37	25	0.003	0.00	94.83	94.83	

Table 48. Effluent data for Control experiment #2 (Single dose of ultrapure water)

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
	Dilution	A ₈₇₀ (mg S/L)			
0.00		0.00			<i>Inoculated, pH=7.56</i>
13.83	50	0.005	0.00	83.86	83.86
24.00	50	0.028	0.70	89.48	90.18
37.00	50	0.051	2.52	78.04	80.56
47.00	50	0.094	5.91	82.33	88.24
60.50	50	0.612	46.76	38.94	85.70
67.00	50	0.823	63.40	29.08	92.48
67.08	100	0.452	68.28	29.28	97.56
67.75					<i>Initiated CSTR</i>
81.25	25	0.623	23.81	78.12	101.93
86.25	50	0.320	23.73	77.84	101.57
91.92	50	0.383	28.70	67.47	96.17
97.83	50	0.451	34.06	50.22	84.28
99.50	50	0.533	40.53	63.68	104.21
104.75	50	0.657	50.31	49.25	99.56
112.83	50	0.755	58.04	45.86	103.89
125.58	100	0.333	49.51	45.01	94.52
125.58	100	0.355	52.98	44.27	97.26
125.58	100	0.345	51.40	44.70	96.11
127.00					<i>Media flow terminated</i>
127.55	50	0.567	43.21	34.54	77.75
128.00	50	0.452	34.14	28.19	62.33
129.00	50	0.281	20.65	19.96	40.62
130.00	25	0.402	15.10	14.96	30.05
131.00	25	0.261	9.54	11.89	21.43
132.00	25	0.138	4.69	9.58	14.27
132.50	25	0.150	5.16	9.27	14.43
133.00					<i>Ultrapure water treatment</i>
134.17	25	0.009	0.00	8.00	8.00
135.00	25	0.000	0.00	7.50	7.50
136.25	25	0.000	0.00	7.40	7.40
138.05	25	0.001	0.00	6.92	6.92
139.67					<i>Resumed media flow</i>
140.25	25	0.004	0.00	25.99	25.99
141.75	25	0.161	5.60	56.17	61.76
143.25	25	0.312	11.55	68.79	80.34
145.25	25	0.619	23.66	68.14	91.79
147.25	50	0.491	37.22	59.06	96.27
149.25	50	0.637	48.73	47.83	96.56
153.75	100	0.383	57.40	47.50	104.90
153.75	100	0.304	44.94	46.77	91.70
153.75	100	0.374	55.98	46.55	102.53
157.33	100	0.319	47.30	47.24	94.54
164.75	100	0.310	45.88	44.92	90.80
165.00	100	0.327	48.56	47.94	96.50
176.75	100	0.348	51.88	44.92	96.80
199.75	100	0.339	50.46	45.08	95.54
199.75	50	0.688	52.75	46.39	99.14
230.50	100	0.348	51.88	46.94	98.82
					<i>Experiment terminated</i>

Table 49. Influent data for Control experiment #3 (Repetitive dose of ultrapure water)

Time (hr)	Dilution	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
		A ₆₇₀	(mg S/L)			
84.75	25	-0.001	0.00	119.84	119.84	
108.75	25	0.000	0.00	172.09	172.09	
120.83	25	-0.008	0.00	113.80	113.80	
125.50	25	0.002	0.00	6.48	6.48	<i>During media washout</i>
131.58	25	0.000	0.00	5.87	5.87	<i>During dosing period</i>
135.25	25	0.000	0.00	107.79	107.79	
143.33	25	0.000	0.00	190.08	190.08	
159.33	25	0.000	0.00	100.29	100.29	
171.25	25	0.000	0.00	141.55	141.55	
182.33	25	0.001	0.00	102.46	102.46	
203.33	25	0.000	0.00	103.98	103.98	
219.75	25	0.000	0.00	103.80	103.80	
223.75	25	0.000	0.00	103.12	103.12	
229.75	25	0.003	0.00	121.35	121.35	
253.75	25	0.001	0.00	104.63	104.63	
279.25	25	0.000	0.00	102.22	102.22	
289.92	25	0.000	0.00	98.45	98.45	

Table 50. Effluent data for Control experiment #3 (Repetitive dose of ultrapure water)

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
	Dilution	A ₆₇₀ (mg S/L)			
0.00		0.00			<i>Inoculated, pH=7.21</i>
16.75	25	0.005	108.37	108.37	
24.00	25	0.014	113.53	113.53	
41.75	25	0.238	95.23	103.86	
48.00	50	0.505	65.10	103.42	
54.17	100	0.273	49.84	89.89	
60.00	100	0.290	47.02	89.74	
60.00	100	0.291	46.55	89.44	
66.17	100	0.341	42.68	93.46	
66.83					<i>Initiated CSTR mode</i>
73.75	25	0.474	83.78	101.71	
84.75	50	0.231	82.90	99.61	
91.17	25	0.653	78.48	103.48	
97.50	50	0.651	56.50	106.34	
108.83	100	0.383	47.80	105.19	
120.75	100	0.339	48.22	98.68	
120.75	100	0.365	46.16	100.72	
121.50					<i>Media flow terminated</i>
122.50	50	0.495	30.58	68.11	
123.50	50	0.282	21.82	42.55	
124.50	25	0.389	14.24	28.82	
125.50	25	0.238	11.34	19.97	
126.50	25	0.141	8.91	13.72	
127.00	25	0.128	8.53	12.83	
127.50					<i>Ultrapure water treatment</i>
128.50	25	0.046	7.32	8.38	
129.50	25	0.013	6.98	6.98	
131.50	25	0.008	6.22	6.22	
133.92	25	0.000	5.90	5.90	
134.25					<i>Resumed media flow</i>
135.25	25	0.121	33.08	37.09	
137.33	50	0.469	45.80	81.28	
139.37	50	0.569	47.56	90.93	
141.25	50	0.546	47.95	89.50	
143.33	50	0.719	47.56	102.75	
147.25	100	0.376	48.71	105.01	
147.25	100	0.370	47.56	102.91	
147.25	100	0.365	48.66	103.22	
159.33	100	0.322	46.72	94.50	
171.25	100	0.336	46.76	96.74	
182.33	100	0.341	47.59	98.36	
203.25	100	0.369	45.94	101.13	
203.25	100	0.386	45.79	103.66	
203.25	100	0.370	46.84	102.18	
203.75					<i>Media flow terminated</i>
204.75	50	0.509	28.82	67.45	
205.75	50	0.337	19.82	44.89	
206.75	25	0.331	14.11	26.41	

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
	Dilution	A ₆₇₀ (mg S/L)			
207.75	25	0.276	10.13	10.63	20.76
208.75	25	0.161	5.60	8.71	14.31
209.75	25			7.56	7.56
210.25					<i>Ultrapure water treatment #2</i>
211.50	25	0.016	0.00	6.81	6.81
212.33	25	0.025	0.23	6.11	6.34
214.25	25	0.029	0.39	5.85	6.24
216.25	25	0.000	0.00	0.00	0.00
216.75					<i>Resumed media flow</i>
217.75	25	0.159	5.52	32.78	38.30
219.75	50	0.352	26.25	51.16	77.41
221.75	50	0.644	49.28	45.75	95.03
223.75	100	0.334	49.67	46.48	96.14
227.75	100	0.372	55.66	47.20	102.86
229.75	100	0.362	54.09	46.57	100.66
253.75	100	0.372	55.66	48.87	104.53
279.25	100	0.328	48.72	47.45	96.17
279.25	100	0.364	54.40	48.57	102.97
279.25	100	0.377	56.45	46.85	103.30
286.00	100	0.364	54.40	46.41	100.81 <i>Experiment terminated</i>

Table 51. Influent data for Dosing experiment #2 (Single dose of glutaraldehyde)

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
	Dilution	A ₆₇₀ (mg S/L)			
0.00			119.16	119.16	
48.67	25	0.000	0.00	115.46	115.46
70.00	25	0.001	0.00	116.52	116.52
88.08	25	0.000	0.00	99.56	99.56
98.50	25	0.000	0.00	0.00	0.00
108.58	25	0.000	0.00	113.91	113.91
113.50	25	0.000	0.00	114.41	114.41
121.25	25	0.000	0.00	112.85	112.85
136.08	25	0.001	0.00	114.13	114.13
154.67	25	0.002	0.00	122.11	122.11
173.50	10	0.001	0.00	117.57	117.57
196.00	25	0.000	0.00	113.42	113.42
210.00	25	0.000	0.00	120.81	120.81 ^s
232.00	25	0.017	0.00	118.26	118.26
258.25	25	0.000	0.00	102.61	102.61

Table 52. Effluent data for Dosing experiment #2 (Single dose of glutaraldehyde)

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
	Dilution	A ₆₇₀ (mg S/L)			
0.00			0.00		<i>Inoculated; pH=7.18</i>
42.00	50	0.784	60.32	50.57	110.89
42.75	100	0.380	56.92	45.55	102.47
42.83					<i>Initiated CSTR mode</i>
48.58	50	0.416	31.30	82.41	113.71
61.75	100	0.249	36.26	54.12	90.38
70.00	100	0.309	45.73	50.11	95.84
76.00	100	0.368	55.03	52.23	107.27
88.00	100	0.373	55.82	50.71	106.53
93.83	100	0.413	62.13	52.24	114.36
93.83	100	0.411	61.81	51.21	113.02
93.83	100	0.381	57.08	50.25	107.34
94.50					<i>Media flow terminated</i>
95.50	50	0.527	40.06	29.92	69.97
96.50	50	0.302	22.31	18.10	40.41
97.50	25	0.474	17.94	11.55	29.49
98.50	25	0.261	9.54	7.66	17.20
99.50	25	0.189	6.70	4.94	11.64
100.00	25	0.079	2.36	4.38	6.74
100.78					<i>Glutaraldehyde Treatment</i>
101.75	25	0.045	1.02	2.73	3.75
102.75	25	0.016	0.00	2.30	2.30
104.75	25	0.004	0.00	2.23	2.23
106.75	25	0.000	0.00	0.00	0.00
107.50					<i>Resumed media flow</i>
108.50	25	0.000	0.00	38.80	38.80
109.50	25	0.001	0.00	66.45	66.45
113.50	25	0.001	0.00	108.98	108.98
117.50	25	0.005	0.00	113.45	113.45
121.25	25	0.007	0.00	115.08	115.08
129.50	25	0.012	0.00	112.94	112.94
136.00	10	0.040	0.33	113.03	113.36
141.50	10	0.041	0.35	122.57	122.91
147.50	10	0.045	0.41	113.74	114.15
154.50	10	0.078	0.93	113.69	114.62
156.50	10	0.081	0.98	113.41	114.39
158.50	10	0.089	1.10	114.91	116.01
164.00	10	0.150	2.06	115.30	117.36
167.50	10	0.233	3.37	116.37	119.75
169.50	10	0.356	5.31	108.56	113.87
173.50	10	0.549	8.36	101.93	110.29
179.00	25	0.540	20.54	92.43	112.97
181.00	50	0.344	25.62	86.03	111.65
183.00	50	0.505	38.32	73.97	112.29
185.00	100	0.301	44.46	59.58	104.04
187.00	100	0.330	49.04	53.63	102.66
188.50	100	0.375	56.14	55.25	111.38
191.75	100	0.351	52.35	50.57	102.92
196.00	100	0.352	52.51	49.11	101.61

Time (hr)	Dilution	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
		A ₆₇₀	(mg S/L)			
210.00	100	0.342	50.93	48.39	99.32	
232.00	100	0.346	51.56	48.97	100.54	<i>New media, pH=7.25</i>
268.00	100	0.355	52.98	47.10	100.08	
268.00	100	0.327	48.56	46.38	94.94	<i>Experiment Terminated</i>

Table 53. Influent data for Dosing experiment #4 (Repetitive dose of glutaraldehyde)

Time (hr)	Dilution	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
		A ₆₇₀	(mg S/L)			
0.00				98.70	98.70	
40.00	25	0.000	0.00	96.42	96.42	
69.08	10	0.000	0.00	94.16	94.16	
84.42	10	0.000	0.00	92.39	92.39	
90.50	10	0.000	0.00	0.00	0.00	
104.25	10	0.000	0.00	98.37	98.37	New sulfide assay reagents
128.00	10	0.000	0.00	91.86	91.86	
152.00	10	0.000	0.00	96.86	96.86	
202.58	10	0.000	0.00	97.45	97.45	
218.33	10	0.000	0.00	84.35	84.35	New media, pH=7.27
219.25	10	0.000	0.00	94.98	94.98	
247.50	10	0.000	0.00	95.60	95.60	
273.00	10	0.002	0.00	96.47	96.47	
299.67	10	0.000	0.00	91.40	91.40	
323.92	10	0.000	0.00	101.07	101.07	
332.25	25	0.000	0.00	96.88	96.88	

Table 54. Effluent sampling data for Dosing experiment #4 (Repetitive dose of glutaraldehyde)

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
	Dilution	A ₆₇₀ (mg S/L)			
0.00		0.00			<i>Inoculated, pH=7.16</i>
12.00	10	0.142	1.94	95.89	97.82
16.50	10	0.249	3.63	96.88	100.51
20.00	10	0.334	4.97	101.52	106.49
26.00	25	0.314	11.63	88.10	99.73
30.00	50	0.330	24.52	60.42	84.94
32.00	50	0.601	45.89	42.86	88.75
36.00	50	0.648	49.60	39.35	88.95
37.25	50	0.708	54.33	37.53	91.86
38.00					<i>Initiated CSTR mode</i>
40.00	25	0.641	24.52	61.29	85.81
41.00	25	0.474	17.94	68.19	86.13
42.00	25	0.431	16.24	71.86	88.10
44.00	25	0.407	15.30	74.09	89.39
45.00	25	0.396	14.86	79.60	94.46
46.00	25	0.479	18.13	74.91	93.04
52.75	25	0.654	25.04	72.73	97.76
57.00	50	0.401	30.12	64.89	95.01
61.00	50	0.582	44.39	55.32	99.71
65.00	50	0.678	51.96	48.93	100.89
69.00	50	0.655	50.15	46.46	96.61
83.00	50	0.654	40.87	47.24	88.11
84.50	50	0.589	36.76	46.66	83.41
84.50	50	0.637	39.79	48.56	88.35
84.50	50	0.639	39.92	46.67	86.59
85.00					<i>Media flow terminated</i>
86.00	50	0.478	29.73	28.13	57.87
87.00	50	0.326	20.12	18.37	38.49
88.00	25	0.384	11.89	13.17	25.06
89.00	25	0.299	9.21	9.89	19.10
90.00	10	0.451	5.61	8.19	13.80
90.50	10	0.362	4.48	7.74	12.22
91.50					<i>Glutaraldehyde treatment</i>
92.00	10	0.185	2.24	7.07	9.31
93.50	10	0.116	1.37	6.10	7.46
95.50	10	0.035	0.34	6.10	6.44
97.75	10	0.000	0.00	0.00	0.00
98.25					<i>Resumed media flow</i>
99.25	10	0.001	0.00	35.02	35.02
104.25	10	0.021	0.17	93.84	94.01
110.75	10	0.040	0.41	106.35	106.75
119.08	10	0.065	0.72	96.68	97.40
124.00	10	0.074	0.84	93.63	94.47
128.00	10	0.088	1.01	93.94	94.95
133.00	10	0.125	1.48	95.88	97.36
137.00	10	0.160	1.92	97.65	99.58
143.25	10	0.263	3.23	91.85	95.08
147.75	10	0.556	6.93	88.23	95.16

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
	Dilution	A ₆₇₀ (mg S/L)			
149.75	10	0.677	8.46	85.91	94.38
152.00	25	0.334	10.31	83.37	93.68
155.00	25	0.675	21.10	70.63	91.73
157.00	50	0.514	32.01	61.23	93.24
159.00	50	0.578	36.06	48.55	84.61
161.00	50	0.628	39.22	47.80	87.02
165.00	50	0.677	42.32	46.64	88.96
170.00	50	0.563	35.11	46.19	81.30
177.00	50	0.701	43.84	44.73	88.57
186.00	50	0.732	45.80	44.32	90.12
202.00	50	0.666	41.63	44.50	86.13
203.00					Media flow terminated
205.00	25	0.617	19.26	14.83	34.09
206.00	25	0.473	14.71	20.98	35.68
207.50	25	0.244	7.47	10.40	17.87
209.08	10	0.222	2.71	7.85	10.56
210.00	10	0.135	1.61	7.74	9.35
210.50					Glutaraldehyde treatment #2
211.50	10	0.019	0.14	6.86	7.00
213.50	10	0.004	0.00	6.19	6.19
216.75	10	0.000	0.00	0.00	0.00
217.28					Resumed media flow
218.28	10	0.004	0.00	35.46	35.46 New media, pH=7.27
219.25	10	0.004	0.00	56.72	56.72
224.50	10	0.010	0.03	89.26	89.29
229.00	10	0.028	0.25	90.41	90.66
233.00	10	0.044	0.46	97.82	98.28
237.00	10	0.052	0.56	92.41	92.97
242.33	10	0.083	0.95	94.38	95.33
247.50	10	0.123	1.46	95.33	96.79
251.50	10	0.170	2.05	91.57	93.62
255.50	10	0.236	2.89	89.67	92.56
259.50	10	0.440	5.47	89.21	94.67
263.50	10	0.812	10.17	83.62	93.79
264.25	25	0.360	11.14	81.73	92.87
265.50	25	0.409	12.69	78.16	90.85
269.00	50	0.352	21.76	69.91	91.67
273.00	50	0.480	29.86	51.15	81.01
277.75	50	0.526	32.77	46.02	78.79
279.50	50	0.654	40.87	45.96	86.83
281.00	50	0.534	33.28	47.10	80.37
288.17	100	0.317	39.10	47.17	86.27
299.67	50	0.534	33.28	46.19	79.46
323.92	50	0.689	43.08	44.40	87.48
332.25	50	0.694	43.40	46.32	89.72
332.25	50	0.669	41.82	44.57	86.38 Experiment terminated

Table 55. Influent sampling data for Dosing experiment #6 (Repetitive dose of nitrite).

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Nitrite			Comments
	Dilution	A ₆₇₀ (mg S/L)			Dilution	A ₅₄₆ (mg N/L)		
0.00				95.51	95.51			
21.50	10	0.000	0.00	103.43	103.43			
83.00	10	0.001	0.00	106.84	106.84			
116.75	10	0.001	0.00	90.35	90.35			
160.00	10	-0.011	0.00	92.87	92.87			
166.17	25	-0.001	0.00	94.60	94.60	100	1.408	111.83 Nitrite Treatment
166.50				89.89	89.89	100	1.403	111.43
167.00				85.47	85.47	100	1.243	98.75
167.50				87.52	87.52	100		
168.00				90.41	90.41	100	1.454	115.47
168.50				91.49	91.49	100	1.462	116.11
169.00						100	1.502	119.28
169.50						100	1.463	116.18
170.00				88.69	88.69	100	1.397	110.95
170.50						100	1.347	106.99
171.00	10	-0.008	0.00	90.53	90.53	100	1.367	108.58
171.50						100	1.458	115.79
172.00				88.84	88.84	100	1.458	115.79
172.50						100	1.368	108.65
173.00						100	1.398	111.03
174.00						100	1.443	114.60
175.00				89.76	89.76	100	1.429	113.49
176.50						100	1.400	111.19
186.75	10	-0.007	0.00	93.23	93.23	100	1.476	117.21
189.00				91.90	91.90	100	1.416	112.46
191.00	10	0.002	0.00	91.02	91.02	50	0.000	0.00 Ceased nitrite
196.00	10	0.001	0.00	90.92	90.92	10	0.002	0.00
209.00	10	0.002	0.00	86.30	86.30			
261.00	10	0.000	0.00	100.98	100.98			New media pH=7.28
262.08	25	0.000	0.00			100	1.469	116.66 Nitrite Treatment
262.17				76.84	76.84	100	1.497	118.88
262.50				82.40	82.40	100	1.437	114.12
263.00				100.93	100.93	100	1.403	111.43
264.00				87.02	87.02	100	1.404	111.51
264.50				88.71	88.71	100	1.486	118.01
265.00				115.44	115.44	100	1.332	105.80
265.50				93.23	93.23	100	1.443	114.60
266.00				90.18	90.18	100	1.385	110.00
266.50				91.28	91.28	100	1.465	116.34
267.00				78.02	78.02	100	1.381	109.69
268.00				58.89	58.89	100	1.454	115.47
269.00				100.76	100.76	100	1.394	110.72
270.00	10	-0.007	0.00	104.07	104.07	100	1.419	112.70
271.00				90.21	90.21	100	1.381	109.69
273.00				90.38	90.38	100	1.356	107.70
280.25				89.96	89.96	100	1.415	112.38
285.00				92.58	92.58	100	1.379	109.53
287.00				91.64	91.64	10	0.000	0.00 Ceased nitrite
294.00	10	0.001	0.00	94.99	94.99			
350.00	10	0.002	0.00	94.81	94.81			

Table 56. Effluent sampling data for Dosing experiment #6 (Repetitive dose of nitrite)

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Nitrite			Comments
	Dilution	A ₆₇₀ (mg S/L)			Dilution	A ₅₄₆ (mg N/L)		
0.00								<i>Inoculated, pH=7.19</i>
46.50	50	0.568	41.22	56.47				
72.25	50	0.634	45.77	47.16				
80.50	50	0.586	42.46	47.77				
81.00								
83.00	25	0.474	17.37	78.00				<i>Initiate CSTR Mode</i>
85.00	25	0.208	8.20	89.81				<i>New media pH=7.24</i>
90.75	10	0.242	3.75	96.08				
94.58	10	0.204	3.23	94.77				
98.75	10	0.164	2.68	98.77				
114.67	25	0.340	12.75	80.86				
116.75	25	0.442	16.27	77.19				
118.75	50	0.255	19.65	77.61				
122.00	50	0.218	17.10	68.00				
129.00	50	0.473	34.67	58.42				
131.00	50	0.544	39.57	56.54				
133.00	50	0.531	38.67	55.95				
134.83	50	0.669	48.18	51.25				
134.83	50	0.676	48.66					
145.50	50	0.646	46.60	47.03				
160.00	50	0.708	50.87	45.69				
164.25	50	0.729	52.32	44.74				
164.25	50	0.749	53.69	39.86				
164.25	50	0.770	55.14	44.42				
166.08								<i>Nitrite Treatment</i>
166.17	50	0.719	51.63	43.51	100	0.031	2.69	
166.50	50	0.610	44.11	49.59	100	0.210	16.87	
167.00	50	0.538	39.15	57.74	100	0.431	34.39	
167.50	25	0.669	24.09	65.25	100			
168.00	25			67.96	100	0.753	59.91	
168.50	25	0.427	15.75	71.80	100	0.821	65.30	
169.00	25	0.345	12.93	73.19	100	1.001	79.57	
169.50	10	0.702	10.09	78.58	100	1.011	80.36	
170.00	10	0.531	7.73	82.01	100	1.124	89.32	
170.50	10	0.466	6.84	84.25	100	1.142	90.74	
171.00	10	0.339	5.09	84.21	100	1.196	95.02	
171.50	10	0.316	4.77	86.67	100	1.213	96.37	
172.00	10	0.248	3.83	87.23	100	1.266	100.57	
173.00	10	0.134	2.26	89.68	100	1.259	100.02	
174.00	5	0.240	1.86	91.48	100	1.309	103.98	
175.00	5	0.164	1.34	88.20	100	1.383	109.84	
176.50	5	0.115	1.00	90.30	100	1.348	107.07	
186.75	5	0.024	0.00	84.47	100	1.378	109.45	
189.00	5	0.033	0.00	90.54	100	1.373	109.05	
190.08								<i>Ceased nitrite</i>
190.17	10	0.014	0.00	91.12	100	1.365	108.42	
190.50	10	0.020	0.00	89.83	100	1.170	92.96	
191.00	10	0.019	0.00	90.34	100	0.850	67.60	

Time (hr)	Sulfide			Sulfate (mg S/L)	Total S (mg S/L)	Nitrite			Comments
	Dilution	A ₆₇₀	(mg S/L)			Dilution	A ₅₄₆	(mg NL)	
191.50						100	0.671	53.41	
192.00	10	0.028	0.00	93.06	93.06	100	0.526	41.92	
192.50						100	0.418	33.36	
193.00	10	0.033	0.00	88.70	88.70	100	0.311	24.88	
193.50						100	0.240	19.25	
194.00	10	0.051	1.12	89.19	90.30	50	0.355	14.18	
194.50						50	0.265	10.62	
195.00	10	0.058	1.21	89.83	91.05	50	0.183	7.37	
195.50						10	0.608	4.84	
196.00	10	0.070	1.38	88.26	89.64	10	0.365	2.92	
196.50						10	0.183	1.47	
197.00	10	0.087	1.61	88.57	90.18	10	0.076	0.63	
197.50						10	0.004	0.00	
198.00	10	0.120	2.07	88.43	90.50	10	0.002	0.00	
198.50						10	0.004	0.00	
199.00	10	0.167	2.72	89.64	92.36				
200.00	10	0.212	3.34	88.26	91.60				
202.00	10	0.276	4.22	86.82	91.04				
205.00	10	0.420	6.20	84.91	91.11				
206.92	10	0.514	7.50	83.93	91.43				
209.00	10	0.656	9.46	82.51	91.97				
211.00	25	0.310	11.72	79.10	90.82				
213.25	25	0.415	15.34	74.62	89.96				
216.25	50	0.321	24.20	68.22	92.41				
218.00	50	0.373	27.78	61.40	89.18				
221.00	50	0.586	42.46	48.44	90.90				
222.00	50	0.634	45.77	47.13	92.90				
223.00	50	0.686	49.35	46.75	96.11				
227.00	50	0.674	48.53	46.17	94.69				
230.00	50	0.738	52.94	43.74	96.67				
239.25	50	0.672	48.39	43.14	91.53				
239.25	50	0.757	54.25	42.56	96.80				
239.25	50	0.778	55.69	43.94	99.63				
243.75	50	0.716	51.42	40.04	91.46				
258.00	50	0.794	56.80	39.50	96.30				
261.00	100	0.394	58.46	41.89	100.34				New media pH=7.28
261.00	100	0.398	59.01	41.52	100.53				
261.00	100	0.399	59.15	41.68	100.82				
262.08	50								Nitrite Treatment #2
262.17	50	0.762	54.59	43.38	97.97	100	0.027	2.37	
262.50	50	0.660	47.56	46.49	94.05	100	0.171	13.78	
263.00	50	0.568	41.22	55.01	96.23	100	0.407	32.49	
264.00	25	0.674	24.26	63.78	88.04	100	0.726	57.77	
264.50	25	0.563	20.44	68.00	88.43	100	0.807	64.19	
265.00	10	1.025	14.54	72.90	87.44	100	0.903	71.80	
265.50	10	0.785	11.24	71.21	82.44	100	0.925	73.54	
266.00	10	0.709	10.19	73.93	84.12	100	1.012	80.44	
266.50	10	0.565	8.20	76.08	84.28	100	1.097	87.18	
267.00	10	0.463	6.80	91.51	98.31	100	1.047	83.21	

Time (hr)	Sulfide			Sulfate (mg S/L)	Total S (mg S/L)	Nitrite			Comments
	Dilution	A ₆₇₀	(mg S/L)			Dilution	A ₅₄₆	(mg N/L)	
267.50	10	0.391	5.80	79.62	85.42	100	1.140	90.58	Ceased nitrite
268.00	10	0.314	4.74	77.67	82.41	100	1.119	88.92	
269.00	10	0.208	3.28	81.73	85.01	100	1.190	94.55	
270.00	10	0.164	2.68	87.51	90.18	100	1.125	89.40	
271.00	10	0.111	1.94	88.11	90.05	100	1.239	98.43	
273.00	10	0.104	1.85	90.04	91.89	100	1.214	96.45	
280.25	10	-0.166	0.00	87.36	87.36	100	1.175	93.36	
285.00	10	0.046	0.00	82.95	82.95	100	1.283	101.92	
286.08									
286.17	10	0.039	0.00	85.36	85.36	100	1.219	96.85	
286.50	10	0.040	0.00	83.19	83.19	100	1.004	79.81	
287.00	10	0.053	0.00	89.96	89.96	100	0.807	64.19	
288.00	10	0.094	1.71	89.17	90.88	100	0.426	33.99	
288.50						100	0.303	24.25	
289.00	10	0.133	2.25	87.86	90.11	100	0.212	17.03	
289.50						50	0.301	12.04	
290.00	10	0.222	3.47	86.17	89.65	50	0.192	7.72	
290.50						50	0.091	3.72	
291.00	10	0.504	7.36	83.22	90.58	50	0.038	1.62	
292.00	10	0.931	13.25	79.46	92.71	10	0.009	0.00	
292.50	25	0.453	16.65	75.40	92.05	10	0.006	0.00	
293.00	25	0.518	18.89	70.28	89.16				
294.00	25	0.678	24.40	66.82	91.22				
295.00	50	0.434	31.98	62.95	94.93				
296.00	50	0.479	35.09	56.27	91.35				
303.00	50	0.611	44.18	51.80	95.98				
308.00	50	0.655	47.22	47.11	94.33				
312.50	50	0.760	54.45	42.03	96.48				
316.50	50	0.776	55.55	42.07	97.62				
332.25	50	0.720	51.70	40.66	92.36				
343.00	50	0.755	54.11	39.97	94.08				
350.00	50	0.778	55.69	39.85	95.54				
350.00	50	0.760	54.45	39.88	94.34				
350.00	50	0.804	57.48	39.86	97.34				

Experiment terminated

Table 57. Influent sampling data for Dosing experiment #8 (Repetitive dose of glutaraldehyde)

Time (hr)	Dilution	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
		A ₆₇₀	(mg S/L)			
73.08	10	-0.001	0.00	117.80	117.80	
94.08	10	0.000	0.00	109.90	109.90	
119.08	10	0.001	0.00	116.71	116.71	
145.58	10	0.004	0.00	111.28	111.28	
159.67	10	0.000	0.00	5.23	5.23	
175.83	10	0.002	0.00	106.71	106.71	
210.17	10	0.000	0.00	115.75	115.75	
224.58						<i>New media, pH=7.23</i>
238.08	10	0.009	0.00	97.34	97.34	
261.83	10	0.001	0.00	102.02	102.02	
286.67	10	0.000	0.00	95.30	95.30	
310.83	10	-0.001	0.00	95.97	95.97	
335.08	10	0.000	0.00	97.62	97.62	
357.33	10	0.001	0.00	104.19	104.19	
389.92	10	-0.001	0.00	104.60	104.60	
413.83	10	0.000	0.00	104.86	104.86	

Table 58. Effluent sampling data for Dosing experiment #8 (Repetitive dose of glutaraldehyde)

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
	Dilution	A ₆₇₀ (mg S/L)			
0.00		0.00			<i>Inoculated, pH=7.07</i>
48.00	50	0.737	52.87	60.59	113.45
60.00	100	0.358	53.49	61.70	115.19
72.00	100	0.384	57.08	53.74	110.82
72.00	100	0.393	58.32	54.09	112.41
72.00	100	0.434	63.97	52.94	116.91
72.08					<i>Initiated CSTR mode</i>
73.08	50	0.594	43.01	73.13	116.14
74.08	50	0.389	28.88	84.54	113.42
77.08	10	0.642	9.26	99.82	109.09
80.08	10	0.273	4.18	106.12	110.30
87.08	10	0.189	3.02		<i>Dil. Water off for ~1hr.</i>
94.08	10	0.281	4.29	110.08	114.37
98.42	10	0.394	5.85	109.00	114.85
102.17	10	0.671	9.66	105.70	115.36
106.33	25	0.429	15.82	89.65	105.47
108.08	25	0.603	21.82	86.79	108.61
109.08	50	0.312	23.58	87.15	110.73
110.08	50	0.382	28.40	78.59	107.00
112.08	50	0.453	33.29	74.37	107.66
114.08	50	0.501	36.60	69.46	106.06
116.08	50	0.596	43.15	62.36	105.51
119.08	100	0.363	54.18	54.15	108.34
121.58	100	0.367	54.73	53.24	107.98
130.08	100	0.308	46.60	56.70	103.30
143.08	100	0.340	51.01	50.27	101.28
145.58	100	0.402	59.56	52.75	112.31
145.58	100	0.376	55.98	51.68	107.66
145.58	100	0.392	58.18	50.43	108.61
146.83					<i>Media flow terminated</i>
147.83	50	0.594	43.01	33.46	76.47
149.83	25	0.468	17.16	15.57	32.74
150.83	25	0.265	10.17		
151.83	25	0.185	7.41	9.37	16.78
152.83	10	0.385	5.72	7.86	13.58
153.42					<i>Glutaraldehyde treatment</i>
153.83	10	0.288	4.38	7.14	11.53
154.83	10	0.171	2.77	5.04	7.81
157.08	10	0.055	1.17	5.71	6.88
159.67	10	0.019	0.00	5.82	5.82
160.17					<i>Resumed media flow</i>
168.33	10	0.000	0.00	110.72	110.72
175.75	10	0.017	0.00	101.39	101.39
184.17	10	0.032	0.86	101.44	102.30
190.67	10	0.034	0.88	104.96	105.84
195.83	10	0.031	0.84	106.45	107.29
201.92	10	0.038	0.94	103.02	103.96
210.17	10	0.073	1.42	102.37	103.79

Time (hr)	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
	Dilution	A ₅₇₀ (mg S/L)			
214.83	10	0.148	2.45	101.25	103.71
219.42	10	0.293	4.45	96.58	101.03
222.92	10	0.379	5.64	93.39	99.03
224.25	10	0.546	7.94	90.47	98.41
226.33	25	0.249	9.62	88.19	97.81
227.33	25	0.333	12.51	86.44	98.95
228.33	25	0.408	15.10	85.61	100.70
229.33	25	0.447	16.44	87.88	104.32
230.33	25	0.605	21.88	84.26	106.15
231.33	25	0.780	27.92	74.10	102.02
233.83	50	0.611	44.18	58.10	102.28
234.83	50	0.635	45.84	53.21	99.05
235.83	50	0.725	52.04	49.98	102.02
236.83	100	0.338	50.74	47.54	98.28
246.83	100	0.320	48.26	46.22	94.47
261.83	100	0.321	48.39	48.03	96.43
265.83	100	0.320	48.26	48.33	96.59
266.83					Media flow terminated
267.83	50	0.373	27.78	31.17	58.95
268.83	25	0.561	20.37	21.09	41.46
269.83	25	0.324	12.20	15.69	27.89
270.83	10	0.727	10.44	11.90	22.34
271.83	10	0.541	7.87	9.55	17.42
273.33	10	0.273	4.18	7.64	11.81
273.42					Glutaraldehyde treatment #2
273.83	10	0.218	3.42	7.94	11.36
274.83	10	0.110	1.93	6.31	8.24
277.08	10	0.046	1.05	10.37	11.42
279.67	10	0.000	0.41	0.00	0.41
280.17					Resumed media flow
286.67	10	0.002	0.00	95.26	95.26
291.33	10	0.000	0.00	97.48	97.48
297.92	10	0.018	0.66	97.96	98.62
304.25	10	0.024	0.75	103.25	103.99
310.83	10	0.023	0.73	99.90	100.63
316.25	10	0.026	0.77	101.19	101.96
322.67	10	0.023	0.73	103.61	104.34
329.92	10	0.032	0.86	109.28	110.14
335.08	10	0.053	1.15		
340.00	10	0.073	1.42	101.42	102.84
344.58	10	0.165	2.69	101.25	103.94
346.33	10	0.216	3.39	100.28	103.67
348.33	10	0.281	4.29	98.11	102.40
351.33	10	0.621	8.97	92.71	101.68
352.33	10	0.649	9.36	92.05	101.41
353.33	25	0.306	11.58	91.36	102.94
354.33	25	0.379	14.10	85.84	99.93
356.33	25	0.425	15.68	80.79	96.47
357.33	25	0.566	20.54	79.57	100.11

Time (hr)	Dilution	Sulfide		Sulfate (mg S/L)	Total S (mg S/L)	Comments
		A ₆₇₀	(mg S/L)			
358.33	25	0.725	26.02	69.99	96.01	
359.33	50	0.467	34.26	68.09	102.35	
360.33	50	0.534	38.88	59.25	98.12	
361.33	50	0.708	50.87	49.30	100.17	
362.33	50	0.607	43.91	48.99	92.90	
363.58	50	0.690	49.63	47.67	97.29	
370.58	50	0.712	51.14	49.21	100.35	
384.08	50	0.736	52.80	46.57	99.37	
389.92	50	0.711	51.08	47.72	98.79	
413.83	100	0.338	50.74	49.73	100.47	
413.83	100	0.353	52.80	49.78	102.58	
413.83	100	0.344	51.56	49.28	100.84	Experiment terminated

APPENDIX G: Biofilm Thickness Data

Table 59. Biofilm thickness data for Control #2

Control #2	Coupon			
	#19	#6	#16	#4
Images	Steady-State (μm)	Before Dose (μm)	After Dose (μm)	Return to Steady-State (μm)
1	1.70	1.18	4.94	0.00
	0.00	2.38	6.13	2.18
	1.80	0.00	4.73	4.26
	0.00	0.00	3.38	5.05
	5.40	1.70	5.01	5.31
	0.00	1.21	4.82	4.73
2	5.25	1.00	4.50	3.58
	1.91	4.99	2.36	4.44
	1.18	6.22	3.07	3.93
	1.70	2.86	3.90	1.72
	4.57	2.23	5.68	0.00
	6.56	1.70	0.00	4.55
3	1.70	0.00	6.56	7.74
	7.57	0.00	5.31	0.00
	4.57	0.00	2.02	3.03
	3.79	1.85	5.09	7.13
	1.72	3.44	0.00	2.88
	4.82	0.00	0.00	1.18
4	4.34	2.55	3.22	3.70
	0.00	2.76	4.75	6.46
	1.80	0.00	5.44	0.00
	4.87	2.02	3.82	0.00
	5.08	4.26	4.44	4.50
	2.76	3.29	3.82	4.19
5	3.17	0.00	3.82	2.92
	3.58	0.00	3.11	0.00
	3.41	0.00	3.67	7.11
	1.95	2.99	3.22	0.00
	2.11	5.40	3.96	5.91
	5.50	5.44	4.04	6.26
6	2.61	4.87	3.07	5.19
	0.85	4.97	3.79	4.04
	3.88	3.51	5.99	4.49
	0.00	5.56	5.48	7.19
	1.21	0.00	0.00	5.48
	0.00	0.00	0.00	0.00
7	7.63	2.77	0.00	7.93
	4.21	2.76	4.82	3.51
	3.07	5.84	0.00	6.59
	1.85	3.44	3.11	6.08
	3.58	1.91	3.22	6.03
	0.00	0.00	4.59	4.03
Max	3.82	3.60	0.00	5.91
	4.87	2.86	2.41	5.93
	3.17	2.86	8.12	7.05
	3.11	3.17	1.67	10.10
	0.00	2.55	6.07	4.07
	4.79	2.99	2.61	0.00
Average Std. Dev. Minimum Maximum	0.00	2.03	4.50	0.00
	17.74	23.71	15.87	10.88
	2.9	2.4	3.6	4.0
	2.1	1.9	2.0	2.6
Minimum Maximum	0.0	0.0	0.0	0.0
	17.7	23.7	15.9	10.9

Table 60. Biofilm Thickness Data for Control #3.

Control #3	Coupon				
	#15	#2	#20	#14	#12
Images	Steady-State (μm)	Before Dose #1 (μm)	Return to Steady-State (μm)	Before Dose #2 (μm)	Return to Steady-State (μm)
1	4.26	0.00	8.04	6.88	0.00
	0.71	3.51	4.05	2.88	6.66
	1.50	2.61	6.15	5.91	4.49
	1.89	2.18	6.95	2.64	5.05
	8.28	5.85	5.80	5.68	9.88
	0.00	0.00	6.57	4.79	6.28
	4.02	0.00	4.07	0.00	7.89
	2.96	0.00	7.64	6.15	13.49
2	0.00	0.00	5.91	0.00	13.59
	5.29	12.75	4.82	6.15	6.89
	5.50	8.75	4.49	10.40	11.91
	7.03	4.36	5.18	8.52	9.23
	10.97	4.03	3.78	8.98	7.05
	8.88	2.41	2.85	8.76	10.44
	8.69	15.13	5.10	6.42	7.60
	6.14	7.33	2.99	6.28	10.44
3	5.91	3.55	3.32	6.89	4.87
	4.97	1.44	0.00	8.29	4.31
	4.57	0.00	6.37	6.39	5.10
	7.33	0.00	6.95	7.13	0.00
	5.46	0.00	5.44	3.44	3.34
	5.51	4.04	6.96	6.87	11.66
	0.00	5.19	5.44	6.03	5.62
	5.31	5.48	4.96	7.51	7.89
4	4.49	2.36	9.46	4.03	4.57
	3.08	7.57	9.30	7.05	11.41
	3.29	2.60	6.77	10.64	8.21
	0.00	1.42	2.85	8.52	5.54
	7.62	6.25	3.22	7.15	8.31
	1.42	9.46	5.56	6.66	8.36
	3.74	7.74	5.93	5.75	14.40
	6.54	4.49	3.67	5.99	11.15
5	7.64	0.00	3.90	6.89	9.97
	5.40	0.00	4.66	7.39	9.49
	1.72	0.00	3.79	7.42	9.69
	5.33	15.95	6.39	6.16	5.76
	5.95	11.59	3.55	4.42	14.66
	3.74	19.95	5.33	6.45	14.97
	4.99	20.13	6.54	5.20	10.64
	5.44	21.06	7.34	5.21	14.22
6	3.44	23.89	5.29	0.00	6.45
	0.00	4.97	7.33	10.64	4.76
	5.60	0.00	1.91	1.50	0.00
	4.08	0.71	0.00	3.58	10.88
	5.23	4.14	7.25	6.82	7.33
	3.91	17.51	4.94	6.42	10.99
	4.05	12.08	6.86	10.88	0.00
	5.20	6.49	5.31	8.51	7.28
7	2.84	1.89	4.73	9.69	9.22
	32.44	33.91	21.73	13.95	24.17
Max	32.44	33.91	21.73	13.95	24.17
Average	4.5	5.9	5.2	6.2	8.0
Std. Dev.	2.5	6.5	2.0	2.6	3.9
Minimum	0.0	0.0	0.0	0.0	0.0
Maximum	32.4	33.9	21.7	14.0	24.2

Table 61. Biofilm Thickness Data for Experiment #2.

Experiment #2	Coupon		
	#8	#12	#2
Images (μm)	Steady-State (μm)	After Dose (μm)	Return to Steady-State (μm)
1	9.60	9.02	3.91
	10.67	4.89	5.83
	16.61	9.15	19.76
	13.29	3.16	6.58
	12.56	6.67	5.45
	10.43	6.52	7.53
	9.96	0.00	4.47
2	13.01	2.44	10.32
	12.09	7.62	12.09
	14.45	6.97	6.65
	9.00	4.66	5.97
	7.58	5.73	3.22
	6.45	0.00	1.71
	5.03	0.00	0.00
3	8.53	5.97	5.00
	7.14	5.30	4.76
	7.35	2.93	5.86
	5.58	5.94	10.94
	1.38	5.26	14.31
	3.37	4.53	17.54
	5.55	4.27	9.10
4	12.58	5.35	5.47
	6.70	6.20	7.35
	5.41	3.16	4.24
	0.00	7.36	5.33
	7.36	6.00	7.11
	5.07	3.62	2.56
	8.40	7.58	4.80
5	6.64	3.92	7.44
	10.52	2.55	8.38
	6.26	5.03	4.37
	6.90	5.25	3.08
	9.86	5.98	6.04
	7.41	6.55	5.85
	6.32	4.36	8.54
6	4.89	6.32	17.38
	6.07	9.48	13.34
	8.14	9.00	7.17
	8.07	0.00	6.79
	6.23	4.95	11.10
	4.29	4.27	8.65
	4.27	8.56	8.59
7	4.66	1.28	7.58
	6.55	4.14	13.25
	7.44	5.77	4.79
	12.41	4.79	0.00
	13.56	7.25	6.82
	10.21	4.43	8.32
	13.75	5.40	8.02
Max	25.43	17.30	23.53
Average	8.2	5.1	7.4
Std. Dev.	3.5	2.4	4.2
Minimum	0.0	0.0	0.0
Maximum	25.4	17.3	23.5

Table 62. Biofilm Thickness Data for Experiment #4.

Experiment #4	Coupon				
	#14	#9	#6	#11	#10
Images	Steady-State (μm)	After Dose #1 (μm)	Return to Steady-State (μm)	After Dose #2 (μm)	Return to Steady-State (μm)
1	3.42	2.37	3.59	5.70	3.89
	1.44	7.82	4.20	4.66	4.03
	4.29	7.91	24.95	3.12	6.16
	5.63	5.99	11.06	6.04	5.58
	4.27	5.77	2.55	5.12	3.08
	3.71	5.94	2.61	8.79	5.62
	3.29	0.00	3.56	4.66	0.00
	1.44	4.98	3.75	0.00	6.12
2	2.37	4.95	3.52	8.37	17.02
	0.00	3.09	5.55	5.58	21.89
	2.65	6.40	9.12	7.22	8.63
	3.09	2.84	12.87	8.71	0.00
	4.24	4.29	4.72	8.16	2.44
	0.00	3.57	6.45	7.53	0.00
	4.15	4.32	4.67	0.00	7.11
	0.00	4.05	4.80	0.00	5.10
3	3.69	5.71	7.07	0.00	11.95
	3.75	3.45	4.53	0.00	14.62
	3.03	2.85	7.82	0.00	9.67
	0.00	4.88	4.43	0.00	8.09
	0.00	2.52	2.89	0.00	10.05
	3.03	4.45	2.97	4.29	10.13
	1.28	1.91	0.00	7.89	18.27
	3.22	5.73	9.43	11.34	9.58
4	3.00	13.79	9.20	0.00	16.29
	0.00	4.50	9.49	2.84	18.74
	3.09	6.89	6.97	4.29	13.29
	3.39	4.88	3.86	3.97	9.44
	2.55	12.09	9.90	6.16	6.06
	2.88	15.64	0.00	5.45	8.07
	4.74	15.19	5.50	4.60	11.46
	2.38	10.67	3.92	5.25	9.68
5	3.91	13.05	5.70	4.43	6.36
	4.66	3.75	3.68	4.56	6.90
	3.30	0.00	2.62	3.55	8.78
	1.66	3.09	4.06	3.09	7.88
	0.00	3.52	5.81	3.30	13.65
	0.00	9.50	6.67	5.63	26.07
	2.47	8.53	6.64	3.68	17.25
	1.18	9.60	5.22	7.57	9.99
6	0.00	4.60	7.35	6.18	10.06
	1.21	4.14	5.40	5.20	10.00
	3.09	0.00	5.92	2.85	12.40
	1.06	3.68	3.55	4.67	9.58
	0.00	7.07	4.75	4.06	8.53
	0.00	11.85	5.73	3.62	14.39
	4.80	3.29	3.33	2.89	13.68
	1.95	3.16	5.18	0.00	9.12
7	2.47	0.00	12.56	10.71	13.14
	19.56	26.92	16.52	18.32	34.76
Max	19.56	26.92	16.52	18.32	34.76
Average	2.4	5.7	5.9	4.4	9.8
Std. Dev.	1.6	3.8	3.9	3.0	5.4
Minimum	0.0	0.0	0.0	0.0	0.0
Maximum	19.6	26.9	25.0	18.3	34.8

Table 63. Biofilm Thickness Data for Experiment #6.

Experiment #6	Coupon				
	#3	#14	#12	#6	#8
Images	Steady-State (μm)	After Dose #1 (μm)	Return to Steady-State (μm)	After Dose #2 (μm)	Return to Steady-State (μm)
1	3.08	4.26	4.97	15.15	9.97
	4.57	3.32	4.82	15.84	10.93
	2.96	5.68	9.05	5.18	13.53
	4.57	3.91	4.59	5.44	10.50
	2.89	6.21	6.54	5.46	19.22
	2.84	1.91	4.73	9.73	26.94
	3.78	4.05	0.00	5.19	11.27
2	0.71	6.59	3.07	19.06	8.04
	0.00	6.73	3.70	14.66	6.66
	2.33	0.00	1.06	16.60	5.85
	3.55	4.79	0.00	7.40	7.09
	4.04	6.88	1.70	6.40	6.39
	4.97	5.18	4.73	7.86	4.42
	4.26	2.61	4.49	8.56	10.04
3	4.50	6.28	11.13	13.88	7.05
	10.68	6.92	10.81	5.46	7.48
	6.86	0.00	15.69	6.77	6.21
	3.07	3.38	7.11	2.86	10.57
	8.28	9.69	4.97	7.11	19.43
	4.96	5.99	3.81	6.24	5.85
	4.59	8.04	4.28	5.84	9.13
4	7.10	3.93	7.70	10.88	21.52
	5.44	5.60	3.44	16.05	9.53
	4.50	8.78	3.58	18.44	4.97
	5.99	8.83	4.14	17.16	5.05
	6.19	5.23	3.69	11.67	5.56
	2.60	0.00	6.26	13.44	5.62
	3.96	0.00	8.90	0.00	4.08
5	3.08	4.13	10.21	0.00	5.52
	5.08	5.23	6.89	0.00	6.54
	3.55	5.22	14.75	0.00	6.56
	10.21	4.04	18.87	9.75	12.77
	7.93	4.97	5.95	5.01	9.72
	5.01	6.81	11.87	7.00	0.00
	5.46	2.60	4.73	9.13	0.00
6	2.84	4.75	10.93	13.72	17.49
	1.67	6.89	7.57	15.13	26.98
	2.13	9.46	8.28	8.76	32.62
	4.73	4.50	4.26	8.75	16.55
	2.47	0.00	0.00	18.01	14.28
	5.44	0.00	9.29	12.59	15.83
	4.99	6.38	8.09	4.73	7.11
7	4.13	5.33	7.87	14.66	5.01
	5.10	3.15	0.00	13.72	4.52
	9.48	5.05	14.00	27.42	14.96
	5.01	8.09	16.32	21.99	6.62
	4.26	7.57	13.01	8.04	5.44
	4.23	3.07	6.62	7.83	4.82
	2.84	0.00	5.03	0.00	4.55
Max	22.30	12.31	31.21	35.05	41.14
Average	4.5	4.7	6.8	9.9	10.0
Std. Dev.	2.2	2.6	4.4	6.1	6.8
Minimum	0.0	0.0	0.0	0.0	0.0
Maximum	22.3	12.3	31.2	35.1	41.1

Table 64. Biofilm Thickness Data for Experiment #8.

Experiment #8	Coupon				
	#4	#15	#14	#19	#12
Images	Steady-State (μm)	After Dose #1 (μm)	Return to Steady-State (μm)	After Dose #2 (μm)	Return to Steady-State (μm)
1	3.73	5.24	7.61	4.48	14.25
	2.41	2.59	6.37	6.41	8.01
	5.04	1.42	6.75	4.98	7.50
	6.48	12.41	5.97	6.13	13.08
	4.65	5.19	6.87	5.38	10.70
	2.17	8.93	8.95	4.98	11.19
	2.46	6.13	7.55	11.75	8.34
2	16.88	6.13	5.67	12.09	10.44
	20.77	0.00	4.41	12.14	4.65
	21.95	3.95	5.63	12.11	7.50
	19.69	11.59	5.61	11.79	0.00
	18.55	0.00	6.88	18.06	8.55
	10.03	0.00	8.36	6.48	0.00
	0.00	0.00	4.25	8.74	7.81
3	4.47	7.38	2.87	6.61	10.91
	4.04	0.00	5.04	7.08	14.74
	2.54	0.00	4.51	5.19	13.26
	3.31	0.00	6.60	6.15	13.22
	2.84	12.27	4.30	4.49	9.16
	2.64	17.26	0.00	2.87	8.12
	4.07	0.00	4.77	0.00	6.56
4	6.66	3.55	3.90	6.96	8.02
	5.66	0.00	5.67	2.83	8.26
	3.43	8.02	2.98	3.21	9.39
	8.25	0.00	6.55	4.07	15.90
	5.43	0.00	4.34	4.25	13.10
	2.84	2.98	6.86	4.20	12.30
	5.90	3.43	8.02	11.39	14.65
5	6.37	2.59	7.55	3.43	0.00
	6.15	0.00	9.20	5.43	17.02
	8.03	2.11	0.00	5.17	23.27
	7.11	1.67	9.68	3.90	13.00
	3.78	0.00	9.72	4.78	5.74
	0.00	3.61	6.61	4.02	5.05
	0.00	0.00	5.57	3.08	9.92
6	7.08	0.00	9.19	3.08	9.67
	4.72	0.00	0.00	3.31	9.44
	5.09	4.86	6.67	5.97	19.46
	4.72	0.00	0.00	4.12	11.56
	4.77	0.00	6.13	2.98	14.15
	1.18	6.06	7.17	2.36	8.70
	8.25	0.00	0.00	12.30	6.15
7	8.02	0.00	7.78	3.31	12.74
	12.05	1.58	6.23	6.15	9.92
	17.71	4.22	7.55	3.10	12.97
	15.33	0.00	4.41	2.92	13.22
	11.32	6.86	9.70	3.30	10.83
	0.00	0.00	7.81	4.63	9.70
	6.39	0.00	7.80	3.95	7.80
Max	49.06	31.65	26.18	32.60	40.87
Average	6.8	3.1	5.8	5.8	10.2
Std. Dev.	5.6	4.1	2.6	3.5	4.5
Minimum	0.0	0.0	0.0	0.0	0.0
Maximum	49.1	31.7	26.2	32.6	40.9

APPENDIX H: Total Organic Carbon Data

Table 65. Total organic carbon and areal density data for Control #2.

Sample Date	Reactor Status	Coupon	Resuspended Volume (ml)	Injection Volume (μl)	TOC Reading (ng/inj vol)	TOC (μg/ml)	Avg. TOC + Std. Dev (μg/ml)	TOC in 10 ml (μg)	Areal Density (μg/cm ²)	Avg. Areal Density + Std Dev (μg/cm ²)
4/9/98	Steady-State	18	10	20	1.714	85.7	86.5	857	38.9	39.2
			10	20	1.745	87.3	1.1	873	39.6	0.5
4/9/98	Before Dose	5	10	50	4.335	86.7	69.5	867	39.3	31.5
			10	50	4.290	85.8	19.4	858	38.9	8.8
	Before Dose	12	10	50	2.571	51.4		514	23.3	
			10	50	2.699	54.0		540	24.5	
4/9/98	After Dose	3	10	20	1.307	65.4	97.5	654	29.7	44.2
			10	20	1.323	66.2	36.6	662	30.0	16.6
	After Dose	17	10	20	2.580	129.0		1290	58.5	
			10	20	2.587	129.4		1294	58.7	
4/11/98	Return to Steady-State	9	10	20	3.026	151.3	170.4	1513	68.7	77.3
			10	20	2.993	149.7	23.1	1497	67.9	10.5
	Return to Steady-State	11	10	20	3.829	191.5		1915	86.9	
			10	20	3.785	189.3		1893	85.9	

Table 66. Total organic carbon and areal density data for Control #3.

Sample Date	Reactor Status	Coupon	Resuspended Volume (ml)	Injection Volume (μl)	TOC Reading (ng/inj vol)	TOC (μg/ml)	Avg. TOC + Std. Dev (μg/ml)	TOC in 10 ml (μg)	Areal Density (μg/cm ²)	Avg. Areal Density + Std Dev (μg/cm ²)
5/4/98	Steady-State	1	10	25	11.590	463.6	412.7	4636	210.4	187.3
			10	20	8.764	438.2	45.5	4382	198.9	20.6
	Steady-State	19	10	20	7.577	378.9		3789	171.9	
			10	20	7.403	370.2		3702	168.0	
5/4/98	Before Dose #1	8	10	20	2.615	130.8	452.6	1308	59.3	205.4
			10	20	2.580	129.0	372.6	1290	58.5	169.1
	Before Dose #1	10	10	20	15.600	780.0		7800	354.0	
			10	20	15.410	770.5		7705	349.7	
5/8/98	Return to Steady-State	11	10	20	20.150	1007.5	581.1	10075	457.2	263.7
			10	20	20.040	1002.0	489.3	10020	454.7	222.0
	Return to Steady-State	6	10	20	3.215	160.8		1608	72.9	
			10	20	3.079	154.0		1540	69.9	
5/8/98	Before Dose #2	3	10	20	2.694	134.7	89.3	1347	61.1	40.5
			10	20	2.476	123.8	46.4	1238	56.2	21.1
	Before Dose #2	9	10	20	1.001	50.1		501	22.7	
			10	20	0.969	48.5		485	22.0	
5/11/98	Return to Steady-State	13	10	20	5.022	251.1	505.2	2511	113.9	229.3
			10	20	4.955	247.8	295.4	2478	112.4	134.0
	Return to Steady-State	17	10	20	15.290	764.5		7645	346.9	
			10	20	15.150	757.5		7575	343.8	

Table 67. Total organic carbon and areal density data for Experiment #2.

Sample Date	Reactor Status	Coupon	Resuspended Volume (ml)	Injection Volume (μl)	TOC Reading (ng/inj vol)	TOC (μg/ml)	Avg. TOC + Std. Dev (μg/ml)	TOC in 10 ml (μg)	Areal Density (μg/cm ²)	Avg. Areal Density + Std Dev (μg/cm ²)
5/19/98	Steady-State	9	10	20	0.425	21.3	62.9	212.5	9.6	28.6
			10	20	0.423	21.2	40.0	211.5	9.6	18.2
	Steady-State	7	10	20	2.190	109.5		1095.0	49.7	
			10	20	2.219	111.0		1109.5	50.3	
	Steady-State	14	10	20	1.153	57.7		576.5	26.2	
			10	20	1.141	57.1		570.5	25.9	
5/19/98	Before Dose	5	10	20	1.607	80.4	79.7	803.5	36.5	36.2
			10	20	1.543	77.2	14.2	771.5	35.0	6.5
	Before Dose	4	10	20	1.956	97.8		978.0	44.4	
			10	20	1.882	94.1		941.0	42.7	
	Before Dose	1	10	20	1.255	62.8		627.5	28.5	
			10	20	1.324	66.2		662.0	30.0	
5/19/98	After Dose	10	10	20	2.699	135.0	62.3	1349.5	61.2	28.3
			10	20	2.652	132.6	55.4	1326.0	60.2	25.2
	After Dose	3	10	20	0.484	24.2		242.0	11.0	
			10	20	0.453	22.7		226.5	10.3	
	After Dose	18	10	20	0.582	29.1		291.0	13.2	
			10	20	0.606	30.3		303.0	13.8	
5/26/98	Return to Steady-State	20	10	20	4.672	233.6	123.9	2336.0	106.0	56.2
			10	20	4.720	236.0	86.8	2360.0	107.1	39.4
	Return to Steady-State	6	10	20	1.591	79.6		795.5	36.1	
			10	20	1.693	84.7		846.5	38.4	
	Return to Steady-State	11	10	20	1.089	54.5		544.5	24.7	
			10	20	1.097	54.9		548.5	24.9	

Table 68. Total organic carbon and areal density data for Experiment #4.

Sample Date	Reactor Status	Coupon	Resuspended Volume (ml)	Injection Volume (μl)	TOC Reading (ng/inj vol)	TOC (μg/ml)	Avg. TOC + Std. Dev (μg/ml)	TOC in 10 ml (μg)	Areal Density (μg/cm ²)	Avg. Areal Density + Std Dev (μg/cm ²)
6/13/98	Steady State	16	10	20	5.573	278.7	209.4 89.7	2786.5	126.5	95.0 40.7
			10	20	5.508	275.4		2754.0	125.0	
	Steady State	2	10	20	5.087	254.4		2543.5	115.4	
			10	20	5.191	259.6		2595.5	117.8	
	Steady State	5	10	20	1.901	95.1		950.5	43.1	
			10	20	1.868	93.4		934.0	42.4	
6/13/98	After Dose #1	3	10	20	3.387	169.4	132.5 39.9	1693.5	76.9	60.1 18.1
			10	20	3.362	168.1		1681.0	76.3	
	After Dose #1	1	10	20	1.627	81.4		813.5	36.9	
			10	20	1.683	84.2		841.5	38.2	
	After Dose #1	8	10	20	2.930	146.5		1465.0	66.5	
			10	20	2.913	145.7		1456.5	66.1	
6/18/98	Return to Steady-State	15	10	20	4.344	217.2	170.1 36.9	2172.0	98.6	77.2 16.7
			10	20	4.263	213.2		2131.5	96.7	
	Return to Steady-State	13	10	20	2.605	130.3		1302.5	59.1	
			10	20	2.789	139.5		1394.5	63.3	
	Return to Steady-State	7	10	20	3.241	162.1		1620.5	73.5	
			10	20	3.165	158.3		1582.5	71.8	
6/18/98	After Dose #2	19	10	20	2.934	146.7	172.7 87.1	1467.0	66.6	78.4 39.5
			10	20	2.950	147.5		1475.0	66.9	
	After Dose #2	17	10	20	5.589	279.5		2794.5	126.8	
			10	20	5.624	281.2		2812.0	127.6	
	After Dose #2	4	10	20	1.825	91.3		912.5	41.4	
			10	20	1.803	90.2		901.5	40.9	
6/23/98	Return to Steady-State	18	10	20	2.114	105.7	81.3 21.1	1057.0	48.0	36.9 9.6
			10	20	2.104	105.2		1052.0	47.7	
	Return to Steady-State	12	10	20	1.651	82.6		825.5	37.5	
			10	20	1.551	77.6		775.5	35.2	
	Return to Steady-State	20	10	20	1.188	59.4		594.0	27.0	
			10	20	1.153	57.7		576.5	26.2	

Table 69. Total organic carbon and areal density data for Experiment #6.

Sample Date	Reactor Status	Coupon	Resuspended Volume (ml)	Injection Volume (μl)	TOC Reading (ng/inj vol)	TOC (μg/ml)	Avg. TOC + Std. Dev (μg/ml)	TOC in 10 ml (μg)	Areal Density (μg/cm ²)	Avg. Areal Density + Std Dev (μg/cm ²)
8/15/98	Steady State	2	10	20	0.277	13.9	58.7 34.5	138.5	6.3	26.7 15.6
			10	20	0.292	14.6		146.0	6.6	
	Steady State	10	10	20	1.614	80.7		807.0	36.6	
			10	20	1.624	81.2		812.0	36.8	
			10	20	1.622	81.1		811.0	36.8	
8/16/98	Steady State	1	10	20	1.619	81.0		809.5	36.7	
			10	20	1.619	81.0		809.5	36.7	
	After Dose #1	15	10	20	1.158	57.9	99.9 63.7	579.0	26.3	45.3 28.9
			10	20	1.135	56.8		567.5	25.8	
			10	20	3.630	181.5		1815.0	82.4	
8/19/98	After Dose #1	20	10	20	3.655	182.8		1827.5	82.9	
			10	20	1.214	60.7		607.0	27.5	
	After Dose #1	11	10	20	1.191	59.6		595.5	27.0	
			10	20	1.286	64.3		643.0	29.2	
			10	20	1.305	65.3		652.5	29.6	
8/20/98	Return to Steady-State	4	10	20	2.514	125.7	26.3	1257.0	57.0	11.9
			10	20	2.416	120.8		1208.0	54.8	
	Return to Steady-State	15	10	20	1.786	89.3		893.0	40.5	
			10	20	1.800	90.0		900.0	40.8	
			10	20	1.863	93.2		931.5	42.3	
8/23/98	After Dose #2	5	10	20	1.850	92.5	102.0 8.5	925.0	42.0	46.3 3.9
			10	20	2.200	110.0		1100.0	49.9	
	After Dose #2	17	10	20	2.264	113.2		1132.0	51.4	
			10	20	1.999	100.0		999.5	45.4	
			10	20	2.062	103.1		1031.0	46.8	
8/23/98	Return to Steady-State	16	10	20	3.955	197.8	130.0 52.5	1977.5	89.7	59.0 23.8
			10	20	3.918	195.9		1959.0	88.9	
	Return to Steady-State	7	10	20	1.725	86.3		862.5	39.1	
			10	20	1.757	87.9		878.5	39.9	
			10	20	2.114	105.7		1057.0	48.0	
	Return to Steady-State	9	10	20	2.125	106.3		1062.5	48.2	

Table 70. Total organic carbon and areal density data for Experiment #8.

Sample Date	Reactor Status	Coupon	Resuspended Volume (ml)	Injection Volume (μl)	TOC Reading (ng/inj vol)	TOC (μg/ml)	Avg. TOC + Std. Dev (μg/ml)	TOC in 10 ml (μg)	Areal Density (μg/cm ²)	Avg. Areal Density + Std Dev (μg/cm ²)
9/9/98	Steady State	18	10	20	1.915	95.8	106.7 10.1	957.5	43.5	48.4 4.6
			10	20	1.891	94.6		945.5	42.9	
	Steady State	9	10	20	2.359	118.0		1179.5	53.5	
			10	20	2.344	117.2		1172.0	53.2	
	Steady State	20	10	20	2.155	107.8		1077.5	48.9	
			10	20	2.141	107.1		1070.5	48.6	
9/10/98	After Dose #1	16	10	20	3.261	163.1	144.9 27.8	1630.5	74.0	65.8 12.6
			10	20	3.235	161.8		1617.5	73.4	
	After Dose #1	13	10	20	3.326	166.3		1663.0	75.5	
			10	20	3.203	160.2		1601.5	72.7	
	After Dose #1	2	10	20	2.196	109.8		1098.0	49.8	
			10	20	2.167	108.4		1083.5	49.2	
9/14/98	Return to Steady-State	17	10	20	2.407	120.4	88.0 26.0	1203.5	54.6	39.9 11.8
			10	20	2.448	122.4		1224.0	55.5	
	Return to Steady-State	7	10	20	1.370	68.5		685.0	31.1	
			10	20	1.345	67.3		672.5	30.5	
	Return to Steady-State	3	10	20	1.512	75.6		756.0	34.3	
			10	20	1.481	74.1		740.5	33.6	
9/15/98	After Dose #2	10	10	20	4.564	228.2	197.5 43.6	2282.0	103.6	89.6 19.8
			10	20	4.633	231.7		2316.5	105.1	
	After Dose #2	8	10	20	2.846	142.3		1423.0	64.6	
			10	20	2.813	140.7		1406.5	63.8	
	After Dose #2	11	10	20	4.418	220.9		2209.0	100.2	
			10	20	4.427	221.4		2213.5	100.4	
9/20/98	Return to Steady-State	1	10	20	1.101	55.1	110.1 43.8	550.5	25.0	50.0 19.9
			10	20	1.093	54.7		546.5	24.8	
	Return to Steady-State	5	10	20	2.936	146.8		1468.0	66.6	
			10	20	2.985	149.3		1492.5	67.7	
	Return to Steady-State	6	10	20	2.541	127.1		1270.5	57.7	
			10	20	2.553	127.7		1276.5	57.9	

APPENDIX I: Planktonic and Biofilm Sulfide Recovery Time Data

Table 71. Planktonic sulfide recovery times to achieve 10 mg S/L and 90% of steady-state.

Experiment	Vial	S-S	Average	0.90	Interpolated								
		Sulfide (mg S/L)	S-S (mg S/L)	S-S (mg S/L)	Time (hr)	Sulfide (mg S/L)	t90% (hr)	t90% (hr)	10 ppm	Time (hr)	Sulfide (mg S/L)	Interpolated (hr)	t<10ppm (hr)
Growth	11/18/98	500.41	455.59	410.03	13.00	293.66	13.80	14	10				0
		521.78			14.00	439.77							
		536.01											
		536.94											
		538.32											
		520.50											
		518.78											
		465.27											
		538.32											
		503.17											
	3/18/99	460.73	475.62	428.05	16.00	384.89	16.32	16	10				0
		486.40			17.00	519.64							
		471.30											
		471.30											
		489.43											
		454.68											
		495.47											
Plank #1	Control	457.00	459.75	413.78	69.00	409.44	69.29	69	10	47.17	6.24	49.08	49
		474.91			72.17	457.00				52.00	15.72		
		483.87											
		432.87											
		450.79											
		459.06											
	50 ppm	456.31	462.71	416.43	168.08	383.94	169.17	1	10	142.67	1.49	143.23	143
		468.71			170.00	441.14				168.08	383.94		
		459.75											
		474.91											
		460.44											
		462.51											
		456.31											

Table 71. Planktonic sulfide recovery times to achieve 10 mg S/L and 90% of steady-state. *Continued*

Experiment	Vial	S-S	Average	0.90	Time (hr)	Sulfide (mg S/L)	Interpolated		10 ppm	Time (hr)	Sulfide (mg S/L)	Interpolated (hr)	t<10ppm (hr)
		(mg S/L)	S-S (mg S/L)	S-S (mg S/L)			t90% (hr)	t90% (hr)					
Plank #2	Control	365.56	362.99	326.69	62.50	235.05	64.67	65	10	49.00	9.52	49.18	49
		360.73			65.58	365.56				51.00	14.92		
		369.18											
		356.50											
Plank #3	Control	349.24	338.52	304.67	57.00	297.28	57.45	57	10	39.08	0.85	42.23	42
		312.99			58.50	322.05				47.00	23.87		
		332.33											
		359.52											

Table 72. Biofilm sulfide recovery times to achieve 10 mg S/L and 90% of steady-state.

Experiment	Steady-State Raw Data Sulfide (mg S/L)	Average S-S (mg S/L)	90% S-S (mg S/L)	Resumed Flow (hr)	Time (hr)	Sulfide (mg S/L)	Interpolated t90% (hr)	t90% (hr)	10 ppm	Time (hr)	Sulfide (mg S/L)	Interpolated (hr)	t<10ppm (hr)
Ctrl2	50.308	53.21	47.89	139.67	147.25	37.22	149.10	9	10	141.75	5.60	142.86	3
	58.036				149.25	48.73				143.25	11.55		
<i>Triplicate</i>	49.511	51.299											
	52.981												
	51.404												
Ctrl3 #1	49.834	53.25	47.92	134.25	141.25	41.55	142.22	8	10	135.25	4.02	135.65	1
	57.397				143.33	55.20				137.33	35.48		
<i>Duplicate</i>	50.457	52.508											
	54.558												
Ctrl3 #2	55.197	53.26	47.93	216.75	219.75	26.25	221.63	5	10	217.75	5.52	218.18	1
	56.293				221.75	49.28				219.75	26.25		
	55.347												
	54.558												
	47.776												
	49.984												
	50.773												
<i>Triplicate</i>	55.189	56.136											
	57.871												
	55.347												
Expt2	55.032	57.06	51.36	107.5	187.00	49.04	187.49	80	10	173.50	8.36	174.24	67
	55.820				188.50	56.14				179.00	20.54		
<i>Triplicate</i>	62.129	60.342											
	61.814												
	57.082												
Expt4 #1	51.964	45.45	40.91	98.25	161.00	39.22	163.17	65	10	149.75	8.46	151.62	53
	50.150				165.00	42.32				152.00	10.31		
	40.867												
<i>Triplicate</i>	36.755	38.821											
	39.791												
	39.918												

Table 72. Biofilm sulfide recovery times to achieve 10 mg S/L and 90% of steady-state (continued).

Experiment	Steady-State	Average	90%	Resumed	Time	Sulfide	Interpolated		10 ppm	Time	Sulfide	Interpolated	t<10ppm
	Raw Data Sulfide (mg S/L)	S-S (mg S/L)	S-S (mg S/L)				t90% (hr)	t90% (hr)					
Expt4 #2	42.321	41.74	37.57	217.28	277.75	32.77	278.79	62	10	259.50	5.47	263.35	46
	35.111				279.50	40.87				263.50	10.17		
	43.839												
	45.800												
	41.626												
Expt6 #1	48.663	49.96	44.96	190.08	221.00	42.46	221.76	32	10	209	9	209.48	19
	46.595				222.00	45.77				211	12		
	50.868												
	Triplicate 52.316												
	53.694												
Expt6 #2	55.141	55.40	49.86	286.08	308.00	47.22	309.65	24	10	291	7	291.45	5
	54.245				312.50	54.45				292	13		
	55.693												
	51.420												
	56.795												
Expt8 #1	Triplicate 58.456	52.89	47.60	160.17	234.83	45.84	235.12	75	10	226	10	226.47	66
	59.008				235.83	52.04				227	13		
	59.145												
	51.013												
	57.905												
Expt8 #2	45.837	49.23	44.31	280.17	360.33	38.88	360.79	81	10	352	9	352.62	72
	52.040				361.33	50.87				353	12		
	50.737												
	Triplicate 48.256												
	48.394												
	48.256												

APPENDIX J: Glutaraldehyde Dosing Data

Table 73. Glutaraldehyde concentration versus time data for Experiment #2.

Dose Period (hr)	Glutaraldehyde		$\ln[C_{\text{glut}}]$	C_{glut}/C_o	$\ln[C_{\text{glut}}/C_o]$
	Area	[mg/L]			
0.47	1703884	507.00	6.23	1.00	0.000
0.72	1276704	380.29	5.94	0.75	-0.288
0.97	1064456	317.34	5.76	0.63	-0.469
1.22	970360	289.43	5.67	0.57	-0.561
1.47	826557	246.77	5.51	0.49	-0.720
1.72	753347	225.06	5.42	0.44	-0.812
1.97	690564	206.43	5.33	0.41	-0.899
2.22	623057	186.41	5.23	0.37	-1.001
2.47	545932	163.53	5.10	0.32	-1.131
2.97	461358	138.45	4.93	0.27	-1.298
3.22	415893	124.96	4.83	0.25	-1.400
3.47	421650	126.67	4.84	0.25	-1.387
3.97	334030	100.68	4.61	0.20	-1.617
4.47	286218	86.50	4.46	0.17	-1.768
4.97	198856	60.59	4.10	0.12	-2.124
5.47	160080	49.09	3.89	0.10	-2.335
5.97	129122	39.90	3.69	0.08	-2.542
7.72	50423	16.56	2.81	0.03	-3.421

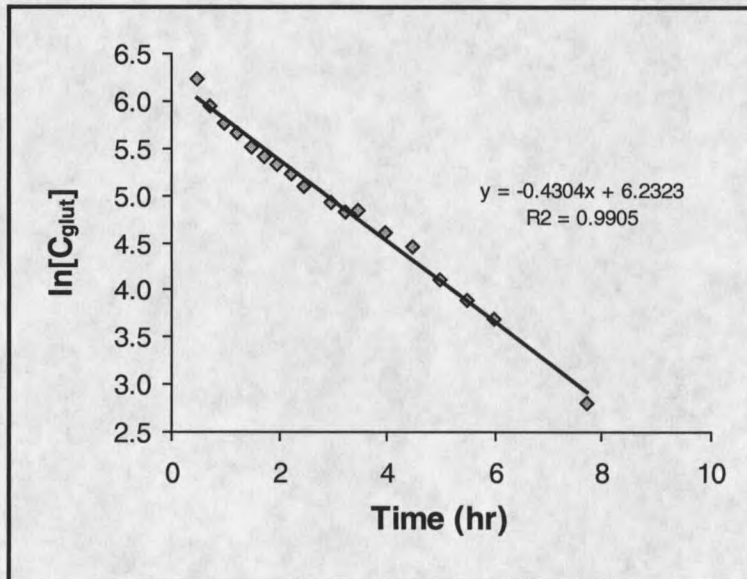


Figure 39. Experiment #2. Glutaraldehyde first-order rate coefficient determination.

Table 74. Experiment #2 linear regression summary for the natural logarithm transformation of the glutaraldehyde concentration versus time data.

Regression Statistics								
Multiple R	0.99525							
R Square	0.99052							
Adjusted R Square	0.98993							
Standard Error	0.08753							
Observations	18							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	12.808	12.808	1671.826	0.000			
Residual	16	0.12257	0.00766					
Total	17	12.930						
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	6.23235	0.03841	162.276	0.000	6.15093	6.31377	6.15093	6.31377
X Variable 1	-0.43036	0.01053	-40.888	0.000	-0.45267	-0.40805	-0.45267	-0.40805

Table 75. Glutaraldehyde concentration vs. time data for Experiment #4 Dose #1.

Dose Period (hr)	Glutaraldehyde		$\ln[C_{\text{glut}}]$	C_{glut}/C_o	$\ln[C_{\text{glut}}/C_o]$
	Area	[mg/L]			
0.25	1410801	442.41	6.09	1.00	0.00
0.50	1151492	361.48	5.89	0.82	-0.20
0.75	1194938	375.04	5.93	0.85	-0.17
1.00	1113464	349.61	5.86	0.79	-0.24
1.28	995537	312.80	5.75	0.71	-0.35
1.50	865674	272.27	5.61	0.62	-0.49
2.00	670564	211.37	5.35	0.48	-0.74
2.50	530955	167.80	5.12	0.38	-0.97
2.75	500237	158.21	5.06	0.36	-1.03
3.00	443347	140.45	4.94	0.32	-1.15
3.25	405482	128.63	4.86	0.29	-1.24
3.50	345217	109.82	4.70	0.25	-1.39
3.75	290455	92.73	4.53	0.21	-1.56
4.00	267156	85.46	4.45	0.19	-1.64
4.25	248841	79.74	4.38	0.18	-1.71
4.75	166398	54.01	3.99	0.12	-2.10
5.25	148747	48.50	3.88	0.11	-2.21
5.75	119723	39.44	3.67	0.09	-2.42
6.25	75406	25.61	3.24	0.06	-2.85

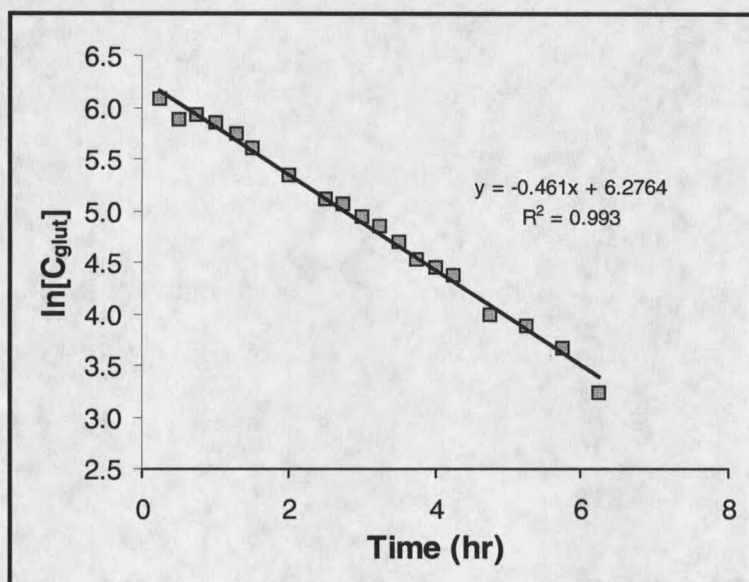


Figure 40. Experiment #4 Dose #1. Glutaraldehyde first-order rate coefficient determination.

Table 76. Glutaraldehyde concentration vs. time data for Experiment #4 Dose #2.

Dose Period (hr)	Glutaraldehyde		$\ln[C_{\text{glut}}]$	C_{glut}/C_o	$\ln[C_{\text{glut}}/C_o]$
	Area	[mg/L]			
0.25	1374899	443.26	6.09	1.00	0.00
0.50	1259013	406.11	6.01	0.92	-0.09
0.75	1116302	360.36	5.89	0.81	-0.21
1.00	934157	301.97	5.71	0.68	-0.38
1.25	900759	291.26	5.67	0.66	-0.42
1.50	868367	280.88	5.64	0.63	-0.46
1.75	765544	247.92	5.51	0.56	-0.58
2.00	676175	219.27	5.39	0.49	-0.70
2.25	610575	198.24	5.29	0.45	-0.80
2.50	536902	174.62	5.16	0.39	-0.93
2.75	489913	159.56	5.07	0.36	-1.02
3.00	421566	137.65	4.92	0.31	-1.17
3.25	355477	116.46	4.76	0.26	-1.34
3.50	340449	111.64	4.72	0.25	-1.38
3.75	309971	101.87	4.62	0.23	-1.47
4.00	264719	87.36	4.47	0.20	-1.62
4.25	248963	82.31	4.41	0.19	-1.68
4.75	187511	62.61	4.14	0.14	-1.96
5.00	167192	56.10	4.03	0.13	-2.07
5.25	142287	48.12	3.87	0.11	-2.22
5.50	133993	45.46	3.82	0.10	-2.28
5.75	121324	41.40	3.72	0.09	-2.37
6.25	90923	31.65	3.45	0.07	-2.64
6.75	82404	28.92	3.36	0.07	-2.73

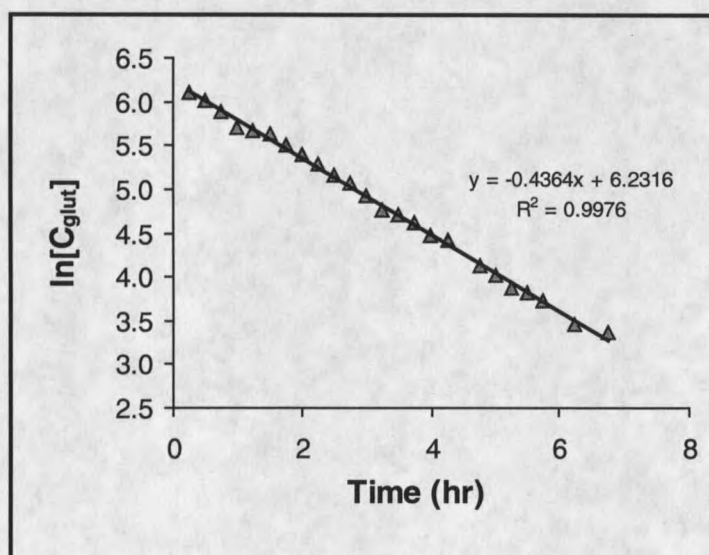


Figure 41. Experiment #4 Dose #2. Glutaraldehyde first-order rate coefficient determination.

Table 77. Experiment #4 linear regression summary for the natural logarithm transformation of the glutaraldehyde concentration versus time data.

Dose #1

Regression Statistics	
Multiple R	0.99650
R Square	0.99302
Adjusted R Square	0.99261
Standard Error	0.07202
Observations	19

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	12.549	12.549	2419.195	0.000
Residual	17	0.08818	0.00519		
Total	18	12.637			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	6.27645	0.03231	194.256	0.000	6.20828	6.34462	6.20828	6.34462
X Variable 1	-0.46102	0.00937	-49.185	0.000	-0.48079	-0.44124	-0.48079	-0.44124

Dose #2

Regression Statistics	
Multiple R	0.99880
R Square	0.99760
Adjusted R Square	0.99749
Standard Error	0.04194
Observations	24

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	16.067	16.067	9135.443	0.000
Residual	22	0.03869	0.00176		
Total	23	16.106			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	6.23159	0.01705	365.513	0.000	6.19624	6.26695	6.19624	6.26695
X Variable 1	-0.43640	0.00457	-95.580	0.000	-0.44587	-0.42693	-0.44587	-0.42693

Table 78. Glutaraldehyde concentration vs. time data for Experiment #8 Dose #1.

Dose Period (hr)	Glutaraldehyde		$\ln[C_{\text{glut}}]$	C_{glut}/C_o	$\ln[C_{\text{glut}}/C_o]$
	Area	[mg/L]			
0.17	1541401	418.80	6.04	1.00	0.00
0.42	1460147	396.80	5.98	0.95	-0.05
0.67	1312622	356.87	5.88	0.85	-0.16
0.92	1300190	353.50	5.87	0.84	-0.17
1.17	1118208	304.23	5.72	0.73	-0.32
1.42	1013961	276.01	5.62	0.66	-0.42
1.67	882325	240.37	5.48	0.57	-0.56
1.92	791846	215.88	5.37	0.52	-0.66
2.17	679850	185.56	5.22	0.44	-0.81
2.42	611271	166.99	5.12	0.40	-0.92
2.67	565830	154.69	5.04	0.37	-1.00
2.92	506148	138.53	4.93	0.33	-1.11
3.17	452315	123.96	4.82	0.30	-1.22
3.50	393551	108.05	4.68	0.26	-1.35
3.67	360999	99.24	4.60	0.24	-1.44
3.92	326580	89.92	4.50	0.21	-1.54
4.17	287967	79.47	4.38	0.19	-1.66
4.42	256190	70.86	4.26	0.17	-1.78
4.67	231486	64.18	4.16	0.15	-1.88
4.92	217536	60.40	4.10	0.14	-1.94
5.17	177890	49.67	3.91	0.12	-2.13
5.42	172279	48.15	3.87	0.11	-2.16
5.67	149194	41.90	3.74	0.10	-2.30
6.17	126921	35.87	3.58	0.09	-2.46

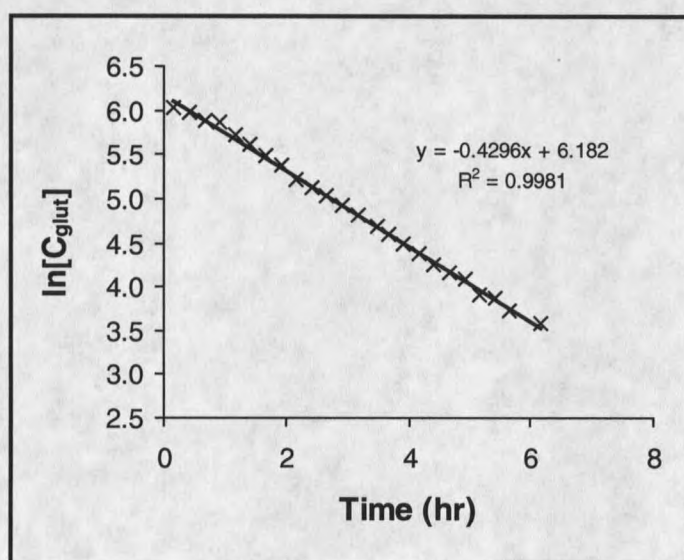


Figure 42. Experiment #8 Dose #1. Glutaraldehyde first-order rate coefficient determination.

Table 79. Glutaraldehyde concentration vs. time data for Experiment #8 Dose #2.

Dose Period (hr)	Glutaraldehyde		$\ln[C_{\text{glut}}]$	C_{glut}/C_o	$\ln[C_{\text{glut}}/C_o]$
	Area	[mg/L]			
0.38	1300860	325.33	5.78	1.00	0.00
0.63	1266104	316.70	5.76	0.97	-0.03
0.88	1234336	308.80	5.73	0.95	-0.05
1.13	1154134	288.87	5.67	0.89	-0.12
1.38	1087073	272.21	5.61	0.84	-0.18
1.63	1011048	253.32	5.53	0.78	-0.25
1.88	934383	234.27	5.46	0.72	-0.33
2.13	811106	203.63	5.32	0.63	-0.47
2.38	732346	184.06	5.22	0.57	-0.57
2.63	662751	166.77	5.12	0.51	-0.67
2.88	566902	142.95	4.96	0.44	-0.82
3.13	536099	135.30	4.91	0.42	-0.88
3.38	485364	122.69	4.81	0.38	-0.98
3.63	427468	108.30	4.68	0.33	-1.10
3.88	387071	98.27	4.59	0.30	-1.20
4.13	349173	88.85	4.49	0.27	-1.30
4.38	313379	79.95	4.38	0.25	-1.40
4.63	285462	73.02	4.29	0.22	-1.49
4.88	253903	65.17	4.18	0.20	-1.61
5.13	223386	57.59	4.05	0.18	-1.73
5.38	200844	51.99	3.95	0.16	-1.83
5.63	179275	46.63	3.84	0.14	-1.94
6.13	143369	37.71	3.63	0.12	-2.15

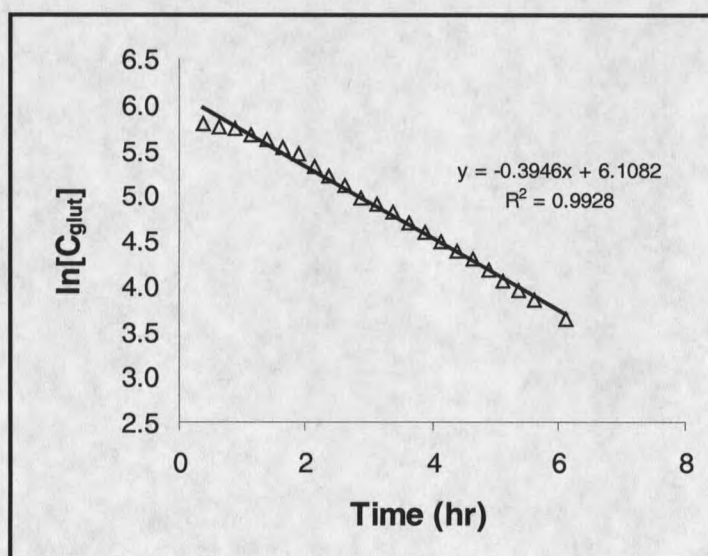


Figure 43. Experiment #8 Dose #2. Glutaraldehyde first-order rate coefficient determination.

Table 80. Experiment #4 linear regression summary for the natural logarithm transformation of the glutaraldehyde concentration versus time data.

Dose #1

Regression Statistics	
Multiple R	0.99904
R Square	0.99808
Adjusted R Square	0.99799
Standard Error	0.03446
Observations	24

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	13.551	13.551	11408.959	0.000
Residual	22	0.02613	0.00119		
Total	23	13.577			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	6.18200	0.01416	436.604	0.000	6.15263	6.21136	6.15263	6.21136
X Variable 1	-0.42955	0.00402	-106.813	0.000	-0.43789	-0.42121	-0.43789	-0.42121

Dose #2

Regression Statistics	
Multiple R	0.99639
R Square	0.99279
Adjusted R Square	0.99245
Standard Error	0.05901
Observations	23

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	10.072	10.072	2892.634	0.000
Residual	21	0.07312	0.00348		
Total	22	10.145			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	6.10818	0.02614	233.633	0.000	6.05381	6.16255	6.05381	6.16255
X Variable 1	-0.39459	0.00734	-53.783	0.000	-0.40985	-0.37933	-0.40985	-0.37933

APPENDIX K: Effective Diffusivity Permeabilities in Water

EFFECTIVE DIFFUSIVITY PERMEABILITY, D_E

The diffusion coefficient characterizes the rate of diffusive transport processes within a biofilm. Empirical correlations have been developed that allow one to predict a liquid diffusion coefficient in terms of the solute and solvent properties. Water is the predominate component within the biofilm. Transport of the solute into the biofilm has often been thought of as an unimpeded motion due to the solvent properties and makeup of the biofilm. Microbial cells, extracellular polymeric substances (EPS), and inorganic materials due, however, hinder the diffusion of the solute into the biofilm and reduce the diffusion coefficient (Stewart, 1998).

A correlation developed for estimating the diffusivities of nonelectrolytes in an infinitely dilute solution will be used to approximate the diffusion of glutaraldehyde in water (Wilke and Chang, 1955). This relationship is represented by equation (1) where D_{AB} is the mass diffusivity of A diffusing through liquid solvent B (cm^2/sec), μ_B is the viscosity of the solution (centipoise), T is the absolute temperature ($^\circ\text{K}$), M_B is the molecular weight of the solvent, V_A is the molal volume of solute at normal boiling point (cm^3/gmol), and Φ_B is the association parameter for solvent B.

$$\frac{D_{AB}\mu_B}{T} = \frac{7.4 \times 10^{-8} (\Phi_B M_B)^{1/2}}{V_A^{0.6}} \quad (1)$$

Rearranging for D_{AB} yields,

$$D_{AB} = 7.4 \times 10^{-8} \left[\frac{(\Phi_B M_B)^{0.5}}{V_A^{0.6}} \right] \left[\frac{T}{\mu_B} \right] \quad (2)$$

The associated parameters with respect to the solvent, water, and the solutes, glutaraldehyde and nitrite, are listed in Table 81.

Table 81. Wilke-Chang effective diffusive permeability parameters

Parameter	Value		Units	Source
$V_{A,glut}$	(2) oxygen @ 7.4 (10) hydrogen @ 3.7 (5) carbon @ 14.8	125.8	cm^3/gmol	LeBas, 1915
Φ_{water}	2.6		---	Reid, 1977
M_{water}	18.01		---	---
T	309.18		$^{\circ}\text{K}$	---
μ_{water}	0.8904		$\text{c g/cm}^2\text{sec}$	CRC

Substituting these values into equation (2) yields

$$D_{glut/water} = 9.67 \times 10^{-10} \text{ m}^2/\text{s}$$

Diffusion coefficients for ions are strongly affected by interactions between diffusing molecules. In the case of sodium nitrite, solute-solute interactions affect the apparent diffusion coefficient. In other words, the sodium ion does not possess the same diffusion coefficient as the nitrite ion. An overall diffusion coefficient for this strong electrolyte can be determined using equation (3) (Cussler, 1997).

$$D_{NaNO_2} = \left[\frac{2}{1/D_{Na^+} + 1/D_{NO_2^-}} \right] \quad (3)$$

The diffusion coefficients for the sodium and nitrite ions in water at 25°C are 1.33×10^{-5} and 1.90×10^{-5} cm²/sec, respectively. The value for the nitrite ion was assumed to be approximately equal to the value for nitrate. Substituting these quantities results in,

$$D_{\text{nitrite/water}} = 1.56 \times 10^{-9} \text{ m}^2/\text{s}$$

APPENDIX L: Glutaraldehyde Inactivation Efficiency

Table 82. Inactivation efficiency for different disinfection concentrations

Source	Organism	Dose	Conc (mg/L)	Duration (hr)	C*t	Efficacy (Log Reduction)
<i>Cheung & Beech #1 (1996)</i>	Portsmouth Harbour SRB	1	50	6	300	1
		1	100	6	600	3
		1	400	6	2400	5
<i>Cheung & Beech #2 (1996)</i>	Indonesian Coast SRB	1	50	6	300	1
		1	100	6	600	4
		1	400	6	2400	5
<i>Eagar (1988)</i>	<i>P.fluorescens</i>	1	100	12.5	1250	2
		2	100	12.5	1250	3
		3	100	12.5	1250	4
		1	500	2.5	1250	1.4
		2	500	2.5	1250	3
		3	500	2.5	1250	4
<i>Grab & Theis #1 (1993)</i>	<i>D.desulfuricans</i>	1	100	1	100	2
		1	100	4	400	4
<i>Grab & Theis #2 (1993)</i>	SRB Isolate	1	220	1	220	4
		1	220	4	880	5
	SRB Isolate	1	550	1	550	5
		1	550	4	2200	5
<i>Ruseska (1982)</i>	SRB	1	50	6	300	1.7
		1	100	6	600	4.8
<i>Our work</i>	Undefined population	1	500	7	295*	0
					295	5**

* Equivalent concentration

** Arbitrary number used to plot vertical line

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