



Rate and stoichiometry of sulfate reducing bacteria in suspended and biofilm cultures
by Satoshi Okabe

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Civil Engineering
Montana State University
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Abstract:

The accumulation and activity of sulfate reducing bacteria (SRB) are of critical concern in many industrial water systems. For example, SRB cause numerous problems in petroleum production including contamination of petroleum with H₂S, injection well plugging, and corrosion. Prediction of SRB behavior in natural and industrial water systems is difficult because microbial activity and growth are strongly dependent on environmental conditions (e.g., nutrient status and physical constraints). Attachment of cells on surfaces may influence their activity and growth due to change in local environment and/or cell metabolism itself. Thus, it is essential to determine effects of these environmental factors on the activity and growth of SRB to develop a comprehensive model and use this model to predict the SRB behavior.

Mono-population *Desulfovibrio desulfuricans* was grown on lactate and sulfate in a chemostat and in a RotoTorque biofilm reactor. Effects of temperature, limiting nutrients (e.g., phosphorous, nitrogen, and sulfate), and sulfide product on rate and stoichiometry of microbial sulfate reduction were determined in the chemostat experiment. Biofilm kinetics and stoichiometry were determined in the RotoTorque reactor and compared with planktonic (chemostat) data.

The Monod kinetic coefficients ($\mu_{\max}$, K_s , and $Y_{x/S}$) were dependent on temperature, but stoichiometry for catabolic reactions was not. The limiting C:P and C:N ratios (w/w) were found to be in the range of 400:1 to 800:1 and 45:1 to 120:1, respectively. Production of extracellular polymeric substance (EPS) increased with increasing both C:P and C:N ratios in the medium. A non-competitive inhibition model adequately described sulfide product inhibition kinetics. Anabolic reactions (cell production), but not the catabolic reactions (energy production), were strongly inhibited by high sulfide concentrations. Maximum biofilm specific growth rate ($\mu_{b\max}$) was essentially the same as $\mu_{\max}$ for planktonic cells. μ_b decreased as biofilm grew even though the biofilm was not substrate-limited. Stoichiometry of the catabolic reactions was the same for biofilm and planktonic cells, but a low cellular yield and a high EPS yield were determined in biofilms. These results suggested that *D. desulfuricans* behave differently in biofilms than in suspension. Thus, rate and stoichiometric data determined from planktonic cells must be used cautiously in developing a model to predict growth and activity in biofilms.

RATE AND STOICHIOMETRY OF SULFATE REDUCING BACTERIA
IN SUSPENDED AND BIOFILM CULTURES

by

Satoshi Okabe

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ABSTRACT

The accumulation and activity of sulfate reducing bacteria (SRB) are of critical concern in many industrial water systems. For example, SRB cause numerous problems in petroleum production including contamination of petroleum with H_2S , injection well plugging, and corrosion. Prediction of SRB behavior in natural and industrial water systems is difficult because microbial activity and growth are strongly dependent on environmental conditions (e.g., nutrient status and physical constraints). Attachment of cells on surfaces may influence their activity and growth due to change in local environment and/or cell metabolism itself. Thus, it is essential to determine effects of these environmental factors on the activity and growth of SRB to develop a comprehensive model and use this model to predict the SRB behavior.

Mono-population *Desulfovibrio desulfuricans* was grown on lactate and sulfate in a chemostat and in a RotoTorque biofilm reactor. Effects of temperature, limiting nutrients (e.g., phosphorous, nitrogen, and sulfate), and sulfide product on rate and stoichiometry of microbial sulfate reduction were determined in the chemostat experiment. Biofilm kinetics and stoichiometry were determined in the RotoTorque reactor and compared with planktonic (chemostat) data.

The Monod kinetic coefficients (μ_{max} , K_s , and $Y_{x/s}$) were dependent on temperature, but stoichiometry for catabolic reactions was not. The limiting C:P and C:N ratios (w/w) were found to be in the range of 400:1 to 800:1 and 45:1 to 120:1, respectively. Production of extracellular polymeric substance (EPS) increased with increasing both C:P and C:N ratios in the medium. A non-competitive inhibition model adequately described sulfide product inhibition kinetics. Anabolic reactions (cell production), but not the catabolic reactions (energy production), were strongly inhibited by high sulfide concentrations. Maximum biofilm specific growth rate (μ_b^{max}) was essentially the same as μ_{max} for planktonic cells. μ_b decreased as biofilm grew even though the biofilm was not substrate-limited. Stoichiometry of the catabolic reactions was the same for biofilm and planktonic cells, but a low cellular yield and a high EPS yield were determined in biofilms. These results suggested that *D. desulfuricans* behave differently in biofilms than in suspension. Thus, rate and stoichiometric data determined from planktonic cells must be used cautiously in developing a model to predict growth and activity in biofilms.

CHAPTER 1

GENERAL INTRODUCTION

Biofilm development in natural and industrial water systems depends on the types and concentrations of electron acceptors (e.g., oxygen, nitrate, and sulfate) and electron donors (organic substrates) and environmental factors including temperature, pH, salinity, and fluid dynamics. Development of sulfate-reducing bacterial (SRB) biofilms in natural and industrial water systems can be expected whenever environmental conditions such as E_h , oxygen tension, and nutrients are suitable for SRB growth. For example, SRB biofilm may develop in anaerobic bulk water systems such as in petroleum producing facilities, but is also found in aerobic bulk water systems such as cooling water systems. In aerobic bulk water systems, anaerobic microniches and/or anaerobic layers exist in biofilms due to depletion of oxygen by aerobic bacterial activity (Jorgensen, 1977; Lee et al., 1992). In all natural and industrial aquatic environments, SRB show a pronounced tendency to adhere to available surfaces and to proliferate to form biofilms (Costerton and Geesey, 1979; Dewar, 1986; Rosnes et al., 1990). Because of this sessile mode of growth, bacteria within these biofilms are often undetected by conventional sampling techniques which analyze bulk water conditions. Nevertheless, it is these biofilm (sessile) SRB that are responsible for much of the activity in natural and industrial water systems.

At present, quantitative prediction of SRB activity and growth in industrial water systems is essentially impossible because rate and extent of SRB growth under

relevant environmental conditions are not available. Therefore, it is necessary to determine effects of environmental factors on the activity and growth of SRB to develop a comprehensive model and use this model to predict the SRB behavior in given environments. By comparing data from a variety of environments, a conceptual model is first developed and includes factors such as growth substrate limitation, temperature, sulfide inhibition, and the effects of attachment. Translation of the conceptual model into a mathematical form requires much more controlled experimentation to determine kinetic and stoichiometric coefficients (e.g., growth rate, yield), and the specific effects of external factors such as temperature on these coefficients.

As will be shown below, the initial conceptual model must include the following:

- effect of temperature
- nutrient availability (including e⁻ donor and acceptor, N, P)
- sulfide inhibition
- attachment to surfaces (i.e. biofilm versus planktonic growth)

Nutrient availability can affect both the growth of the organisms (through energy limitation or through limitations in synthesis precursors) as well as the amounts and types of products (e.g., cellular material versus extracellular products). Temperature can affect both kinetics and stoichiometry via phenomena ranging from thermodynamic activity changes through physical enzyme configuration changes. Product inhibition (sulfide) reduces biochemical activity through numerous mechanisms. Finally, attachment to surfaces has been shown to affect bacterial metabolism in a variety of ways, although it is difficult to specify whether these changes are due to a

physiological response to attachment or to an altered extracellular environment resulting from diffusion limitations (Van Loosdrecht et al., 1990).

Research Goal and Objectives

The goal of this research is to investigate the effects of nutritional and physical factors on the rate and stoichiometry of microbial sulfate reduction to incorporate the data into future conceptual models.

The objectives to achieve this goal are as follows:

- 1) Determine the effects of temperature on the rate and stoichiometry of microbial sulfate reduction by *Desulfovibrio desulfuricans*.
- 2) Determine the effects of phosphorous, nitrogen, and sulfate concentrations on the rate and stoichiometry of microbial sulfate reduction by *D. desulfuricans*.
- 3) Determine the effects of sulfide concentration on the growth and activity of *D. desulfuricans*.
- 4) Determine stoichiometry and rate of microbial sulfate reduction in *D. desulfuricans* biofilm cells in comparison with those for planktonic cells.

Process Analysis and Modeling

SRB biofilm accumulation is a complex phenomenon resulting from several processes occurring in parallel and in series. The rate and extent of these processes, in turn, are influenced by numerous physical, chemical, and biological factors. Thus, a process analysis must be applied to solve biofilm-related problems. The process

analysis generally requires (1) development of a mathematical model and (2) experimental testing of the model.

Process Analysis

The most important results, from the viewpoint of a process analysis of a reaction system, are expressions that quantitatively describe the rate and extent (stoichiometry) of the fundamental processes contributing to biofilm accumulation. Stoichiometry indicates the relationship between the extent of microbial growth and the uptake and production of the chemical species involved. Rate describes how fast the reactions will occur. Both stoichiometry and rate must be known to effectively design and control technical scale processes. The stoichiometric relationships are important since they permit estimation of the rate and extent of biomass and product formation (e.g., hydrogen sulfide) by measuring change in sulfate concentration with time.

A conceptual model describing biofilm accumulation processes would be beneficial in interpreting available historical data and be invaluable in designing future experiments. If the conceptual model could be stated in mathematical terms, a mathematical simulation of biofilm accumulation can be performed on the computer at considerably less expense than laboratory experiments. Furthermore, the influence of process variables such as temperature and substrate concentrations can be determined on the computer *prior to* conducting laboratory experiments. The mathematical description of the individual processes can be combined to develop models to extrapolate and generalize experimental results. Many of these fundamental processes have been described mathematically (Characklis and Marshall, 1990).

Experimental Approach

It is important to proceed in stages beginning with the pure culture work where precisely defined growth conditions and conclusions relevant to those conditions can be made. Understanding of the behavior of single species leads to a more rational image of a mixed population behavior.

Rate and stoichiometry are often determined in chemostat experiments. Analysis of rate and stoichiometry of processes within a biofilm are frequently complicated by significant mass transfer resistances in the liquid or diffusional resistances within the biofilm. Rate coefficients of microbial sulfate reduction are difficult to measure in a batch culture because pH, sulfide concentration, and limiting substrate cannot be maintained at the same levels over many generations. After rate and stoichiometry are determined from the pure culture chemostat experiments (planktonic cells), trials with biofilms (sessile cells) are needed to establish whether or not rate and stoichiometry of the planktonic cells can be used to predict bacterial behavior within the biofilm. Once factors affecting growth and activity of planktonic cells are determined in the chemostat, their quantitative effect on biofilm must be determined. Finally, all these data can be incorporated into a model which will permit prediction of SRB behavior in various environments. The model will also lead to the development of means to control SRB growth and activity.

Background

Physiology of SRB

The SRB are a metabolically diverse group of strictly anaerobic organisms using sulfate as terminal electron acceptor, reducing it to sulfide. Most SRB can grow with sulfite or thiosulfate as electron acceptors instead of sulfate (Badziong and Thauer, 1978; Cypionka et al., 1985; Jorgensen and Bak, 1991). Some species can optionally use nitrate or fumarate as electron acceptor (Liu and Peck, 1981; McCready et al., 1983; Postgate, 1984). In the absence of sulfate or other inorganic electron acceptors, several types of SRB can grow by fermentation of special organic substrates (Laanbroek et al., 1982; Widdel and Pfennig, 1981; Traore et al., 1983; Postgate, 1984). The range of carbon sources available for cell growth is very wide. Growth is possible on CO₂ (Brysch et al., 1987), on a range of organic compounds including benzoate but excluding sugars and hydrocarbon, and on fatty acids from stearate. SRB can be classified into two broad nutritional groups. Species of the first group carry out an incomplete oxidation of organic substrates with acetate as an end product. Species of the second group oxidize organic substrates, including acetate, completely to CO₂. Recently, Aeckersberg et al. (1991) isolated SRB from an oil production plant which oxidize saturated hydrocarbons (n-Hexadecane).

The optimum temperature for most SRB is about 20 to 40°C (Widdel, 1988). Relatively few types of SRB are known to be able to grow at high temperature range 55 to 80°C (Rozanova and Khudyakova, 1974; Postgate, 1984; Cochrane et al.,

1988; Rosnes et al., 1991). The physiology of SRB and their ecological impact have been described by Postgate (1984). Nine genera of SRB have now been recognized, and representative species are described in the literature (Postgate, 1984; Widdel, 1988).

Ecology

Significant activities of SRB are measured in salt-marsh or marine sediments because of the high sulfate concentration of seawater ($28 \text{ mmol} = 2.7 \text{ g SO}_4^{2-}/\text{L}$) (Jorgensen, 1983). Despite the inhibitory effect of oxygen on SRB, SRB are sometimes active in aerobic aquatic environments, where SRB thrive in anaerobic microniches (Canfield and Des Marais, 1991; Lee, 1992). Formation and maintenance of such microniches is explained by two factors. First the respiration of aerobic bacteria scavenge oxygen and favor growth conditions for SRB. Second, H_2S produced by SRB is a reductant that reacts with oxygen; thus, if once established, colonies of SRB can protect themselves against oxygen (Cypionka et al., 1985). SRB have been observed in unfavorable pH environments because the metabolic products of SRB represent buffers - the $\text{H}_2\text{S}/\text{HS}^-$ and the $\text{CO}_2/\text{HCO}_3^-$ systems - that protect against extreme pH values.

SRB play a role in the terminal stage of anaerobic degradation of organic materials. In marine and other sulfate containing environments, mineralization involves three broad groups of organisms: heterotrophs that are aerobic, facultative, or anaerobic and are capable of at least partial breakdown of primary substrates; hydrogen-producing acetogenic bacteria; and SRB. In marine sediment slurries, sulfate reduction contributes about 50% of the total mineralization of organic material as a

whole (Sorensen et al., 1981; Jorgensen, 1982; Christensen, 1984). Other studies demonstrated potential hydrogen oxidation by SRB in eutrophic lake sediments (Ingvorsen et al., 1981; Smith and Klug, 1981). In addition to the oxidation of hydrogen, potential significance of lactate, propionate, and amino acids are demonstrated in eutrophic lake sediments (Smith and Klug, 1981), whereas there is evidence that neither acetate or lactate support sulfate reduction (Ingvorsen et al., 1981).

SRB Biofilms and Models

The most important aspect of biofilm accumulation is development of anaerobic conditions within the biofilm even under aerobic bulk water (Lee et al., 1992). When a biofilm reaches a thickness of 10-25 μm under aerobic environment, conditions at the base of biofilm become anaerobic (Culter and Russell, 1976; Costerton and Geesey, 1979).

There is very little quantitative information available related to the rate and extent of SRB biofilm accumulation. Nielsen (1987) conducted a mixed population SRB biofilm study in an annular biofilm reactor. He reported that when the biofilm thicknesses reached 300-400 μm , it was no longer fully penetrated by sulfate at sulfate concentration of about 100 mg/L. The sulfide production from biofilms grown on domestic wastewater was modeled using biofilm kinetics and agreed with experimental results (Nielsen and Hvitved-Jacobsen, 1988). Sulfate limitation in a typical sewer biofilm, with a thickness of 200-300 μm , was shown to occur at sulfate concentrations of 3-5 mg SO_4^{2-} -S/L.

Lee (1990) reported that mixed population SRB biofilm accumulation was

strongly dependent on substrate loading rate. The thickness of the mixed population SRB biofilm easily reached about 1000 μm at high substrate loading ($100 \text{ mg-C m}^{-2} \text{ h}^{-1}$), whereas the thickness was about 5 μm at low substrate loading rate ($5.4 \text{ mg-C m}^{-2} \text{ h}^{-1}$).

To design new wastewater treatment systems to minimize sulfide production or to efficiently control sulfide production in industrial water systems, a reliable method that predicts the sulfide production rate is needed. Empirical models for the prediction of sulfide production from sewer systems have been published (Holder et al., 1984; Holder, 1986; Nielsen and Hvitved-Jacobsen, 1988). However, the biofilm kinetics and effects of nutritional and physical factors are not taken into account in these models. More quantitative and comprehensive prediction models for sulfide production are necessary for more accurate prediction.

Industrial Problems Associated with SRB Activity

Sulfate reducing bacteria (SRB) are very important microorganisms from an industrial standpoint. The anaerobic corrosion of metals, enhanced by the activities of SRB, is one of the best known of the economic problems caused by SRB. The cost related to SRB-mediated corrosion is estimated to be 1 to 2 billion dollars per year in the U. S (Lee, 1990). The cost to the industries with regard to biofouling (SRB biofilm) including corrosion was estimated to be 300-500 million pounds per year for the U.K. (Tiller, 1982). Extensive sulfide corrosion problems with concrete sewer pipes and wastewater treatment were also reported (Witzgall et al., 1990).

In the petroleum industry, SRB cause serious problems including corrosion of

installation, plugging of the petroleum formation, and reservoir souring (contamination of petroleum with H_2S) (Dewar, 1986; Herbert, 1986; Cochrane et al., 1988; Burger et al., 1991; Frazer and Bolling, 1991). Sulfide production by SRB increases the sulfur content of the crude oil which decreases its value and increases refining costs. Costs for downtime, resulting in loss of production, to clean and replace fouled or corroded equipment easily extend to \$ 10 million per day (Characklis, 1991). Hydrogen sulfide production by SRB leads to the corrosion of down-hole drill strings and casings as well as production facilities (Sanders and Hamilton, 1983). SRB growth in seawater injection systems can lead to corrosion as well as contamination of oil and gas with H_2S and viable SRB. Cord-Ruwisch et al. (1987) reported that an increase in H_2S was observed during several years of operation at an oil field in northern Germany, and that as a result of H_2S formation the injection well was plugged by FeS flocs. Comprehensive references regarding SRB causing problems in petroleum industries were reported by Postgate (1982, 1984), Sanders and Hamilton (1983), and Hamilton (1985).

Biofilm accumulation also increases capital costs for equipment in power plants. For example, a nuclear power plant had to replace a condenser after approximately 6 years operation because of severe corrosion attributed partially to microbial activity (Characklis, 1991).

Control Strategies

Biocides

Extensive research has been conducted to develop effective biocides with the

goal of inhibiting SRB growth and hence sulfide production. For example, in the secondary production of petroleum, injection water used in flooding operations is treated routinely with a biocide (typically glutaraldehyde) to control SRB growth in the injection well, reservoir, and piping (Brunt, 1986). Eagar et al. (1986) reported that glutaraldehyde was an effective agent for controlling *Pseudomonas fluorescens* biofilm growth and activity. Also, the results of field study indicated that glutaraldehyde was sufficiently persistent in the distribution system to remain at an efficacious level, and reduced the corrosion rate to an acceptable rate. Gaylarde and Johnston (1983) strongly recommended that biocide test methods for SRB activity should employ mixed sessile SRB in the presence of metal coupons, because sessile SRB on the metal coupon surfaces survived at twice the recommended dose for both biguanide and nitropropanediol.

Biocide addition is often of limited effectiveness since SRB are associated with other anaerobic bacteria in biofilms which coat the surfaces of pipes and other materials. Within these biofilms, SRB are somewhat protected because biocides do not effectively penetrate through the biofilm. All of the reported data have shown the bacteria within biofilms are much more difficult to control with biocides than their planktonic counterparts in these systems (Gaylarde and Johnston, 1983; Ege et al., 1985; Dewar, 1986). Thus, biocide treatment may not be an ultimate means to control SRB activity because of rapid microbial regrowth, cost effectiveness, and environmental concerns.

Nutrient Removal

The reduction of the concentration of an essential nutrient (e.g., phosphorous,

nitrogen, and/or sulfate) to below the limiting concentration is a possible means of controlling SRB activity because the essential nutrients reduce activity and growth of SRB when they become limiting. Maree and Strydom (1987) reported the feasibility of microbial sulfate removal from industrial effluent using an upflow packed bed reactor with photosynthetic sulfur oxidation to prevent the emission of sulfide and confirmed the successful performance of reactor. There is no information in the literature which addresses control of SRB activity and growth by removing required nutrients. Nutrient removal may be a possible means of controlling SRB activity and growth. This would be of benefit both in environmental and economic terms.

Microbial Competition

Microbial control of sulfide production by SRB using *Thiobacillus denitrificans* has attracted considerable attention lately (McInerney et al., 1986; Buisman et al., 1990; Montgomery et al., 1990). *T. denitrificans* is an autotroph and a facultative anaerobe which oxidizes sulfide to sulfate using oxygen or nitrate as the electron acceptor. The introduction of viable cells of *T. denitrificans* into environments with SRB has the potential of controlling sulfide production so long as nitrate concentration remains high. The application of this method is to control sulfide production at or near the water injection well in an oil reservoir. A practical difficulty is efficiently inoculating *T. denitrificans* into the well-bore area. A mutant of *T. denitrificans* (strain F) resistant to glutaraldehyde and sulfide was obtained by McInerney et al. (1986). This mutant strain would allow a combined microbial and biocide (glutaraldehyde) treatment of SRB contaminated industrial systems. Sublette and Sylvester (1987a, 1987b, 1987c) and Sublette (1987) have demonstrated that *T. denitrificans* may be readily cultured

aerobically and anaerobically in batch and continuous reactors on gaseous H_2S under sulfide-limiting conditions. A microbial process for the removal of H_2S from gases has been proposed based on mixing the gas with a culture of *T. denitrificans* (Sublette and Sylvester, 1987a).

The competition for the available electron donors between SRB and methane producing bacteria (MPB) has also received considerable attention (Abram and Nedwell, 1978; Krisjansson et al., 1982; Schönheit et al., 1982; Isa et al., 1986; Toda et al., 1987). The SRB apparently have a higher affinity (low k_m) for hydrogen and acetate relative to the methane producing bacteria (MPB). Thus, SRB are normally dominate both in natural ecosystems, such as freshwater and marine sediments, and in anaerobic digesters, where methanogenesis was found to be inhibited by the presence of sulfate. Yoda et al. (1987) reported that in an anaerobic fluidized bed the methane production rate and MPB biomass decreased after several months of operation at low acetate concentration whereas sulfate reduction rate increased. On the other hand, MPB were able to form a biofilm faster than SRB at high acetate concentrations presumably due to MPB's higher ability to adhere to carrier surfaces than SRB. Hilton and Oleszkiewicz (1989) reported that SRB are more sensitive than MPB to the elevated total sulfide concentrations, while both are sensitive to elevated molecular H_2S concentrations. Thus, at high total sulfide concentrations and high pH the MPB should be able to outcompete the SRB for substrate.

Aeration

Oxygen is the cheapest and most effective inhibitor of SRB activity. If any system can be maintained in an aerated condition even though the dissolved oxygen

concentration is vanishingly small, SRB remain dormant but are not killed (Hardy and Hamilton, 1981; Cypionka et al., 1985). This measure is of limited effectiveness, however, because SRB are generally associated with other bacteria in biofilms. Oxygen does not effectively penetrate through these biofilms due to consumption of oxygen by aerobes (Jorgensen, 1977). As clearly demonstrated by Lee et al. (1992), SRB activity at the substratum beneath a biofilm can be extensive, even at high dissolved oxygen in the bulk water. Furthermore, introducing oxygen into some industrial water systems increases the corrosion of facilities (Lee et al., 1992).

Ultraviolet Radiation

The use of ionizing radiation to control SRB activity and growth has recently attracted attention. Ultraviolet radiation was used to kill SRB in injection waters by Ege et al. (1985). Gamma radiation was also applied to control SRB at the bottom of the well bores as the water enters the oil reservoir (Agaev et al., 1985).

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CHAPTER 2

EFFECTS OF TEMPERATURE AND PHOSPHOROUS CONCENTRATIONS ON
MICROBIAL SULFATE REDUCTIONIntroduction

Sulfate reducing bacteria (SRB) are very important microbes from an environmental and industrial standpoint. For example, in petroleum technology, SRB cause serious problems including corrosion of installations, plugging of the formation and contamination of petroleum with H_2S (souring) in the formation (Postgate, 1984; Hamilton, 1985; Sanders and Hamilton, 1985). Cochrane et al. (1988) report that the presence and growth of thermophilic SRB at temperatures greater than $60^\circ C$ was a major source of sulfide production in a North Sea oil field and that seawater injection results in the appropriate balance of sulfate, temperature and organic nutrient status for growth in the reservoir. Herbert et al. (1986) report that substantial levels of short-chain fatty acids, and ammonia present in many formation waters can be used directly by SRB as a source of energy. However, quantitative description of reservoir souring is essentially impossible because coefficients for rate and extent of SRB growth under relevant environmental conditions are not available.

The microbial environment varies widely through the formation. The temperature varies from that of the cold injection water to that of the hot formation. The system temperature can have a major influence on SRB activity. Most mesophilic

SRB have an optimum growth temperature in the range of 25 to 40°C. Temperature outside this range may account for variation in SRB activity. Temperature gradients in the formation cause changes in SRB growth and associated variables. Biocide treatments must be tailored to these microbial growth patterns.

Concentrations of sulfate, substrate (carbon source), and essential nutrients (e.g., phosphorous) in the formation vary as they are depleted by microbial activity or are mediated by the formation, itself. A determination of the limiting nutrient concentration may be useful to control and predict SRB activity in industrial systems. Essential nutrients (e.g., phosphorous and nitrogen) affect the rates of SRB activity and growth when they become limiting. Seawater has sufficient nutrients to support an active but minimal SRB population (e.g., total P in sea water is 0.001 to 0.1 mg/L (Altman and Pittmer, 1964)). Therefore, the reduction of the concentration of an essential nutrient to below the limiting concentration is a possible means of controlling SRB. Ironically, water treatment chemicals may enrich the system in C, N, and P.

The goal of this chapter is to determine effects of temperature and phosphorous concentration on rate and stoichiometry of microbial sulfate reduction by *Desulfovibrio desulfuricans*.

Background

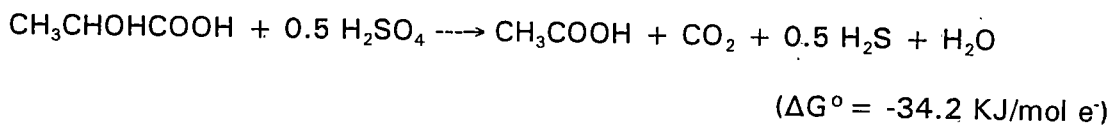
Stoichiometry and Rate

There are two characteristics of microbial reactions of importance to process design and control: stoichiometry and rate. Stoichiometry indicates the changes which will occur and their extent. Rate describes how fast the changes will occur. Both

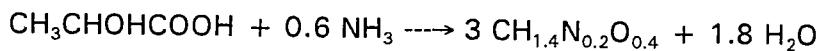
process stoichiometry and rate must be known to effectively design and control technical scale processes.

Stoichiometry. Dissimilatory sulfate reduction can be represented by a pair of stoichiometric equations that describe the oxidation of an organic carbon source and the synthesis of bacterial cells. For example, if lactate is the electron donor and energy source, approximate stoichiometric equations for sulfate reduction are as follows:

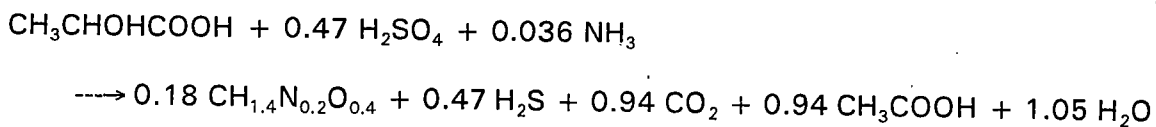
Energy



Synthesis



Overall stoichiometry

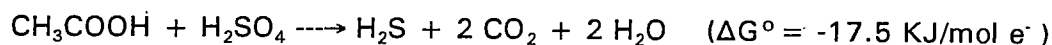


The overall stoichiometric equation was balanced using the experimental determinations of overall yield and carbon dioxide production by *D. desulfuricans* (Traore et al., 1982). D'Alessandro et al. (1974) reported very similar stoichiometry for sulfate reduction by *D. vulgaris*. In both cases, lactate and sulfate were consumed in a 2:1 molar ratio.

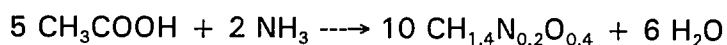
If acetate is the electron donor and energy source, approximate stoichiometric

equations for sulfate reduction are as follows:

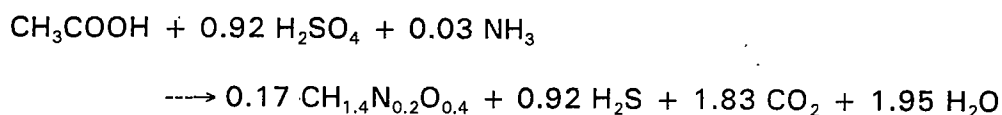
Energy



Synthesis



Overall stoichiometry



The experimental overall yield obtained by Middleton and Lawrence (1977) was used to balance the overall stoichiometric equation. Each molecule of lactate or acetate transfers 4 and 8 electrons, respectively. The yields for SRB growth with lactate, acetate, butyrate, and propionate as sole energy and carbon sources have been reported (Tables 1, 2, and 3). The stoichiometric relationships are important since they permit estimation of the rate and extent of biomass and product formation by measuring changes in sulfate concentration with time.

The reporting of yield requires further definition. This paper reports yield data from various sources which are not directly comparable. Generally, yield is expressed as the ratio of product formed to reactant consumed. In biological reactors, yield generally refers to the ratio of particulate matter produced to soluble substrate consumed. In an SRB chemostat with sterile feed containing only dissolved

components, the extent of particulate products formed may include cells, extracellular polymeric substances (EPS), and sulfide precipitates (e.g., FeS). The EPS may be immobilized on the cells, released into the bulk liquid phase, or both. The sulfide precipitates may be suspended in the bulk liquid (if sufficient mixing is provided) or may be adsorbed on the cells or EPS. Thus, the means of measuring product formation will operationally define the yield. For purposes of this paper, three (3) yields are defined:

Overall yield, $Y_o = (\text{Cells} + \text{EPS} + \text{Precipitates}) / (\text{Substrate consumed})$

Biomass yield, $Y_b = (\text{Cells} + \text{EPS}) / (\text{Substrate consumed})$

Cell yield, $Y_c = (\text{Cells}) / (\text{Substrate consumed})$

Y_o is obtained when gravimetric (suspended solids) determinations are employed. Precipitates may or may not exist in the samples so further analysis of the solids is recommended. Y_b can be estimated by measurement of the particulate organic carbon. Finally, this chapter describes a method for independently estimating the cells and EPS produced. The method combines organic carbon analysis and cell size measurements accomplished by image analysis (Robinson et al., 1984).

The limiting nutrient or substrate considered in this chapter can be the energy source or electron donor (e.g., lactate) or the electron acceptor (sulfate). As a consequence, the yield symbol differentiates between yield based on electron donor (e.g., $Y_{c/Lac}$ for cell yield based on lactate consumption) or electron acceptor (e.g., $Y_{b/Sul}$ for biomass yield based on sulfate consumption).

Rate. The rate of a microbial reaction may be described by the Monod expression as a function of limiting substrate concentration (e.g., lactate).

$$\mu = \frac{(\mu_{\max} S)}{(K_{Lac} + S)} \quad (1)$$

where, μ = specific growth rate (t^{-1}), $\mu_{\max}$ = maximum specific growth rate (t^{-1}), S = lactate concentration ($M_s L^{-3}$), K_{Lac} = half-saturation coefficient for lactate ($M_s L^{-3}$).

$\mu_{\max}$ and K_s for SRB growth with lactate and acetate as energy and carbon source have been reported (Tables 1 and 2). Lactate-utilizing SRB are capable of doubling times as low as 3 to 6 hours at 30°C, whereas acetate-utilizing SRB grow more slowly with doubling times longer than 20 hours (Postgate, 1984). However, the data presented in Tables 1, 2, and 3 show wide variations and are also very incomplete. Thus, it is very difficult to draw further conclusions from these data.

Mathematical Description for the Chemostat

Microbial transformations in chemostats can be mathematically described by mass balance equations assuming the two fundamental processes occurring are growth and maintenance. Growth rate depends upon substrate (e.g., lactate) concentration according to Eq(1). Lactate is assumed to be partitioned into cellular and EPS mass (biomass = cellular + EPS). Mass balances for lactate, cellular, and EPS in the liquid phase are as follows:

Lactate

$$\frac{dS}{dt} = D (S_i - S) - \frac{X \mu}{Y_{c/Lac}} - \frac{R_p X}{Y_{p/Lac}} - m X \quad (2)$$

Table 1. Reported SRB rate and stoichiometric parameters for lactate-utilizing SRB at 30°C.

Investigator	Organisms	$\mu_{\max}$ (h ⁻¹)	K _{Lac} (mg/L)	K _{Sul} (mg/L)	Y _{o/Lac} (g/g)	Y _{o/Sul} (g/g)	pH	B/C ^{a)}
Cappenberg (1975)	<i>D. desulfuricans</i>	0.360	4.4	-	0.343	-	7.4	C
Traore et al. (1982)	<i>D. desulfuricans</i>	0.104	-	-	0.046	-	-	B
	<i>D. africans</i>	0.060	-	-	0.019	-	-	B
	<i>D. gigas</i>	0.092	-	-	0.042	-	-	B
Traore et al. (1981)	<i>D. vulgaris</i>	-	-	-	0.075	-	7.2	B
Ingvorsen & Jorgensen (1984)	<i>D. vulgaris</i>	0.011	-	0.5	-	0.074	7.2	B
	<i>D. sapovorans</i>	0.007	-	0.7	-	0.091	7.2	B
	<i>D. salexigens</i>	0.021	-	7.4	-	0.083	7.2	B

^{a)} Refers to batch or continuous (chemostat) reactor.

Table 2. Reported SRB rate and stoichiometric parameters for acetate-utilizing SRB.

Investigator	Organisms	$\mu_{\max}$ (h ⁻¹)	K _{Ace} (mg/L)	K _{Sul} (mg/L)	Y _{o/Ace} (g/g)	Y _{o/Sul} (g/g)	Temp. B/C ^{a)} (°C)
Midleleton & Lawrence (1977)	Mixed population	0.007	250	-	0.065	-	20 B
		0.019	92	-	0.065	-	25 B
		0.022	5.7	-	0.065	-	31 B
Ingovorsen et al.(1984)	<i>Desulfobactor postgatei</i>	0.030	-	4.2	-	0.158	30 B
Widdel & Pfenning (1981)	<i>Desulfobactor postgatei</i>	0.035	-	-	0.074	-	32 B
Schauder et al.(1986)	<i>Desulfobactor postgatei</i>	0.025	-	-	-	-	30 B
		0.046	-	-	-	-	30 B
Widdel & Pfenning (1977)	<i>Desulfotomaculum acetoxidans</i>	0.058	-	-	0.095	-	36 B
Schauder et al. (1986)	<i>Desulfotomaculum acetoxidans</i>	0.014	-	-	-	-	30 B
		0.032	-	-	-	-	37 B

^{a)} Refers to batch or continuous (chemostat) reactor.

Table 2. Reported SRB rate and stoichiometric parameters for acetate-utilizing SRB. (Continued)

Investigator	Organisms	$\mu_{\max}$ (h ⁻¹)	K _{Ace} (mg/L)	K _{Sul} (mg/L)	Y _{o/Ace} (g/g)	Y _{o/Sul} (g/g)	Temp. B/C ^{a)} (°C)
Schauder et al.(1986)	<i>Desulfobacter hydrogenophilus</i>	0.039	-	-	-	-	30 B
Widdel (1986)	<i>Desulfobacter hydrogenophilus</i>	0.038	-	-	-	-	25-28 B
Widdel et al.(1983)	<i>Desulfonema</i> sp.	0.023	-	-	-	-	30 B
		0.0069	-	-	-	-	30 B
Widdel (1986)	<i>Desulfobacter curvatus</i>	0.033	-	-	-	-	25-28 B

^{a)} Refers to batch or continuous (chemostat) reactor.

Table 3. Reported SRB rate and stoichiometric parameters for propionate and butyrate-utilizing SRB.

Investigator	Organisms	$\mu_{\max}$ (h ⁻¹)	K _{Pro} (mg/L)	Y (g/g)	Temp. (°C)	B/C ^{a)}
Nanninga et al.(1987)	<i>Desulfobulbus propionicus</i>	0.110	-	-	35	B
Widdel & Pfenning (1982)	<i>Desulfobulbus propionicus</i>	0.069	-	0.071 ^{b)}	39	B
Widdel & Pfenning (1977)	<i>Desulfomaculum acetoxidans</i>	0.046	-	-	36	B
Schauder et al.(1986)	<i>Desulfovibrio</i> ^{c)} <i>baarsi</i>	0.017	-	-	30	B
Nanninga et al.(1987)	<i>Desulfovibrio</i> ^{c)} <i>sapovorans</i>	0.066	-	-	35	B
Hunter(1989)	Mixed population	0.070	90.0	0.022 ^{d)}	35	C

a) Refers to batch or continuous (chemostat) reactor.

b) growing on 5 mM of propionate, Y_{o/Pro}.

c) growing on butyrate.

d) Y_{c/Pro}.

Cellular

$$\frac{dX}{dt} = D (X_i - X) + \mu X \quad (3)$$

EPS

$$\frac{dP}{dt} = D (P_i - P) + R_p X \quad (4)$$

where S_i = influent lactate concentration ($M_s L^{-3}$), X = cell concentration ($M_x L^{-3}$), X_i = influent cell concentration ($M_x L^{-3}$), $Y_{c/Lac}$ = cell yield coefficient on lactate ($M_x M_s^{-1}$), $Y_{p/Lac}$ = EPS yield coefficient on lactate ($M_p M_s^{-1}$), P = EPS concentration ($M_p L^{-3}$), P_i = influent EPS concentration ($M_p L^{-3}$), R_p = specific EPS formation rate ($M_p M_x^{-1} t^{-1}$), D = dilution rate (t^{-1}), m = maintenance coefficient ($M_s M_x^{-1} t^{-1}$), and t = time (t).

For a sterile feed, $X_i = 0$, $P_i = 0$, and at steady state, Eqs.(2), (3), and (4) become

$$\frac{D (S_i - S)}{X} = \frac{\mu}{Y_{c/Lac}} + \frac{R_p}{Y_{p/Lac}} + m \quad (5)$$

$$D = \mu \quad (6)$$

$$D P = R_p X \quad (7)$$

All quantities on the left side of Eqs.(5) (6), and (7) are measurable. Equation (6) indicates that μ can be controlled by the experimenter. Eq.(5) simplifies to the following expression if EPS formation is negligible:

$$\frac{D (S_i - S)}{X} = \frac{\mu}{Y_{c/Lac}} + m \quad (8)$$

Experimental Materials and Methods

Experimental System

The rate and stoichiometric coefficients were determined at several temperatures and phosphorous concentrations in a chemostat consisting of a pyrex cylindrical beaker ($0.45 \times 10^{-3} \text{m}^3$ volume) with a Teflon lid sealed using an O-ring (Figure 1). The chemostat was equipped with an inverted plastic funnel attached to metal rods that served to remove wall growth during long runs. Heavy wall butyl rubber tubing (Cole-Parmer, Masterflex neoprene tubing) was used to minimize oxygen flux. The flow rates were controlled by the pump and speed controller (Cole-Parmer, Chicago, IL.).

The pH was maintained at 7.0 ± 0.2 by a pH controller which automatically added sterile, oxygen-free, 1.0 N HCl or NaOH solutions stored under nitrogen atmosphere. The temperature was maintained by a thermoregulator and heating blanket. The slow continuous nitrogen purge (3 L/h) of the reactor maintained anaerobic conditions and prevented H_2S accumulation. Traces of oxygen in the nitrogen feed gas were removed by a reducing column containing copper wire maintained at 370°C . The gas was sterilized by a cotton filter. The flow rate of nitrogen gas was approximately 3 L/h.

Desulfovibrio desulfuricans (ATCC 5575) was grown in Postgate medium G (Postgate 1984), including hemi-calcium lactate (L-lactic acid, SIGMA, No.L-2000) as the sole carbon and energy source. Trace elements and vitamins were added (Table 4). The stock solutions were mixed with base medium after autoclaving and cooling under nitrogen purge. 0.5 g/L of Na_2SO_4 was used for the temperature effect experiments. Sterile $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ was added as a reductant until a vigorously growing culture was established. The final concentration of sodium hydrosulfide was 300 mg/L. For long-term storage, *D. desulfuricans* was preserved at -70°C .

The entire chemostat assembly was washed with chromic sulfuric acid and rinsed out with redistilled water and then autoclaved for 15 minutes at 121°C . After cooling down, the autoclaved culture medium was fed into the reactor under nitrogen purge. After about 4-5 hours, the test organisms were introduced into the reactor. The reactor was operated as a batch culture until turbidity of the medium was clearly observed. Then, continuous flow of media commenced. Steady state conditions were periodically checked by measuring cell number and sulfate concentration in the effluent.

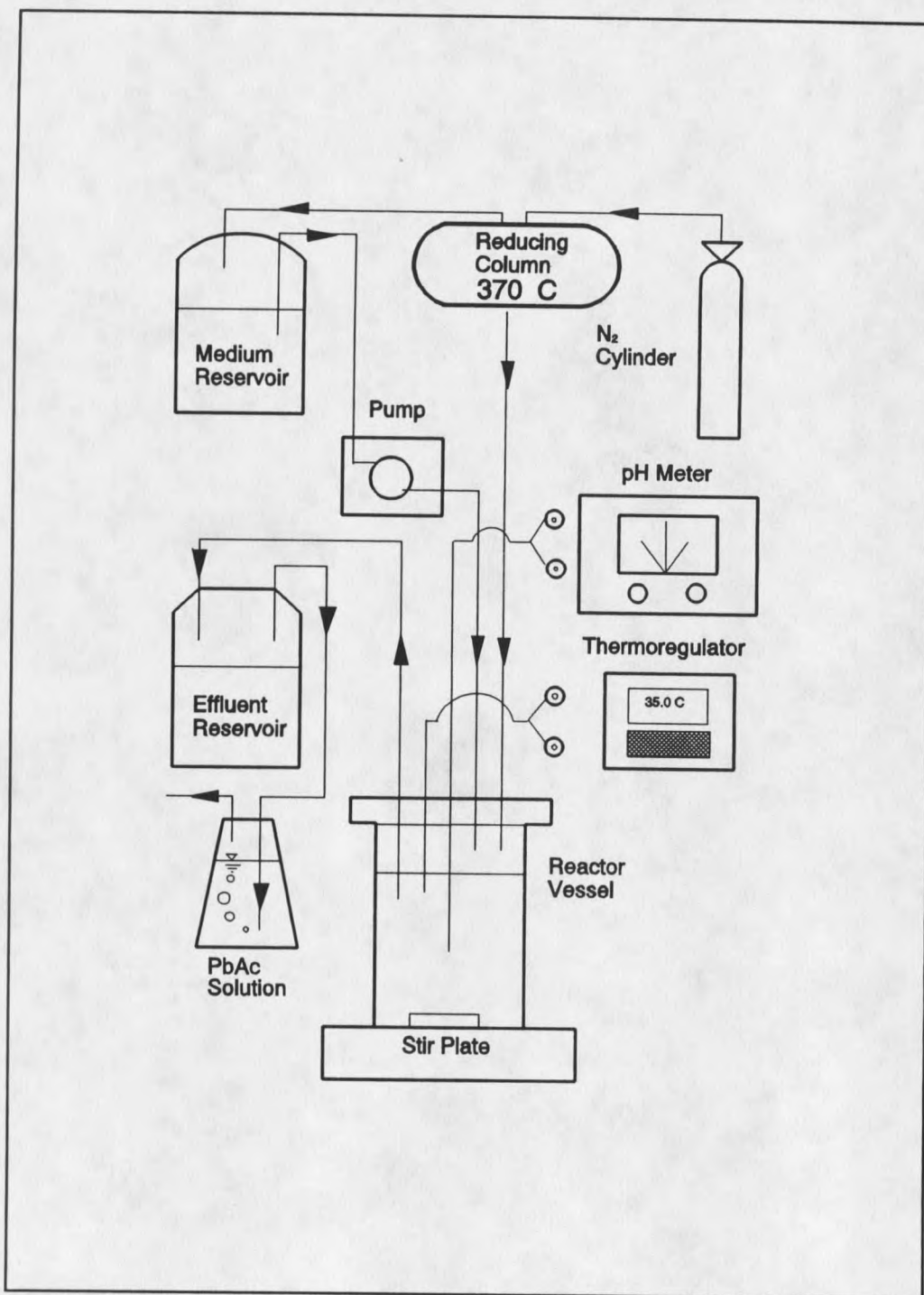


Figure 1. Diagram of chemostat system.

Table 4. Composition of Postgate medium G (Postgate, 1984)

Base medium		
Distilled water		996.0 ml
Na ₂ SO ₄ ^{a)}		3.0 g
NaCl		1.2 g
KCl		0.3 g
NH ₄ Cl		0.3 g
MgCl ₂ · 6H ₂ O		0.4 g
KH ₂ PO ₄		0.2 g
CaCl ₂ · 2H ₂ O		0.15 g
Calcium lactate		0.3125g
Trace element solution	1.0 ml	
Distilled water		993 ml
HCl (25%)		6.5 ml
FeCl ₂ · 4H ₂ O		1.5 g
H ₃ BO ₃		0.06 g
MnCl ₂ · 4H ₂ O		0.1 g
CoCl ₂ · 6H ₂ O		0.12 g
ZnCl ₂		0.07 g
NiCl ₂ · 6H ₂ O		0.025 g
CuCl ₂ · 2H ₂ O		0.015 g
Na ₂ MoO ₄ · 2H ₂ O		0.025 g
Selenite solution	1.0 ml	
Distilled water		1000 ml
NaOH		0.5 g
Na ₂ SeO ₃		0.003 g
Vitamin solution	1.0 ml	
Distilled water		1000 ml
Biotin		0.01 g
p-Aminobenzoic acid		0.05 g
Vitamin B ₁₂		0.05 g
Thiamine		0.1 g
Na-dithionite solution	1.0 ml	
Oxygen-free		
distilled water		100 ml
Na ₂ S ₂ O ₄		3.0 g
Sodium sulfide solution ^{b)}	3.0 ml	
Oxygen-free		
distilled water		100 ml
Na ₂ S · 9H ₂ O		12.0 g

a) 0.5 g/L of Na₂SO₄ was used for temperature effect experiments.

b) This solution was only used until a vigorously growing culture was established.

Analytical Methods

At steady state, effluent samples were obtained for the following analyses: (1) total organic carbon (TOC); (2) soluble organic carbon (SOC); (3) total bacterial counts and cell size; (4) sulfate; (5) sulfide; (6) lactate; (7) acetate; (8) phosphorous; (9) suspended solids. The samples for SOC, lactate, acetate, sulfate, and phosphorous analyses were obtained by filtering an aliquot of the chemostat effluent through 0.20 μm Nuclepore filters.

TOC and SOC were measured with a Dohrmann Carbon Analyzer DC-80 (Dohrmann, Santa Clara, CA.). Unfiltered samples for TOC and filtered samples for SOC were frozen until analyses. Five ml samples were acidified with concentrated phosphoric acid and then bubbled with O_2 gas for a few minutes. Total bacterial counts were determined using an image analyzer (Cambridge/Olympus Quantment 10) by the epifluorescence method described by Hobbie et al. (1977). Sulfate concentration was measured by the barium-sulfate turbidimetric method (APHA, 1989). Lactate concentration was measured by a specific enzymatic method (L-lactic measurement, Boehringer Mannheim, Germany) as described by Cappenberg (1974). Gas chromatography with flame ionization detector (Varian 3700 model) was used to measure acetate concentration. A 2 m $\times$ 2 mm glass column packed with 80/120 Carbowax 20 M (Supelco Bellefonte, PA.) was used. The column was maintained at 175°C. Samples were prepared by adding oxalic acid and internal standard solution (Trimethylacetic acid) to bring the final concentration to 0.06M and 200 mg/L, respectively. Samples were frozen until analyses. The methylene blue

method described by Cline (1969) was used to measure dissolved sulfide concentration. The volatile sulfide was measured by trapping the gaseous sulfide in 1.0 N NaOH solution. Phosphorous concentration was measured as orthophosphorous using the modified ammonium molybdate-ascorbic acid method described by Harwood et al. (1969). Total suspended solids was determined by filtering 20 ml samples through prewashed, dried, and preweighed 0.20 μm Nuclepore filters. Samples were rinsed twice with 5 ml membrane-filtered water and dried at 103°C for 1 hour, and reweighed. Biovolumes were estimated by measuring the cell size with the image analyzer. Biovolumes were converted into cellular carbon estimates using the following conversion factors: 1.07 g wet cell weight/cm³ (Doetsch and Cook, 1973), 0.22 g dry cell weight/g wet cell weight (Luria, 1960), and 0.465 g cell carbon/g dry cell weight (Postgate, 1984). The EPS carbon was calculated by subtracting the calculated cellular carbon from the total biomass carbon (effluent TOC - effluent SOC).

Results

Effects of Temperature

Steady State Cellular Carbon and Lactate Concentrations. Classical behavior was generally observed for the steady state dependence of cellular carbon and lactate concentration on dilution rate at 25°C (Figure 2). EPS carbon concentrations were not significant at any dilution rates for the carbon-limiting experiments.

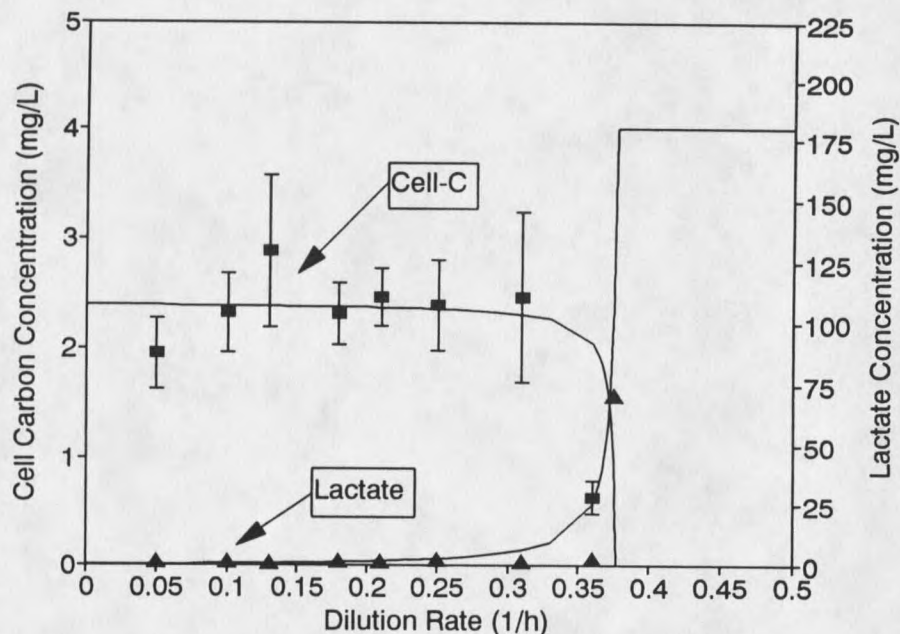


Figure 2. Experimental dependence of the steady state cellular carbon and lactate concentrations on the dilution rate at 25°C. The solid lines are drawn using chemostat mass balance equations. The influent lactate concentration was 180 mg/L. Error bars represent the standard deviation of triplicate measurements.

Therefore, cellular carbon concentrations were calculated as differences between effluent TOC concentration and effluent SOC concentration. The maintenance coefficient was negligible although it may become important at dilution rates less than 0.05 h^{-1} . The effluent sulfate concentrations were in the order of 200 to 300 mg/L (depending on dilution rate) so that this chemostat system was limited by the lactate supply.

Estimation of Monod Growth Parameters. Estimates of $\mu_{\max}$ and K_{Lac} were computed from the effluent lactate concentrations and dilution rates using the following non-linear regression form of Eq.(1):

$$S = \frac{(K_{Lac} \mu)}{(\mu_{max} - \mu)} \quad (9)$$

The non-linear regression of the Monod equation was performed using MSU SAS® (statistical software). The program produce estimates of μ_{max} and K_{Lac} from given data pairs of limiting substrate concentration (S) and specific growth rate ($\mu=D$). The standard error and 95 % confidence interval associated with the estimate of each parameter were also determined. The resulting μ_{max} and K_{Lac} , along with their respective standard error, at each temperature are presented in Table 5. The highest maximum growth rate of 0.55 h^{-1} was obtained at 43°C . Below 25°C and above 43°C , the maximum specific growth rate decreased dramatically to 0.059 h^{-1} at 12°C and 0.115 h^{-1} at 48°C , respectively. The activation energy for μ_{max} was 14 KJ/mole in the range $25^{\circ}\text{C} - 43^{\circ}\text{C}$ and 104 KJ/mole below 25°C (Figure 3). The half-saturation coefficient (K_{Lac}) was minimum at 25°C (Figure 4). The activation energy for K_{Lac} above 25°C was 47 KJ/mole and below 25°C was -52 KJ/mole . The highest cell yield ($Y_{c/Lac}$) was observed in the optimum temperature range for growth $35^{\circ}\text{C} - 43^{\circ}\text{C}$ (Figure 5).

The obtained steady-state experimental results for μ_{max} , K_{Lac} , and $Y_{c/Lac}$ can be summarized by the linear interpretation shown in Figures 3, 4, and 5, which are given specifically by the following functions:

$$\mu_{max}(T) = 10^{6.3686} \cdot 10^{\left(\frac{-2094.52}{T(K)}\right)} \quad (35^{\circ}\text{C} < T < 43^{\circ}\text{C}) \quad (10)$$

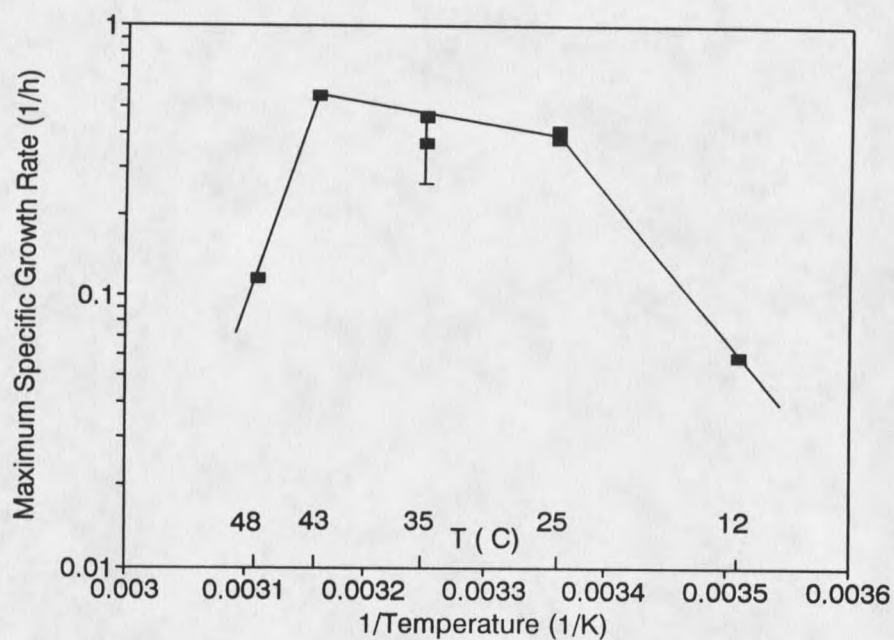


Figure 3. Temperature dependence of the maximum specific growth rate ($\mu_{\max}$). The activation energy for $\mu_{\max}$ were 14 KJ/mole in the range 25-43°C and 104 KJ/mole below 25°C. Error bars represent the standard error of the estimated $\mu_{\max}$. The error bar was not given except one point at 35°C because their standard errors are so small (Table 5).

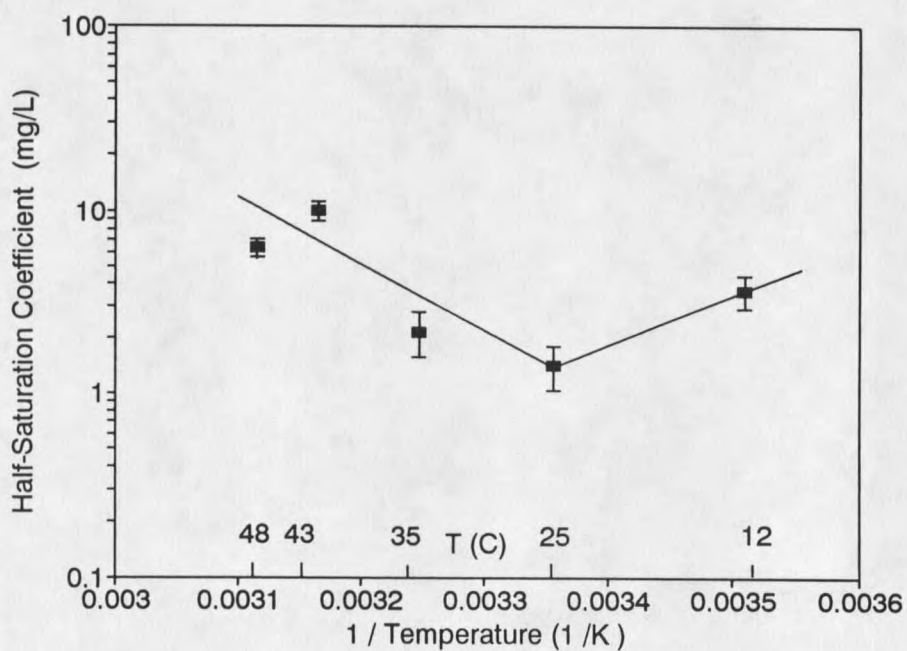


Figure 4. Temperature dependence of the half-saturation coefficient (K_{Lac}). The activation energy was 47 KJ/mole above 25°C and -52 KJ/mole below 25°C. Error bars represent the standard error of the estimated K_s .

Table 5. Experimentally determined kinetic parameters for *D. desulfuricans* growing on lactate as the sole carbon and energy source (estimated parameter value $\pm$ SE).

Temp. (°C)	$\mu_{\max}$ (h ⁻¹)	K_{Lac} (mg/L)	$Y_{\text{c/Lac}}$ (g/g)	m (g/g.h ⁻¹)
12	0.059 ± 0.001	3.7 ± 0.75	0.017 ± 0.001	-0.35 ± 0.54
25 (RUN 1)	0.38 ± 0.002	1.4 ± 0.39	0.025 ± 0.002	0.23 ± 0.36
25 (RUN 2)	0.41 ± 0.001	10.2 ± 0.24	0.025 ± 0.004	2.12 ± 4.13
35 (RUN 1)	0.37 ± 0.004	2.2 ± 0.60	0.024 ± 0.006	0.45 ± 1.08
35 (RUN 2)	0.46 ± 0.11	3.6 ± 2.48	0.036 ± 0.004	0.82 ± 1.14
43	0.55 ± 0.003	10.0 ± 1.22	0.032 ± 0.001	0.28 ± 0.05
48	0.115 ± 0.001	6.4 ± 0.75	0.023 ± 0.001	0.27 ± 0.47

$$\mu_{\max}(T) = 10^{-43.894} \cdot 10^{\left(\frac{13788.53}{T(K)}\right)} \quad (43^{\circ}\text{C} \leq T < 48^{\circ}\text{C}) \quad (11)$$

$$K_{\text{Lac}}(T) = 10^{11.41} \cdot 10^{\left(\frac{-3363.73}{T(K)}\right)} \quad (35^{\circ}\text{C} \leq T < 48^{\circ}\text{C}) \quad (12)$$

$$Y_{\text{c/Lac}}(T) = 10^{1.1053} \cdot 10^{\left(\frac{-814.23}{T(K)}\right)} \quad (12^{\circ}\text{C} < T < 42^{\circ}\text{C}) \quad (13)$$

$$Y_{c/Lac}(T) = 10^{-10.5735} \cdot 10^{\left(\frac{2868.44}{T(K)}\right)} \quad (42^\circ C \leq T < 48^\circ C) \quad (14)$$

Maintenance coefficients (m) were determined by graphical methods using Eq.(5) but its quantitative measure was not statistically significant (Table 5).

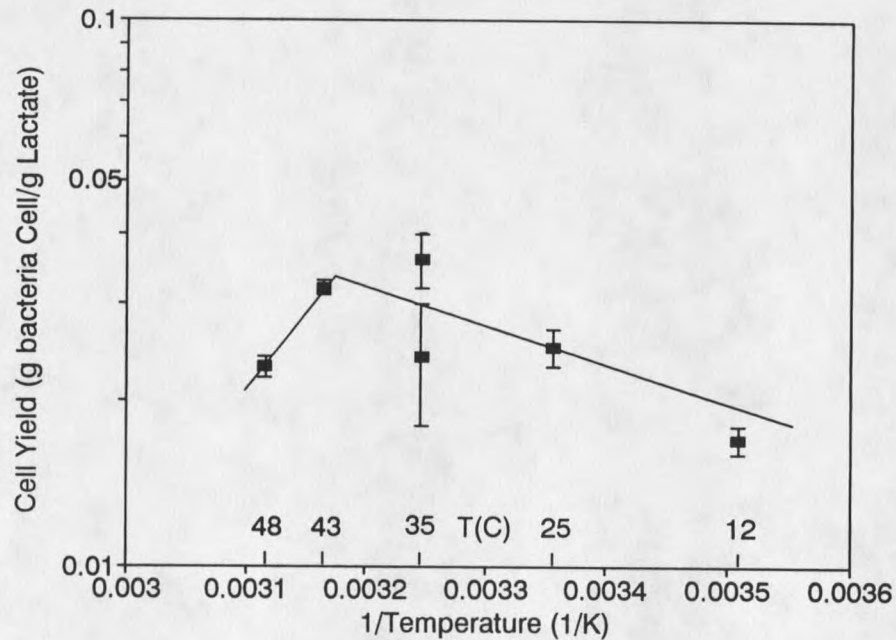


Figure 5. Temperature dependence of the cell yield coefficient ($Y_{c/Lac}$). Error bars represent the standard error of the estimated $Y_{c/Lac}$.

Transient Response to Step Changes in Temperature. To consider the dynamic response of the population to temperature, the effects of step changes in temperature were observed at a dilution rate = 0.10 h^{-1} . The transient response in lactate utilization $[(S_i - S)/S_i]$ was observed for step increases in temperature from 35 to 43 to 53°C followed by a decrease to 48 °C (Figure 6). At 4.5 hour, the temperature was increased from 35°C to 43°C and lactate utilization did not change. However, the

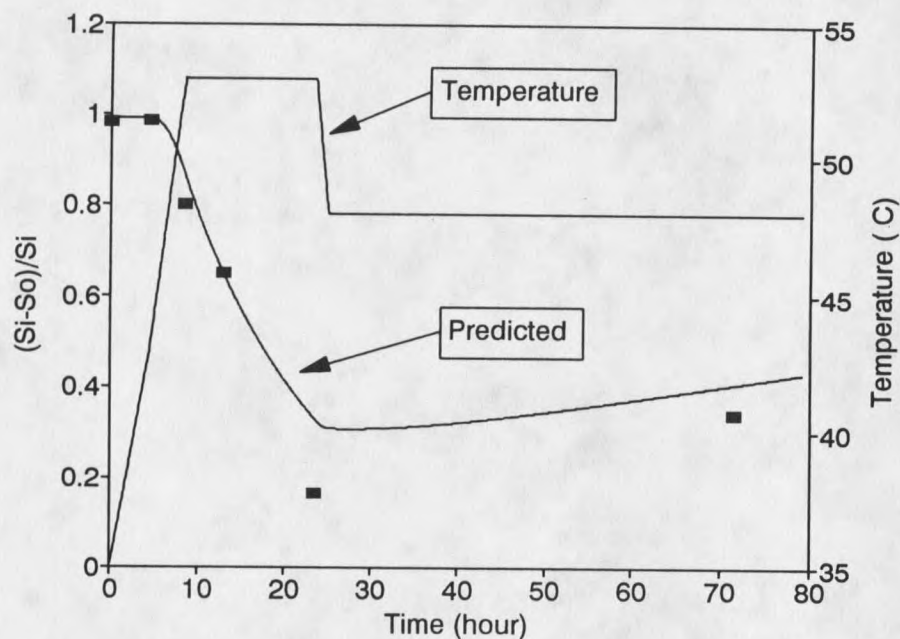


Figure 6. Transient response of lactate oxidation $[(S_i - S)/S_i]$ to step up change in temperature from 35°C to 53°C: (■) experimental points. Theoretical curve according to $\mu_{\max}(T)$, $K_{\text{Lac}}(T)$, and $Y_{c/\text{Lac}}(C)$: $D = 0.10 \text{ h}^{-1}$, $\text{pH} = 7.0$, $S_i = 200 \text{ mg/L}$.

lactate utilization rapidly decreased after the temperature was shifted from 43°C to 53°C. Lactate utilization continued to decrease until the temperature was shifted from 53°C to 48°C. At the high dilution rate ($D = 0.10 \text{ h}^{-1}$), the reactor was near washout at higher temperature. The response of lactate utilization $[(S_i - S)/S_i]$ to a step change in temperature was simulated using the mass balance Eqs.(2), (3), and (4) and temperature functions for parameters $\mu_{\max}(T)$, $K_{\text{Lac}}(T)$, and $Y_{c/\text{Lac}}(T)$ represented by Eqs.(10), (11), (12), (13) and (14). The simulation assumes that $\mu_{\max}(T)$, $K_{\text{Lac}}(T)$, and $Y_{c/\text{Lac}}(T)$ take their new steady-state values immediately after the temperature change.

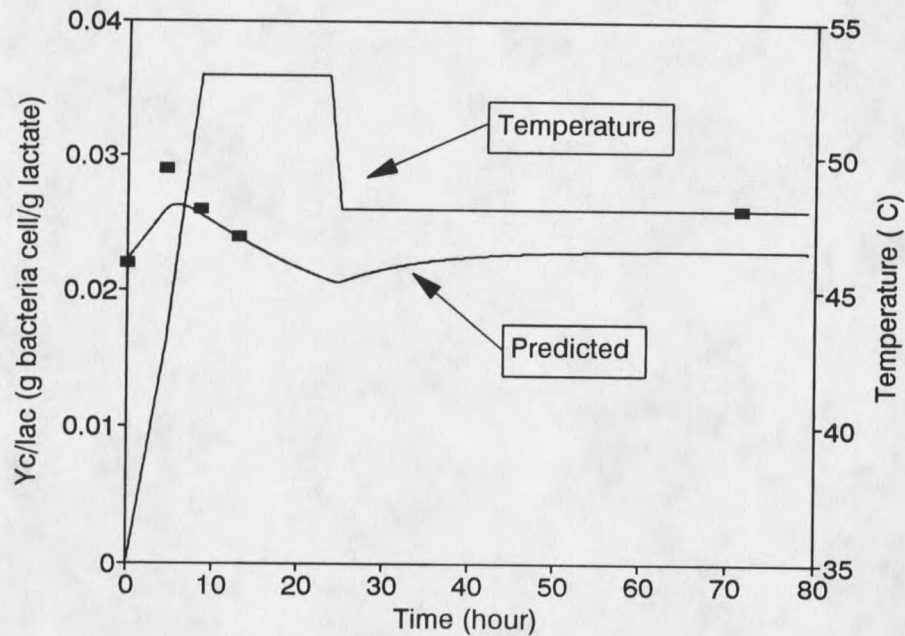
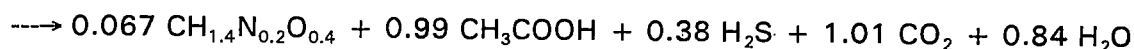
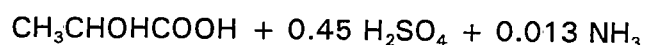
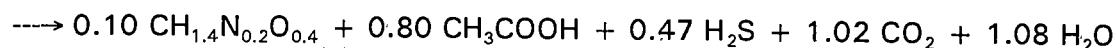
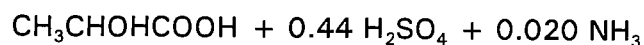
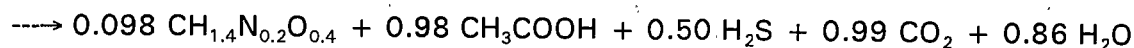
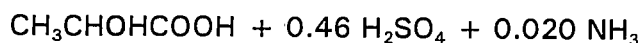
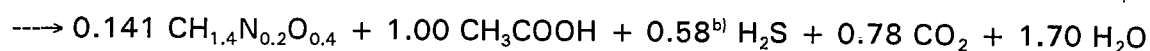
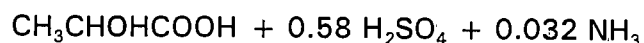
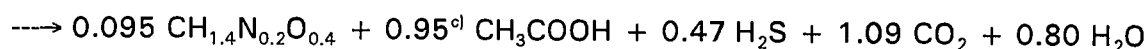
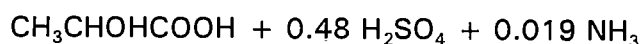
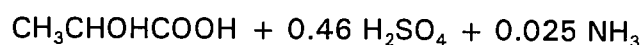
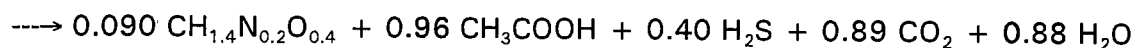
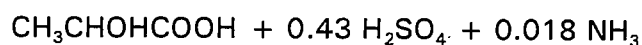


Figure 7. Transient response of cell yield ($Y_{c/Lac}$) to step up change in temperature from 35°C to 53°C: (■) experimental points. Theoretical curve based on $\mu_{max}(T)$, $K_{Lac}(T)$, and $Y_{c/Lac}(C)$: $D = 0.10 \text{ h}^{-1}$, $pH = 7.0$, $S_i = 200 \text{ mg/L}$.

The results indicate that parameters determined at steady state can accurately describe effects of temperature transition within the ranges tested. The response of cell yield coefficient ($Y_{c/Lac}$) to a step change in temperature was simulated in the same way (Figure 7).

Estimation of Stoichiometric Coefficients. The stoichiometry for microbial sulfate reduction was developed from the experimental data obtained at different temperature (Table 6). The stoichiometric coefficients were balanced by assuming the following: (1) empirical formulation ($CH_{1.4}N_{0.2}O_{0.4}$) for bacterial cells, (2) nitrogen source is only NH_3 , (3) amount of other end products of lactate oxidation besides acetate and

Table 6. Influence of temperature on the stoichiometry of microbial sulfate reduction with lactate.At 12°C (102 %) ^{a)}At 25°C (RUN 1) (91 %) ^{a)}At 25°C (RUN 2) (102 %) ^{a)}At 35°C (RUN 1) (97 %) ^{a)}At 35°C (RUN 2)At 43°C (110 %) ^{a)}At 48°C (97 %) ^{a)}

^{a)} Percentage of carbon recovery.

^{b)} This value is calculated from consumed sulfate concentration.

^{c)} This value is calculated from carbon mass balance.

CO₂ are negligible. The molar conversion of lactate, sulfate, bacterial cells, and acetate was obtained from independent measurements of each component. Bacterial cell production was calculated from the difference between effluent TOC and effluent SOC because EPS carbon concentrations were negligible. The conversion of carbon dioxide was calculated by assuming that the difference between the influent and effluent TOC is CO₂ because the only route for loss of organic carbon from the system is through conversion to inorganic carbon. Stoichiometric coefficients were determined from normalized molar conversions of each individually measured component compared to lactate. The stoichiometric coefficient for water was obtained using the oxygen balance. The percentages of carbon recovery in the stoichiometric equations at each temperature were within 90 % of the amounts of lactate-carbon added.

Effects of Phosphorous Concentration

D. desulfuricans was grown at a dilution rate of 0.2 h⁻¹ at 35°C and at various phosphorous concentrations of 48.47, 4.60, 0.39, and 0.03 mg P/L to determine the effects of phosphorous concentration on stoichiometry. Steady state results, along with their respective standard deviations, are presented in Table 7.

EPS and Cellular Carbons. Mean values for effluent cellular and EPS carbon, along with their respective standard deviations, are given in Figure 8. With decreasing phosphorous concentration, EPS carbon concentration increased from 0 mg EPS C/L at 48.47 mg P/L to 2.1 mg EPS C/L at 0.03 mg P/L. In contrast, cellular carbon concentration decreased from 4.0 mg cellular C/L at 48.47 mg P/L to 1.1 mg cellular C/L at 0.03 mg P/L. EPS yield increased and cell yield decreased with decreasing

phosphorous concentration (Figure 9).

Table 7. Steady state results of liquid phase parameters of the continuous culture of *D. desulfuricans* with different influent phosphorous concentrations at a dilution rate of 0.20 h^{-1} .

INFLUENT			EFFLUENT		
phosphorous	lactate	sulfate	phosphorous	lactate	Sulfate
48.47 ± 0.08	402.4 ± 5.3	2560 ± 12	47.97 ± 0.04	0.0 ± 0.0	2341 ± 3
4.60 ± 0.02	437.4 ± 29.8	2574 ± 18	4.13 ± 0.0	3.7 ± 1.3	2341 ± 15
0.39 ± 0.01	411.0 ± 24.9	2533 ± 7	0.06 ± 0.0	5.1 ± 0.7	2297 ± 5
0.03 ± 0.0	382.9 ± 7.8	2541 ± 35	0.00 ± 0.0	113.3 ± 3.4	2405 ± 10

Values given are the mean of triplicate measurements ± 1 SD (in mg/L).

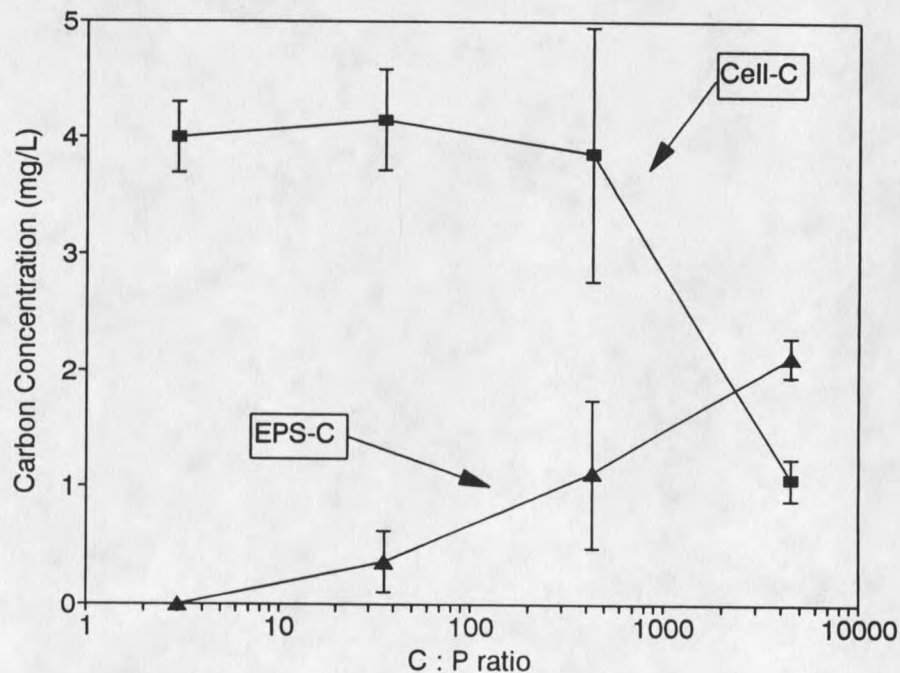


Figure 8. Change in cell and EPS carbon concentrations with changing C:P ratio. Error bars represent the standard deviation of triplicate measurements.

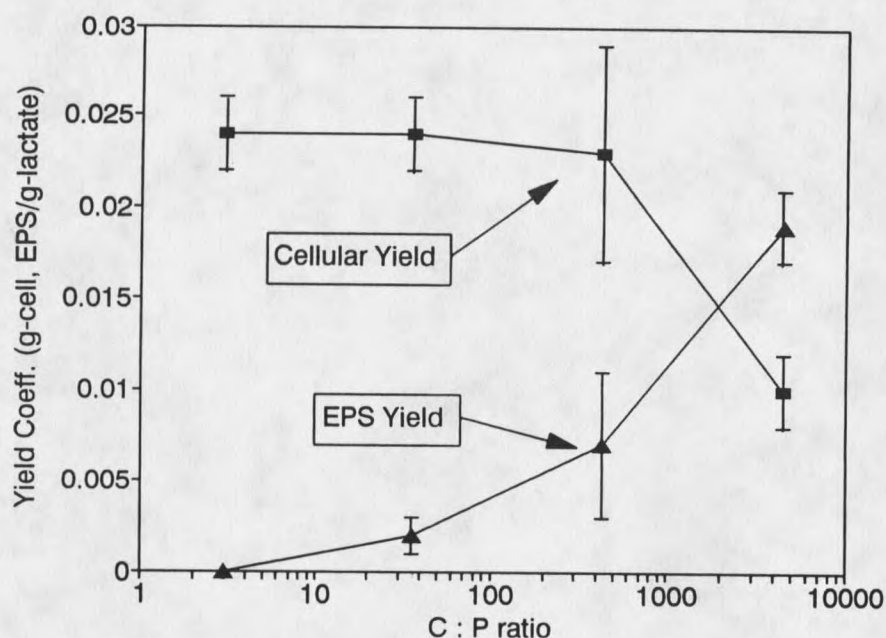


Figure 9. Change in cell and EPS carbon yield coefficients with changing C:P ratio. Error bars represent the standard deviation of triplicate measurements.

Estimation of Phosphorous Requirement. The effluent lactate concentrations increased from 0.0 mg lactate/L at 48.47 mg P/L to 113.3 mg lactate/L at 0.03 mg P/L (Figure 10). The effluent phosphorous concentration at 0.39 mg P/L was still measurable. Thus, at 0.39 mg P/L of phosphorous, medium was low in phosphorous. At lower phosphorous levels, the culture shifted from lactate-limited to phosphorous-limited.

The stoichiometries for microbial sulfate reduction changed with changing phosphorous concentrations (Table 8). Triplicate measurements of all reactant and product concentrations were within 15 % of their respective mean values at each phosphorous loading concentration. $\text{CH}_{1.4}\text{N}_{0.2}\text{O}_{0.4}$ and CH_2O were used as an empirical

formula for bacterial cell and EPS, respectively (Characklis, 1990). The recovery of carbon in the four stoichiometric equations was within 90 % of the amounts of lactate-carbon added. The stoichiometries were in good agreement until phosphorous concentration reached 4.60 mg P/L. At 0.39 mg P/L, there was less phosphorous consumption. The cell yield, however, was the same at phosphorous concentrations of 48.47 and 4.60 mg P/L. The evidence suggests that the bacteria adapted to more efficient phosphorous utilization. At 0.03 mg P/L, phosphorous limitation was evidenced by: (1) significant amounts of lactate in the effluent, (2) lower cell yield, and (3) complete phosphorous removal.

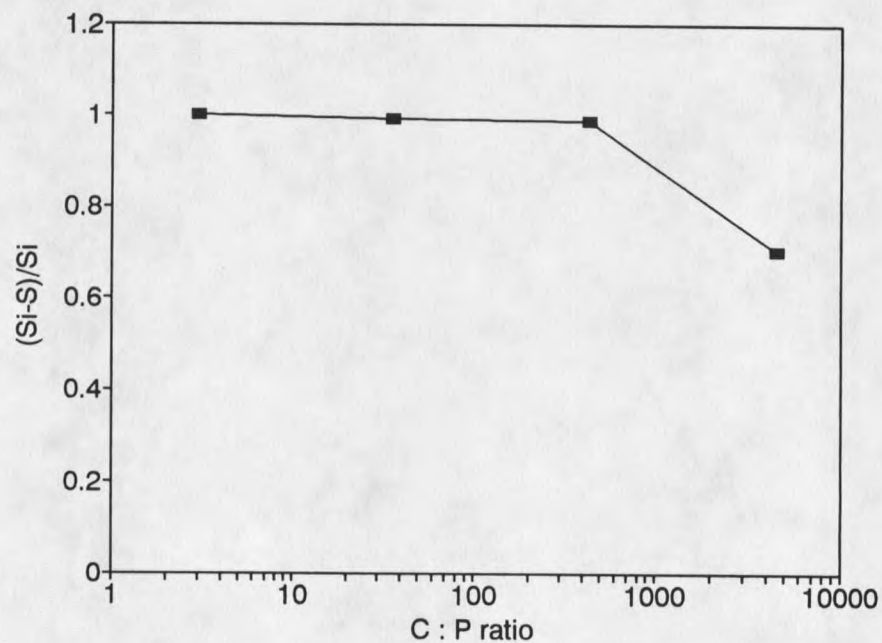
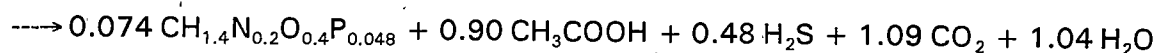
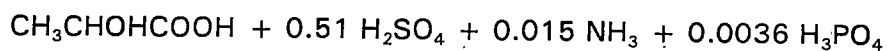


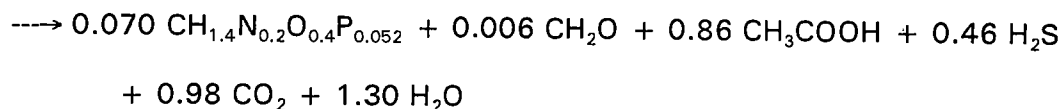
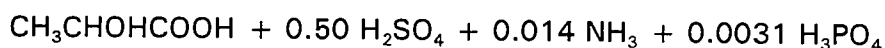
Figure 10. Response of lactate oxidation $[(S_i - S)/S_i]$ by *D. desulfuricans* to change in the phosphorous concentration: $D = 0.20 \text{ h}^{-1}$, Temp. = 35°C . The limiting C:P ratio is in the range 400:1 to 800:1.

Table 8. The stoichiometries obtained from the experimental data at different phosphorous concentrations.

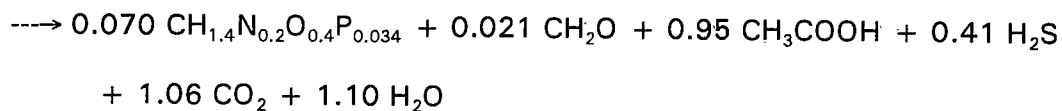
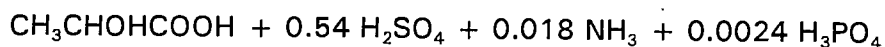
At 48.47 mg-P/L (99 %^{a)})



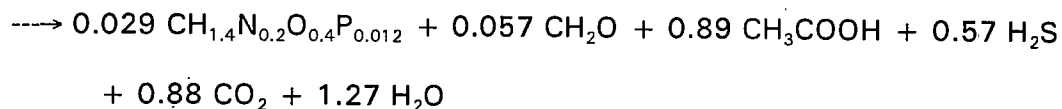
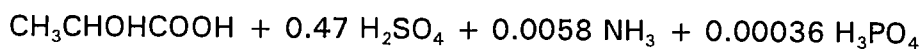
At 4.60 mg-P/L (93 %^{a)})



At 0.39 mg-P/L (102 %^{a)})



At 0.03 mg-P/L (92 %^{a)})



^{a)} Percent recovery of carbon.

Discussion

Effects of Temperature

Cell Yield Coefficient. Cell yield coefficients were determined to be in the range between 0.040 ± 0.006 and 0.017 ± 0.001 g bacteria cell/g lactate in the temperature range from 12°C to 48°C. Thus, temperature does affect cell yield. The measured yields are lower than those reported in the literature. Senez (1962) observed that the overall yields for *D. desulfuricans* (strain Berre S) and *D. desulfuricans* (strain Canet 41) grown on lactate-sulfate synthetic medium with NH_4^+ as nitrogen source in batch systems at 32°C were 0.065 g SS/g lactate and 0.111 g SS/g lactate, respectively. The overall yield was relatively constant up to the optimum temperature of 37°C, and then decreased with increasing temperature. Traore et al. (1982) reported the overall yield for *D. desulfuricans* in batch culture at 30°C of 0.046 g SS/g lactate. Finally, Cappenberg (1975) determined that the overall yield for *D. desulfuricans* grown in the continuous culture at 30°C was 0.34 g SS/g lactate.

The cell yield coefficients obtained in the present study are relatively low for at least two reasons: 1) no yeast extract was used and 2) cell-associated EPS was not considered in yield calculations. The yield coefficient determined using biomass dry weight, which includes both cellular mass and EPS, would be high compared to our data. Based on this finding, yield coefficients reported by others for *D. desulfuricans* should be cautiously interpreted since no distinction was made between cellular and EPS. Robinson et al. (1984) reported that the determination of cell yield using

suspended solids leads to overestimation of 100 % for aerobic *Pseudomonas aeruginosa*. Furthermore, based on our data (data are not shown), the calculation of yield coefficient for *D. desulfuricans* using suspended solids in iron-containing water may lead to significant higher values because of attachment of iron sulfide on the surface of bacteria.

Monod Kinetic Coefficients ($\mu_{\max}$ and K_{Lac}). The kinetics of *D. desulfuricans* growth on lactate as a function of temperature are presented in Figure 11 based on experimentally determined rate coefficients. The highest $\mu_{\max}$ ($0.55 \pm 0.003 \text{ h}^{-1}$) was observed at 43°C at which the highest cell yield was obtained. However, Topiwala and Sinclair (1971) reported that the highest $\mu_{\max}$ was observed at 40°C , while the highest overall yield was obtained at 25°C for aerobic *Aerobacter aerogenes*. Furthermore, Muck and Grady (1974) reported that the highest growth rate for an aerobic mixed population was observed at 30°C , while the highest overall yield was observed at 20°C . They concluded that the variation in observed overall yield was caused by the temperature dependence of the maintenance energy and the rate of bacterial decay. However in this study, the maintenance coefficient was not significant. This may be a reason why the highest cell yield was obtained at the optimum temperature for bacterial growth.

The maximum specific growth rates obtained at 43°C in this study are relatively high compared to values obtained by others. Cappenberg (1975) obtained $\mu_{\max}$ and K_{Lac} for *D. desulfuricans* grown in the continuous culture at 30°C of 0.36 h^{-1} and 4.4 mg lactate/L , respectively. Traore (1982) reported that $\mu_{\max}$ for *D. desulfuricans* grown in the batch culture at 30°C was 0.104 h^{-1} . In addition, Yagisawa et al. (1977)

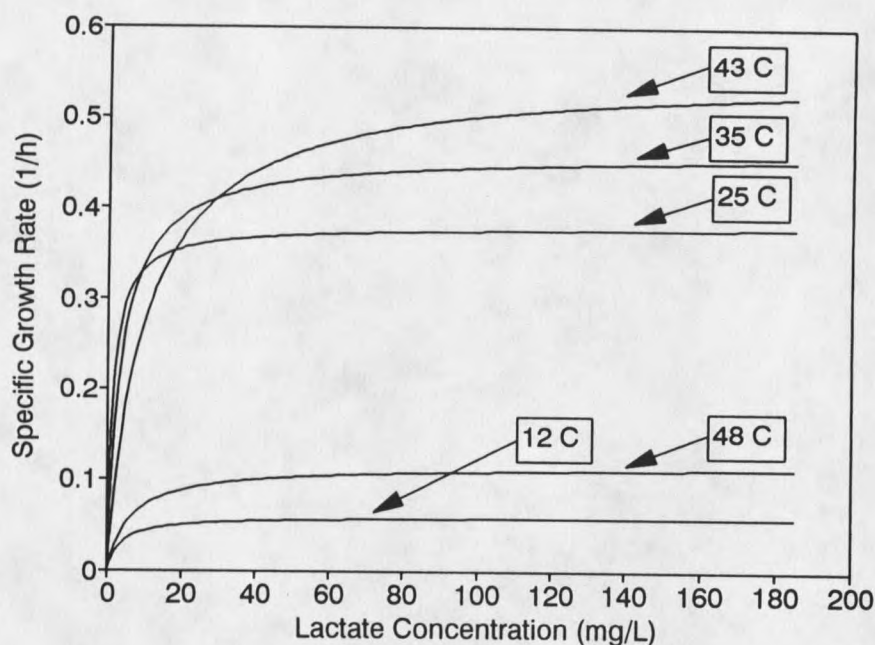


Figure 11. Results of model simulation. Kinetic parameters at steady state were determined from experimental data (e.g., Fig.2).

determined $\mu_{\max}$ for mixed continuous culture of SRB grown in lactate-sulfate medium at 30°C was 0.541 h⁻¹. Possible reasons for the relatively high values for $\mu_{\max}$ in this study include the following: 1) a selective medium for *D. desulfuricans* was used, 2) the continuous nitrogen gas purge removed hydrogen sulfide which may inhibit the growth of SRB at high concentration, and 3) enzymatic activities of SRB were modified during the long culture period, 4) selection of faster growing *D. desulfuricans*.

The observed activation energies for $\mu_{\max}$ were 104 KJ/mole in the range 12°C - 25°C and 14 KJ/mole in the range 25°C - 43°C, respectively. Senez (1962) reported the activation energy for $\mu_{\max}$ for *D. desulfuricans* was 29.1 KJ/mole in the temperature range 24.8°C - 37°C. This evidence indicates that above 43°C and below 25°C the

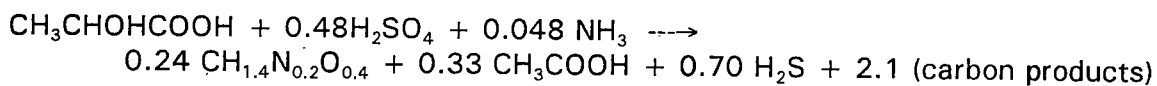
activity of *D. desulfuricans* decreases dramatically.

Although the half-saturation coefficient (K_{Lac}) is not really a rate coefficient, it may be related to substrate transport process into the cells. As a consequence, K_{Lac} is plotted on an Arrhenius plot. Below 25°C, K_{Lac} decreased with increasing temperature, whereas above 25°C, K_{Lac} increased with increasing temperature. Knowles et al. (1965) reported increasing K_s values over the temperature range of 8°C - 30°C when *Nitrosomonas* and *Nitrobacter* were grown in the batch culture of river water. Also, Lawrence and McCarty (1970) reported the value for K_{Ace} decreased slightly when the temperature was increased from 10°C to 20°C, and rose significantly when the temperature was increased to 30°C in the study on the continuous culture of methanogenic bacteria growing on acetate medium. Characklis and Marshall (1990) report positive activation energy for batch K_s data and negative for continuous reactor data.

The growth parameters were determined at 25°C again after all experiments were conducted to evaluate the reproducibility of results. The reproducibility for μ_{max} was reasonably good, whereas that for K_{Lac} was not. Therefore, there is evidence of hysteresis. The first values (RUN 1) for μ_{max} and K_{Lac} were obtained after the temperature was shifted down from 35°C to 25°C, whereas the second values (RUN 2) were obtained after the temperature was shifted up from 12°C to 25°C. Topiwala and Sinclair (1971) reported that the lag due to the physiological readjustment is less with the step down change than with the step up change.

Stoichiometry. The stoichiometry for lactate oxidation by *D. desulfuricans* is not temperature dependent except for cell yield. Two moles of lactate are consumed

for every mole of sulfate reduced at all temperatures. Furthermore, a mole of lactate was converted to approximately a mole of acetate and carbon dioxide. The percentage of recovery of carbon added as lactate indicated that the accuracy of this study is satisfactory. The recovery of sulfur (S) in this study is not good because hydrogen sulfide is volatile and reactive with oxygen and metals in the solution. The tendency of hydrogen sulfide to accumulate in the reactor as FeS was occasionally observed. The stoichiometric equations are consistent with those developed by Traore et al. (1982) and D'Alessandro et al. (1974). Lee (1990) reported that the stoichiometry of microbial sulfate reduction of a mixed population SRB anaerobic biofilm at 0.04 h^{-1} of dilution rate was the following:



SRB dominated the biofilm and 95 % of the total SRB in the reactor were observed in the bulk liquid at a dilution rate of 0.04 h^{-1} . The ratio of lactate to sulfate consumption is 2 : 1 as net expected but acetate production is less than predicted. The reduced net production of acetate, as compared to monopopulation observations, may be the result of GAB (general anaerobic bacteria) utilizing the acetate produced by SRB in the biofilm. The stoichiometric coefficient for bacterial cells was calculated from the number of SRB obtained from most probable number (MPN) method counts by converting the cell numbers to cellular carbon concentration. The stoichiometric coefficient for bacterial cells is relatively high compared to that obtained from this experiment (suspended biomass reactor) and is probably due to accumulation of cells in biofilm. The stoichiometric balance for sulfur (S) is not balanced in this equation. The accumulation of sulfide as FeS in the biofilm caused this discrepancy. The

stoichiometric ratio between lactate and sulfate in the chemostat may be applied to SRB in biofilm systems. However, community interaction influences the other stoichiometric coefficients.

Steady state experimental results predicted the transient response to temperature changes. The calculated transient response curves, based on the steady state experimental results at different temperatures, fit the experimental transient data reasonably well (Figure 6 and 7). It can be seen by comparing the theoretical curves and the experimental points that there is no significant lag between the two responses. Thus, for example, SRB activity in an oil reservoir can be predicted despite changing temperature with distance and time. However, this result can be applied to only planktonic SRB, not necessarily to SRB biofilms. Therefore, it is essential to determine if SRB biofilm systems behave similarly to changes in temperature.

Effects of Phosphorous Concentration

Phosphorous Requirement. The stoichiometric limiting ratio of phosphorous to lactate for *D. desulfuricans* is approximately 1 mg P to 1000 - 2000 mg lactate for complete lactate utilization and maximum cell production. Using TOC values, this would be TOC : P ratio of 400:1 to 800:1. Paul et al. (1988) suggested that the stoichiometric limiting C : P ratio for *D. desulfuricans* grown in continuous culture was 250 in terms of complete lactate utilization and maximum cell production. The stoichiometric limiting C : P ratio for anaerobic SRB obtained in this study is higher than that for aerobic mixed populations because *D. desulfuricans* produces large quantities of acetate from lactate. Incomplete oxidation of lactate to acetate with sulfate as electron acceptor yields - 14 KJ of free energy (ΔG°), about 6 % of the free

energy (ΔG°) of complete oxidation of lactate with oxygen as electron acceptor ($\Delta G^\circ = -247$ KJ). Thus, anaerobic biomass production from lactate is approximately 10 times less than in aerobic systems.

Conclusions

Based on the results of this research, it can be concluded that:

- 1) All stoichiometries indicate that two moles of lactate are oxidized for every mole of sulfate reduced by *D. desulfuricans* and the ratio is independent of temperature.
- 2) The optimum temperature for *D. desulfuricans* growth and yield was approximately 43°C.
- 3) The maximum specific growth rate ($\mu_{\max}$), half-saturation coefficient (K_{Lac}), and the cell yield ($Y_{\text{c/Lac}}$) for *D. desulfuricans* are dependent on temperature.
- 4) The limiting carbon : phosphorous ratio for *D. desulfuricans* is in the range 400 : 1 to 800 : 1.

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CHAPTER 3

FACTORS AFFECTING MICROBIAL SULFATE REDUCTION
: LIMITING NUTRIENTS AND SULFIDE CONCENTRATIONIntroduction

In the petroleum industry, sulfate reducing bacteria (SRB) cause serious problems including corrosion of installations, plugging of the petroleum formation, and contamination of petroleum with H_2S (souring) in the formation. Cochrane et al. (1988) reported that the presence and growth of thermophilic SRB at temperatures greater than $60^\circ C$ was a major source of sulfide production in a North Sea oil field and that seawater injection results in the appropriate balance of sulfate, temperature, and organic nutrient status for growth in the reservoir. Herbert et al. (1986) reported that substantial levels of short-chain fatty acids and ammonia present in many formation waters can be used directly by SRB for metabolism. Ligthelm et al. (1991) recently published a one-dimensional analytical model of H_2S generation and transport within an oil reservoir in which the souring is attributed to SRB activity. Their simulation results indicate that generation of H_2S by SRB occurred in the mixing zone between injected seawater and formation water where spatial gradients in environmental factors such as temperature and nutrients exist. However, effects of temperature and limiting nutrients on SRB activity were not considered by this model. At present, quantitative description of reservoir souring is essentially impossible because coefficients for rate

and extent of SRB growth under relevant environmental conditions are not available.

The environment for microbial growth varies widely throughout the petroleum formation. The temperature varies from that of the cold injection water temperature (-5°C - 20°C) to that of the hot formation temperature (40°C - 100°C) and can have a major influence on SRB activity. Okabe and Characklis (1992) reported that the maximum specific growth rate (μ_{max}) of *D. desulfuricans* was relatively constant (0.38 - 0.55 h^{-1}) between 25°C to 43°C and dramatically decreased outside this temperature range. However, the stoichiometry of microbial sulfate reduction was not temperature dependent. More information is needed on the effects of various relevant environmental factors on SRB activity.

The ultimate goal of our SRB research program is the development of effective means to control SRB activity in various industrial systems including petroleum production. Some researchers are focusing on determination of the effectiveness of various biocides on planktonic and sessile SRB population (Christopher and Oresta, 1991; Roseska et al., 1982). Biocide treatment, however, may not be an ultimate means to control SRB activity in oil fields because of rapid microbial regrowth, plugging of the formation, environmental concerns, and cost effectiveness. Concentrations of sulfate, substrate (carbon sources), and essential nutrients such as phosphorous and nitrogen in the formation vary as they are depleted by microbial activity or are mediated by the formation itself. The reduction of the concentration of an essential nutrient to below the limiting concentration is a possible means of controlling SRB. Thus, determination of the limiting nutrient concentration may be useful to control and predict SRB activity. Okabe and Characklis (1992) reported that the limiting C : P ratio (w/w) for growth of *D. desulfuricans* is in the range of 400 : 1 to 800 : 1. Extensive

EPS production was observed as phosphorous became limiting. Microorganisms also need assimilable nitrogen usually present in the range of a few mg/L (nitrate level is not detectable) in the produced water (Van der Wende, 1991). Thus, nitrogen is a promising nutrient for control of SRB activity in the oil formation. Also, in the case where fresh water is used as injection water, sulfate may be a limiting substrate for SRB activity. Ironically, scale and corrosion inhibitor chemicals may enrich the system in N, P, or SO_4^{2-} .

Despite the importance of microbial sulfate reduction in industrial water systems, limited experimental information on rate and stoichiometry of microbial sulfate reduction under sulfate-limiting conditions is available. Also, our understanding of nutrient requirements for SRB is limited. Although many have studied the effects of sulfide on anaerobic waste water treatment to enhance treatment performance, no SRB product inhibition data has been reported. It is essential to understand how SRB behave under high sulfide concentration environment such as a petroleum reservoir.

This chapter will describe the effects of limiting nutrients concentrations such as nitrogen and sulfate as well as the influence of inhibitory sulfide concentration on rate and stoichiometry of microbial sulfate reduction by *D. desulfuricans*.

Experimental Materials and Methods

Mathematical Model of Chemostat System

Microbial transformations in a chemostat can be mathematically described by mass balance equations assuming the two fundamental processes occurring are growth and maintenance. The rate of microbial growth can be described by the Monod

expression as a function of limiting substrate concentration (e.g., sulfate).

$$\mu = \frac{\mu_{\max} S}{(K_{Sul} + S)} \quad (15)$$

where, μ = specific growth rate (t^{-1}), $\mu_{\max}$ = maximum specific growth rate (t^{-1}), S = sulfate concentration ($M_s L^{-3}$), K_{Sul} = half-saturation coefficient for sulfate ($M_s L^{-3}$). The details of the mathematical description of the chemostat system are described in Chapter 2.

Experimental System

The rate and stoichiometric coefficients at several limiting nutrient (e.g., sulfate and nitrogen) and sulfide concentrations were determined in a chemostat consisting of a pyrex cylindrical beaker ($1.5 \times 10^{-3} m^3$ volume). The chemostat was equipped with a butyl rubber biofilm scraper continuously rotated by a electric motor to remove wall growth. A constant pH and temperature were maintained using a pH control system with sterile 1.0 N HCl and NaOH solutions and thermoregulator, respectively. The slow continuous nitrogen purge of the reactor maintained anaerobic conditions and prevented H_2S accumulation. Traces of oxygen in the nitrogen feed gas were removed by a reducing column containing copper wire maintained at $400^\circ C$. The gas was sterilized by a cotton filter. The flow rate of nitrogen gas was approximately $3 L h^{-1}$.

Desulfovibrio desulfuricans (ATCC 5575) was grown in Postgate medium G (Postgate 1984), including hemi-calcium lactate (L-lactic acid, SIGMA, No.L-2000) as the sole carbon and energy source. Trace elements and vitamins were added. A sterile $Na_2S_2O_4$ solution was added as a reductant until a vigorously growing culture was

established.

Analytical Methods

At steady state, effluent samples were obtained for the following analyses: (1) total organic carbon (TOC); (2) soluble organic carbon (SOC); (3) total bacterial counts and cell size; (4) sulfate; (5) sulfide; (6) lactate; (7) acetate; (8) ammonium nitrogen. The samples for SOC, lactate, acetate, sulfate, and ammonium nitrogen analyses were obtained by filtering an aliquot of the chemostat effluent through 0.22 μm Nuclepore filters. The phenate method was used for ammonium nitrogen analysis (APHA, 1989). The details of the rest of the chemical analytical methods are described in Chapter 2.

Results

Effects of Sulfate Concentration

Steady State Cellular Carbon and Sulfate Concentrations. Classical behavior was generally observed for the steady state dependence of cellular carbon and sulfate concentration on dilution rate (Figure 12). EPS carbon concentrations were not significant at any dilution rates for the sulfate-limiting experiments. Therefore, cellular carbon concentrations were calculated as differences between effluent TOC concentration and effluent SOC concentration. Maintenance energy requirements became important at dilution rates less than 0.05 h^{-1} .

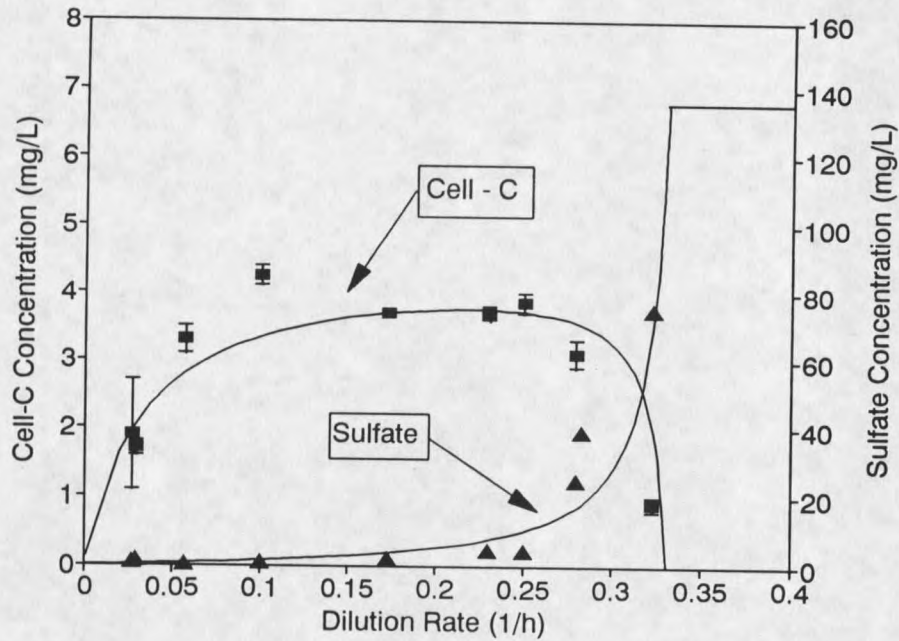


Figure 12. Steady state cellular carbon and sulfate as a function of dilution rate at 35°C. The solid lines reflect kinetic coefficients determined by non-linear regression. The influent sulfate concentration was 135 mg/L. Error bars represent the standard deviation of measurement ($n = 2$).

Estimation of Monod Growth Parameters. Estimates of $\mu_{\max}$ and K_{Sul} were computed from the effluent sulfate concentrations and dilution rates using the following non-linear regression form of Eq.(15):

$$S = \frac{K_{Sul} \mu}{(\mu_{\max} - \mu)} \quad (16)$$

The non-linear regression of the Monod equation was performed using MSU SAS® (statistical software). The program provides estimates of $\mu_{\max}$ and K_{Sul} from given data pairs of limiting substrate concentration (S) and specific growth rate ($\mu = D$). The 95 % confidence interval associated with the estimate of each parameter was also

determined. The maximum growth rates of $0.344 \pm 0.007 \text{ h}^{-1}$ and $0.352 \pm 0.003 \text{ h}^{-1}$ were obtained at 35 and 43°C, respectively. The half-saturation coefficient for sulfate (K_{Sul}) was calculated to be 1.8 ± 0.3 and $1.0 \pm 0.2 \text{ mg/L}$ at 35 and 43°C, respectively. The maintenance energy requirement was determined using graphical methods based on a mathematical model (Characklis, 1990) (Figure 13). Significant maintenance coefficients based on lactate consumption were determined under sulfate-limiting conditions (1.20 ± 0.53 and $1.98 \pm 0.51 \text{ g lactate (g cell)}^{-1} \text{ h}^{-1}$ at 35°C and 43°C, respectively). The summary of rate coefficients under sulfate-limiting conditions are presented along with those reported under lactate-limiting conditions in Table 9.

Estimation of Stoichiometric Coefficients. The stoichiometry for microbial sulfate reduction was developed from the experimental data obtained from a sulfate-limiting continuous culture (Table 10). The stoichiometric coefficients were balanced by assuming the following: (1) an empirical formulation ($\text{CH}_{1.4}\text{N}_{0.2}\text{O}_{0.4}$) for bacterial cells (Characklis, 1990), (2) the only nitrogen source is NH_3 , (3) the amount of other end products of lactate oxidation besides acetate and CO_2 are negligible. The molar conversion of lactate, sulfate, bacterial cells, and acetate was obtained from independent measurements of each component. Bacterial cell production was calculated from the difference between effluent TOC and effluent SOC because EPS carbon concentrations were negligible. The molar conversion of carbon dioxide was calculated by assuming that the difference between the influent and effluent TOC is CO_2 as C because the only route for loss of organic carbon from the system is through conversion to inorganic carbon. Stoichiometric coefficients were determined from normalized molar conversions of each individually measured component compared to

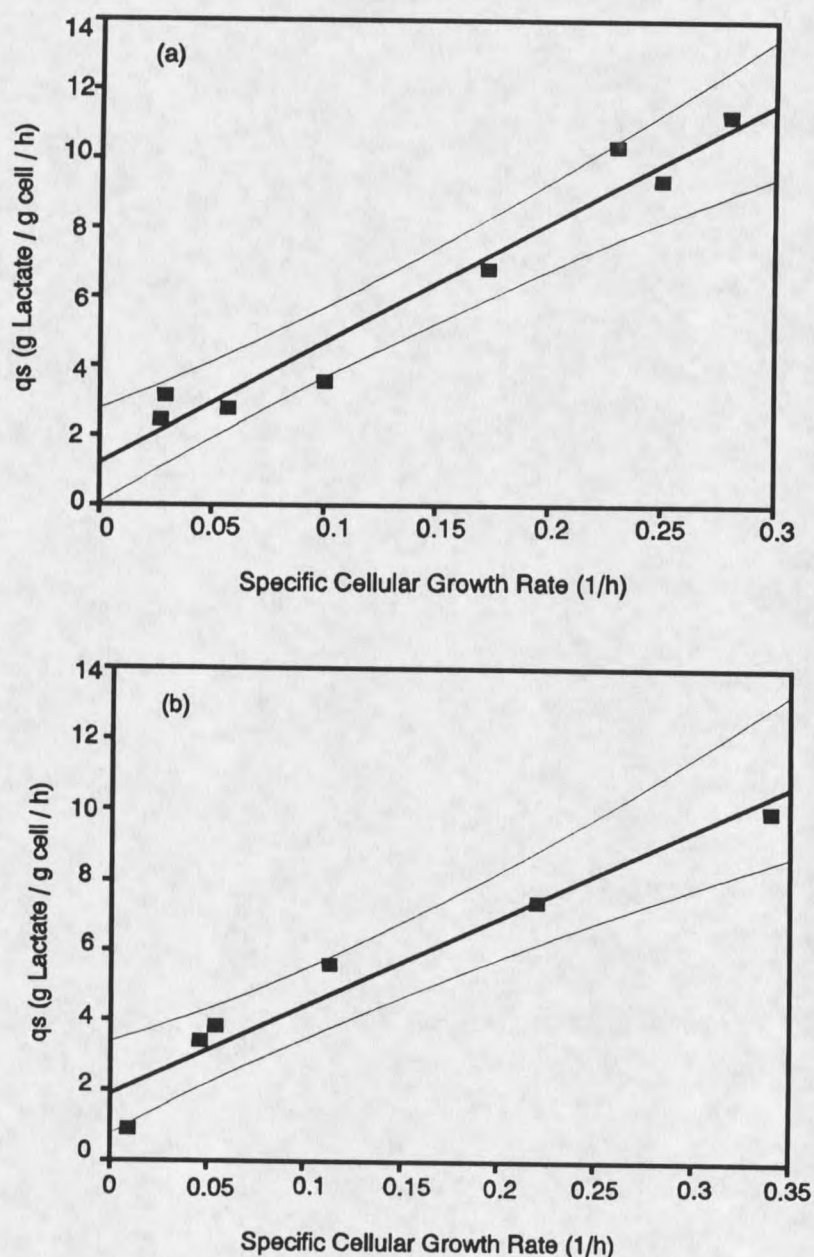


Figure 13. The relationship between specific lactate removal rate (q_s) and dilution rate (D). The maintenance coefficient (m) is the specific lactate removal rate at $D=0$ (the y-intercept). The intrinsic cell yield is a reciprocal of the slope. The linear regression lines along with 95 % confidence intervals are presented. (a): the maintenance coefficient of 1.20 ± 0.53 g lactate (g cell) $^{-1}$ h $^{-1}$ and intrinsic cell yield coefficient of 0.029 ± 0.003 g cell (g lactate) $^{-1}$ were determined at 35°C : $r^2=0.96$. (b): the maintenance coefficient of 1.98 ± 0.51 g lactate (g cell) $^{-1}$ h $^{-1}$ and intrinsic cell yield coefficient of 0.041 ± 0.005 g cell (g lactate) $^{-1}$ were determined at 43°C : $r^2=0.95$.

Table 9. Summary of rate coefficients for *D. desulfuricans* obtained under lactate-limiting and sulfate-limiting conditions. Values given are the estimated parameter $\pm$ the standard error. The maintenance coefficient under sulfate-limiting condition is based on lactate consumption for comparison purposes (see Figure 13).

Temp. (°C)	$\mu_{\max}$ (h ⁻¹)	$K_{\text{Lac}}, K_{\text{Sul}}$ (mg L ⁻¹)	m (g _{Lac} ·g _{Cell} ⁻¹ h ⁻¹)	$Y_{\text{c/Lac}}^{\text{Intr a)}$ (g _{Cell} g _{Lac} ⁻¹)	$Y_{\text{c/Lac}}^{\text{b)}$ (g _{Cell} g _{Lac} ⁻¹)	Reference
<u>Lactate-limiting</u>						
35	0.37 ± 0.004	2.2 ± 0.6	0.45 ± 1.08	0.028 ± 0.008	0.024 ± 0.006	Chapter 2
43	0.55 ± 0.003	10.0 ± 1.2	0.28 ± 0.05	0.031 ± 0.007	0.032 ± 0.001	Chapter 2
<u>Sulfate-limiting</u>						
35	0.344 ± 0.007	1.8 ± 0.3	1.20 ± 0.53	0.029 ± 0.003	0.020 ± 0.003	this study
43	0.352 ± 0.003	1.0 ± 0.2	1.98 ± 0.51	0.041 ± 0.005	0.017 ± 0.003	this study

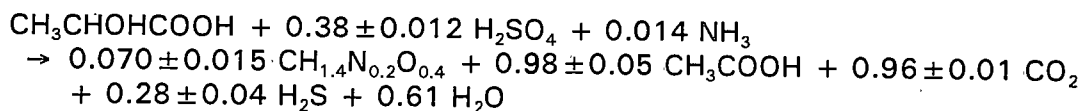
a) $Y_{\text{c/Lac}}^{\text{Int}}$ = intrinsic cell yield on lactate

b) $Y_{\text{c/Lac}}$ = observed cell yield on lactate

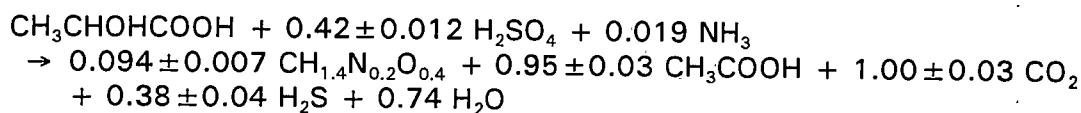
Table 10. Stoichiometries of microbial sulfate reduction under lactate- and sulfate-limiting conditions.

At 35°C

Sulfate-limiting ($S < K_{Sul}$)

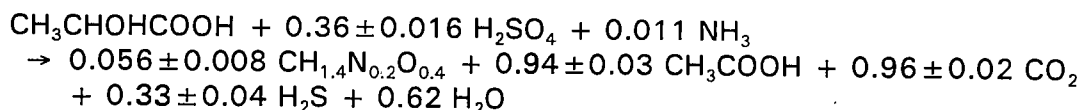


Sulfate-saturated ($S > K_{Sul}$)

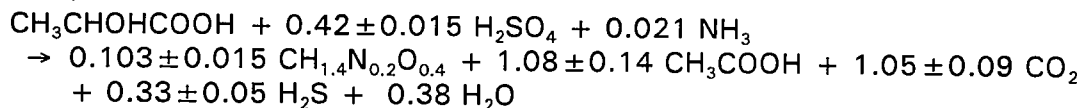


At 43°C

Sulfate-limiting ($S < K_{Sul}$)



Sulfate-saturated ($S > K_{Sul}$)



lactate. The stoichiometric coefficient for water was obtained using the oxygen balance. The percentages of carbon recovery in the stoichiometric equations at each temperature were more than 95 % of the amounts of lactate-carbon added.

At low levels of sulfate (below K_{Sul}), the SO_4^{2-} /lactate stoichiometric ratio decreased to approximately 0.30 from 0.45 as observed above the K_{Sul} value (Figure 14). The SO_4^{2-} /lactate ratio = 0.45 is similar to one obtained under lactate-limiting conditions in Chapter 2.

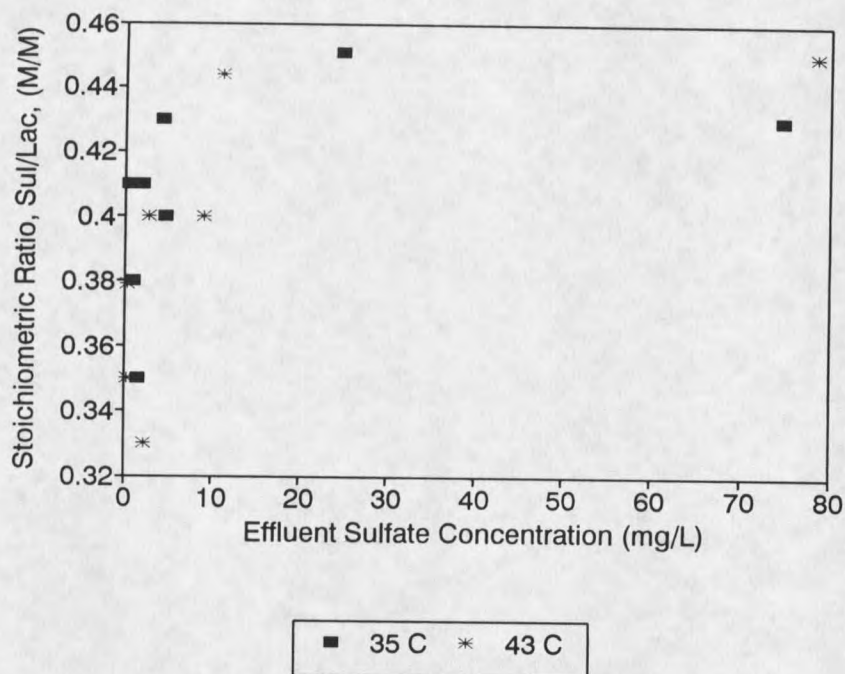


Figure 14. The effect of sulfate concentration on the stoichiometric ratio of SO_4^{2-} /lactate.

Effects of Nitrogen Concentration

D. desulfuricans was grown at a dilution rate of 0.20 h^{-1} , at 35°C , and at various C:N ratios to evaluate effects of nitrogen concentration on lactate oxidation and biomass synthesis. Sulfate was in excess. Steady state results, along with their respective standard deviations of measurement, are presented in Table 11.

Estimation of Nitrogen Requirement. The lactate utilization rate decreased from 99% at C:N=2.2:1 (w/w) to 85% at C:N=120:1 and then decreased to 40% at C:N=230:1 (Figure 15). The effluent nitrogen concentration at C:N=45:1 was 0.29 mg N/L. Thus, medium at C:N=45:1 was low in nitrogen. At lower influent nitrogen

Table 11. Steady state results of liquid phase parameters of the continuous culture of *D. desulfuricans* with different influent ammonium nitrogen concentrations at a dilution rate of 0.20 h^{-1} . The values given are the mean of duplicate measurements of two samples $\pm$ the standard deviation (in mg/L).

INFLUENT			EFFLUENT		
$\text{NH}_4^+\text{-N}$	Lactate	Sulfate	$\text{NH}_4^+\text{-N}$	Lactate	Sulfate
<u>C:N = 2.2:1</u>					
65.59 ± 0.55	376.9 ± 3.6	645.5 ± 0.0	53.74 ± 3.87	2.5 ± 0.7	452.3 ± 6.5
<u>C:N = 22:1</u>					
6.39 ± 0.11	353.7 ± 1.2	644.5 ± 4.5	4.13 ± 0.34	0.9 ± 0.7	455.5 ± 14.4
<u>C:N = 45:1</u>					
3.32 ± 0.01	389.6 ± 1.8	674.5 ± 5.5	0.29 ± 0.01	1.3 ± 0.3	477.7 ± 8.3
<u>C:N = 120:1</u>					
1.22 ± 0.04	375.6 ± 3.7	611.8 ± 0.9	0.15 ± 0.02	56.2 ± 13.1	445.0 ± 9.0
<u>C:N = 230:1</u>					
0.64 ± 0.01	372.0 ± 2.4	638.2 ± 1.8	0.05 ± 0.01	224.9 ± 12.8	531.4 ± 21.5

levels, the metabolism shifted from lactate-limited to nitrogen-limited as evidenced by: (1) significant amounts of lactate in the effluent and (2) lower cell yield. The stoichiometries for microbial sulfate reduction changed with changing C:N ratio assuming nitrogen content of the cells was constant according to the empirical formula $(\text{CH}_{1.4}\text{N}_{0.2}\text{O}_{0.4})$ (Characklis, 1990) (Table 12). CH_2O was used as an empirical formula for EPS (Characklis, 1990). The recovery of carbon in the five stoichiometric equations was more than 95 % of the amounts of lactate carbon added except for C:N = 120:1.

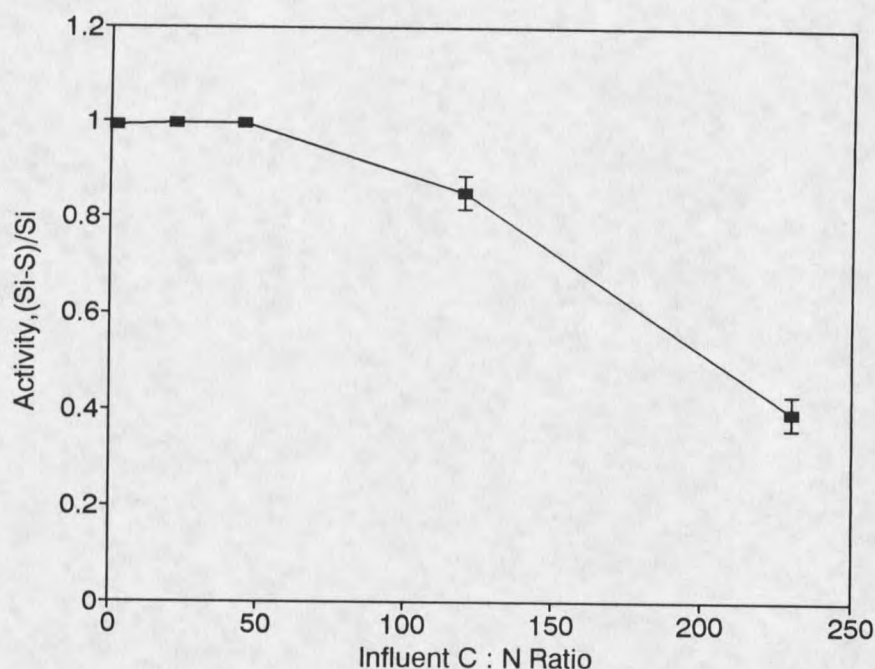


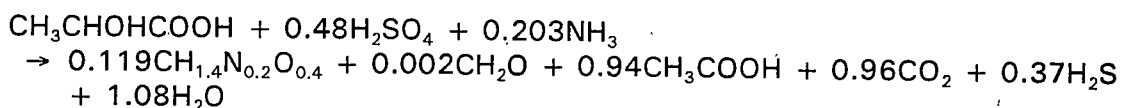
Figure 15. Response of lactate oxidation $[(S_f - S)/S_i]$ to change in the carbon (C):nitrogen (N) ratio: $D = 0.20 \text{ h}^{-1}$, Temp. = 35°C . The limiting C:N ratio (w/w) is in the range 45:1 to 120:1. Error bars represent the standard deviation of measurement ($n=2$). The error bar is not presented except for two points at 120:1 and 230:1 because the other standard deviation are so small (Table 12).

EPS and Cellular Carbons. The cell size of *D. desulfuricans* asymptotically decreased with decreasing influent nitrogen concentration (Figure 16). The cell size decreased by about 30% at C:N=45:1 and remained relatively constant thereafter. With decreasing nitrogen concentration, cell carbon concentration also decreased from 6.3 mg C/L at C:N=2:1 to 1.3 mg C/L at C:N=230:1 (Figure 17). Even at C:N=22:1, cell carbon concentration decreased by 16%. In contrast, EPS carbon concentration increased from 0 mg EPS C/L at C:N=2:1 to 2.11 mg EPS C/L at C:N=45:1, then decreased in parallel to the cell carbon drop. At C:N=45:1, EPS carbon concentration was approximately equal to cell carbon concentration. EPS yield increased and cell

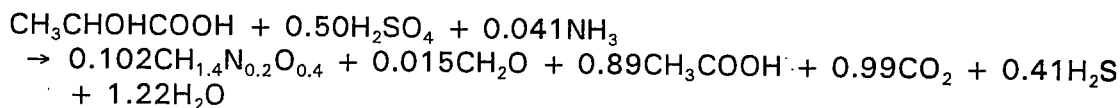
yield decreased with decreasing nitrogen concentration (Figure 18). Above C:N = 45:1, the cell yield was the same as the EPS yield. Note that total biomass yield (cell + EPS) remains constant for all C:N ratios.

Table 12. The effect of nitrogen concentration on the stoichiometry of microbial sulfate reduction.

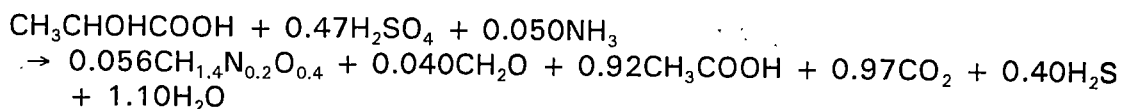
C:N = 2.2:1 (98.7 %) ^{a)}



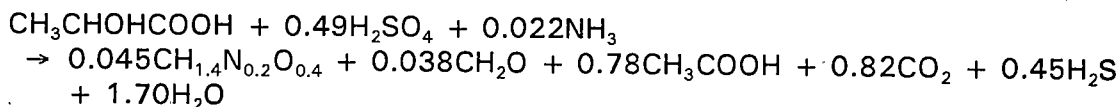
C:N = 22:1 (96.2 %) ^{a)}



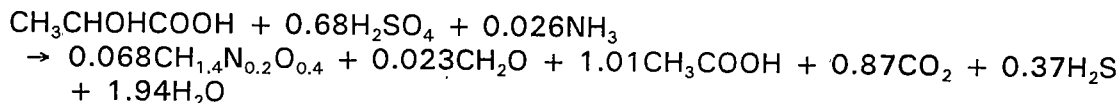
C:N = 45:1 (96.9 %) ^{a)}



C:N = 120:1 (82.0 %) ^{a)}



C:N = 230:1 (99.4 %) ^{a)}



^{a)} percentage of carbon recovery.

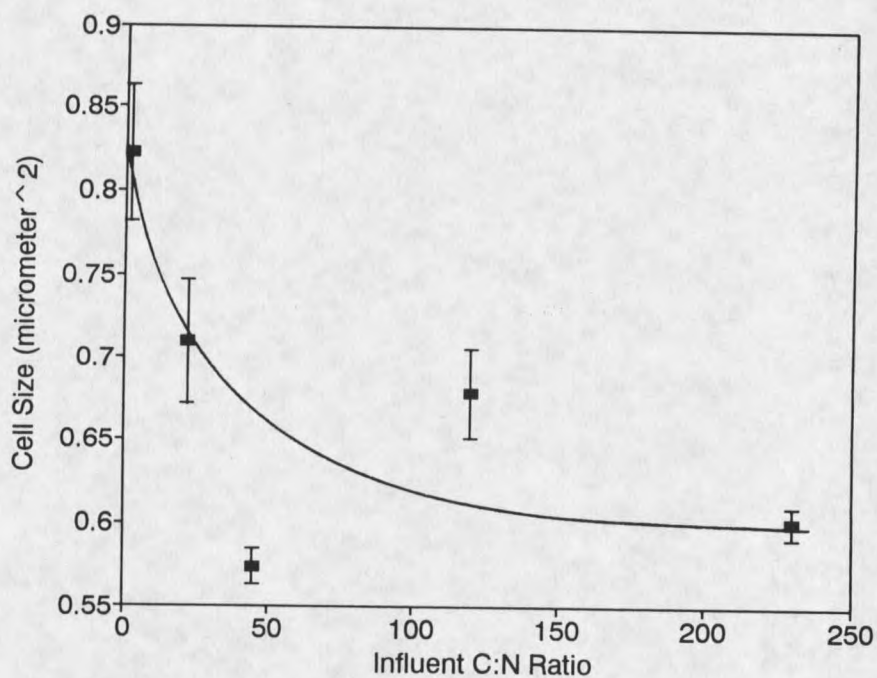


Figure 16. Change in cell size of *D. desulfuricans* with increasing C:N ratio. The cell size was determined using an image analyzer by the epifluorescence method. Error bars represent the standard deviation of measurement ($n = 2$).

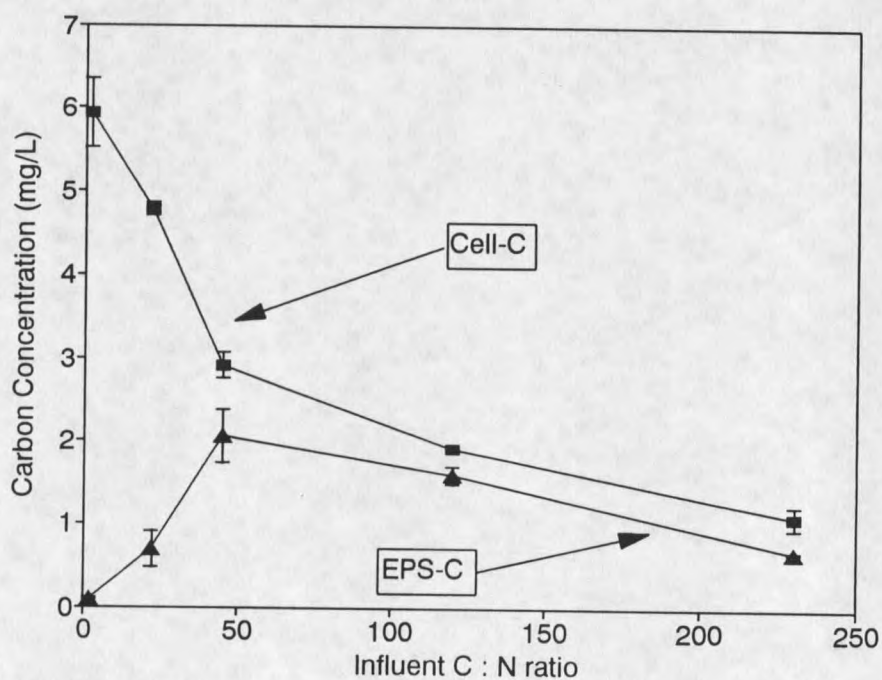


Figure 17. Change in cellular and EPS carbon concentrations with changing C:N ratio. Error bars represent the standard deviation of measurement ($n = 2$).

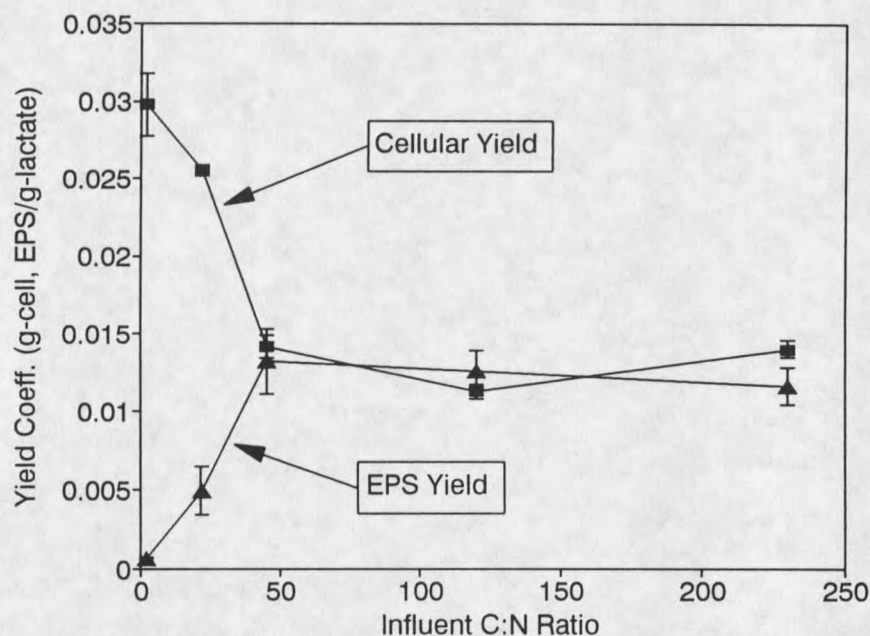


Figure 18. Change in cell and EPS yield coefficients with changing C:N ratio. Error bars represent the standard deviation of measurement ($n = 2$).

Effects of Sulfide Concentration

D. desulfuricans growing at a dilution rate of 0.20 h^{-1} and at 35°C was exposed to various sulfide concentrations (Table 13). Sulfide concentration was measured as total sulfide concentration in the liquid phase. The chemostat pH was strictly maintained at 7.0 with sterile 1.0 N NaOH and HCl solutions. Treatment with 150 mg/L total sulfide slightly decreased lactate utilization and cellular production (Figures 19 and 20). Total sulfide of 280 mg/L dramatically decreased cellular production and increased EPS production. Finally, lactate utilization and cellular production were strongly inhibited at total sulfide of 600 mg/L. The fraction of EPS carbon increased with increasing total sulfide concentration. Overall, cell size decreased with increasing

total sulfide concentration to about $0.7 \mu\text{m}^2$ at 280 mg/L of total sulfide and remained relatively constant thereafter (Figure 21). After treatment with 600 mg/L of total sulfide, 50 mL of culture medium was transferred to 450 mL of the fresh culture medium without sulfide, the cell numbers were monitored to examine the recovery of *D. desulfuricans* from sulfide inhibition (Figure 22). Cell numbers slowly increased without a lag phase and reached the same cell number as the control. The doubling time of the sulfide-treated culture was approximately one third of the control.

Table 13. Steady state results of liquid phase parameters of the continuous culture of *D. desulfuricans* with different influent total sulfide concentrations at a dilution rate of 0.20 h^{-1} . The values given are the mean of duplicate measurements of two samples $\pm$ the standard deviation (in mg/L).

Sulfide-S	Influent Lactate	Effluent Lactate
40.9 ± 0.1	382.9 ± 1.2	2.5 ± 0.7
150.2 ± 1.4	417.7 ± 9.2	7.4 ± 0.2
277.6 ± 4.9	393.9 ± 2.4	31.9 ± 4.2
596.3 ± 44.7	400.0 ± 6.1	272.3 ± 14.1

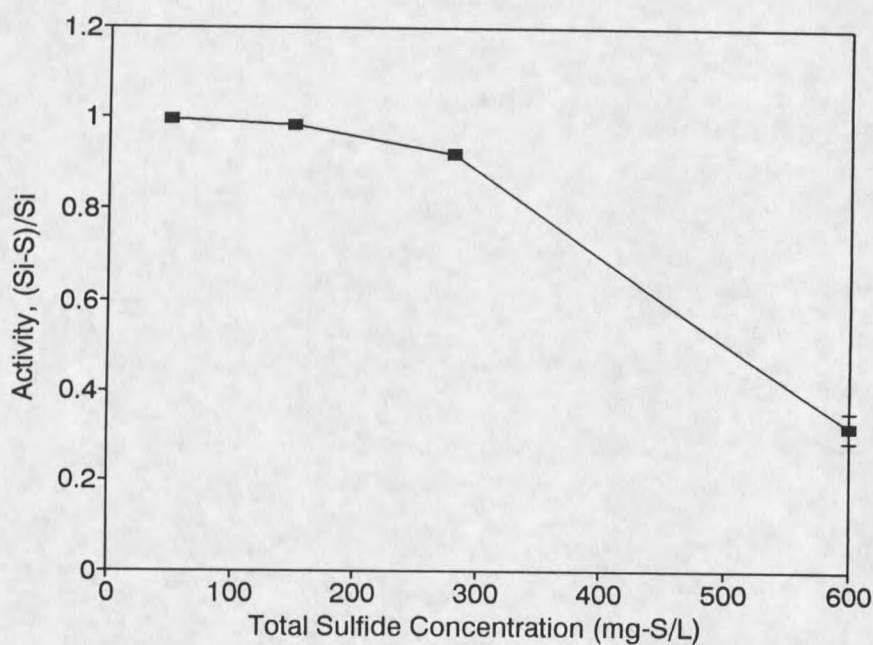


Figure 19. Response of lactate oxidation $[(S_i-S)/S_i]$ to change in total sulfide concentration: $D = 0.20 \text{ h}^{-1}$, Temp. = 35°C , pH = 7.0. Error bars represent the standard deviation of measurement ($n = 2$). The error bar is not presented except for 600 mg TS/L because the other standard deviation are so small (Table 13).

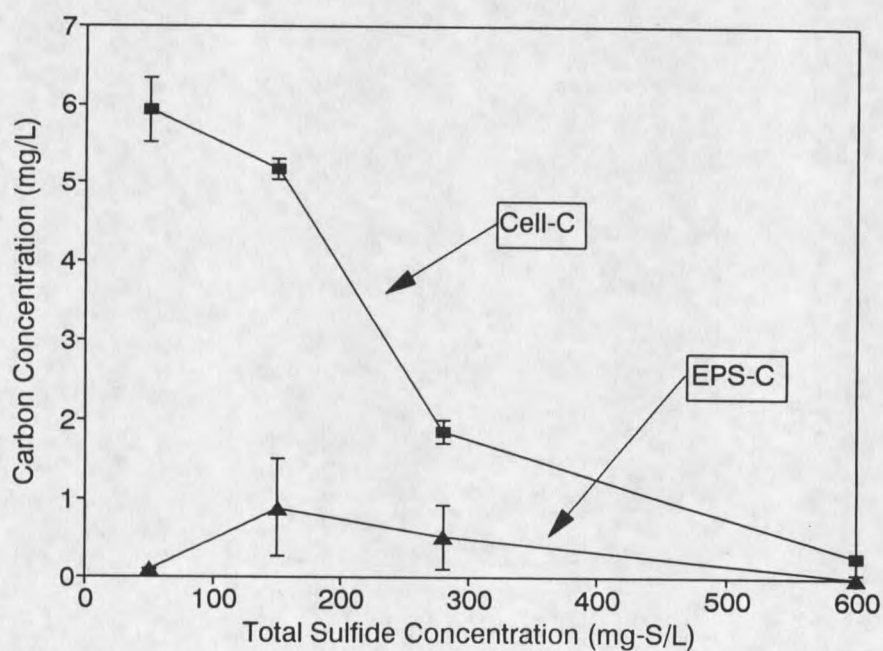


Figure 20. Change in cellular and EPS carbon concentrations with changing total sulfide concentration. Error bars represent the standard deviation of measurement ($n = 2$).

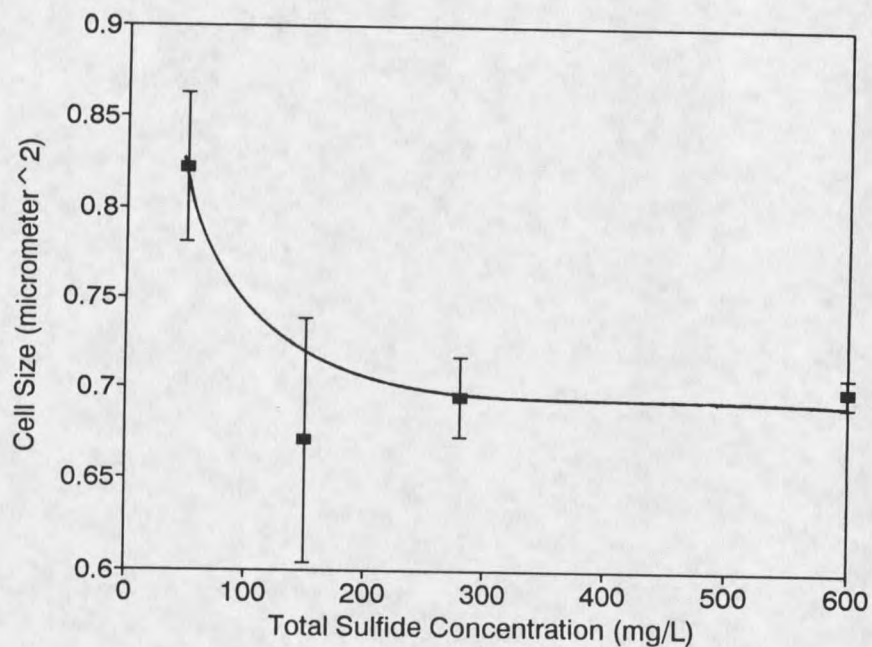


Figure 21. Change in cell size of *D. desulfuricans* with changing total sulfide concentration. Error bars represent the standard deviation of measurement ($n = 2$).

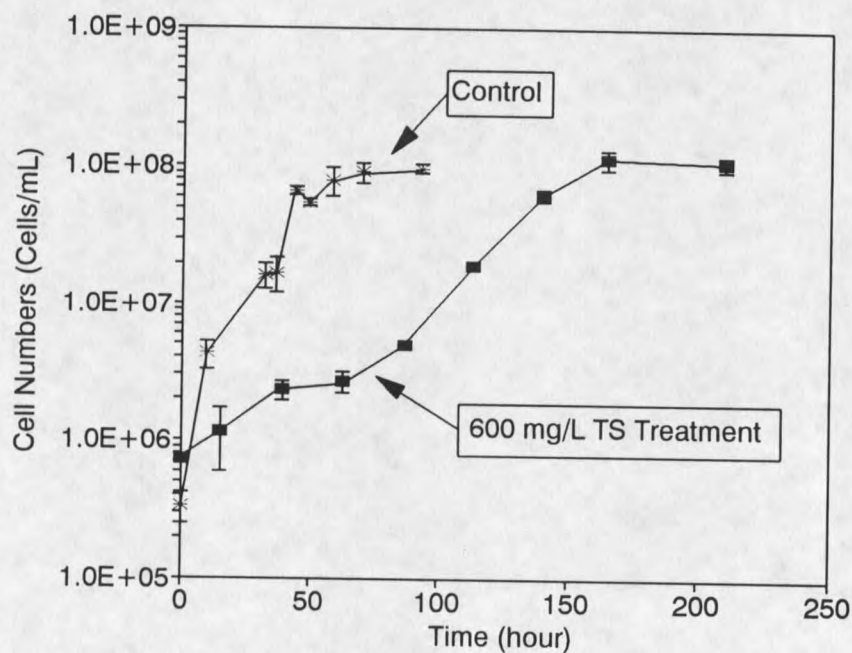


Figure 22. Recovery of *D. desulfuricans* from sulfide inhibition after 600 mg/L total sulfide treatment. Error bars represent the standard deviation of measurement ($n = 3$).

Discussion

Effects of Sulfate Concentration

Maintenance Coefficient. The maintenance coefficients under sulfate-limited conditions are significant (Figures 13(a) and 13(b)). The maintenance coefficient (m) is determined in continuous culture experiments (not in batch experiments) and is the specific substrate removal rate at $D=0$ (the y-intercept in Figures 13(a) and 13(b)). Thus, the effect of m is generally observed at low specific growth rate (at low dilution rate). Significant maintenance coefficients were not observed in the lactate-limiting experiments (Table 5 in Chapter 2). Under sulfate-limiting conditions, the significant maintenance coefficients were a result of operating the chemostat at low dilution rates ($D < 0.05 \text{ h}^{-1}$). The chemostat was not operated at a dilution rate less than 0.05 h^{-1} under lactate-limiting conditions (Chapter 2). Cappenberg (1975) reported that the maintenance coefficient for *D. desulfuricans* grown with lactate-limited growth was $0.53 \text{ g lactate (g dry wt.)}^{-1} \text{ h}^{-1}$ (Table 14). The reported maintenance coefficients in this paper are high compared to the values in the literature primarily because cell-associated EPS and possible precipitates (e.g., FeS) are not considered in the calculation of the maintenance coefficient. The maintenance coefficient determined using biomass dry weight, which includes cellular mass, EPS, and precipitates, would be low compared to our data because of high biomass concentration. The overall yields (Chapter 2), $(\text{cells} + \text{EPS} + \text{precipitates})/(\text{substrate consumed})$, of *D. desulfuricans* in previous studies were about two or three times higher than the cell yields (Appendix B).

Table 14. The ATP balance and growth yields for SRB grown on different substrate and sulfate.

Energy Source	Limiting Substrate	Organisms	Net ATP Generation ^{a)}	$Y_{c/Sul}$ (g g ⁻¹)	$Y_{o/Sul}$ (g g ⁻¹)	$m_{Sul}^{b)}$ (g g ⁻¹ h ⁻¹)	$m_{Lac}^{c)}$ (g g ⁻¹ h ⁻¹)	Reference
H ₂ , SO ₄ ²⁻	SO ₄ ²⁻	<i>D. vulgaris</i>	1	—	0.132	0.17	-	(16)
H ₂ , SO ₄ ²⁻	Ac	<i>D. vulgaris</i>	1	—	0.130	0.54	-	(16)
Lac., SO ₄ ²⁻	Lac.	<i>D. desulfuricans</i>	3	—	-	-	0.53 ^{d)}	(4)
Lac., SO ₄ ²⁻	SO ₄ ²⁻	<i>D. desulfuricans</i>	3	0.047	-	-	1.20	this study
Lac., SO ₄ ²⁻	SO ₄ ²⁻	<i>D. desulfuricans</i>	3	0.043	-	-	1.98	this study
Lac., SO ₄ ²⁻	Lac.	<i>D. desulfuricans</i>	3	0.051	-	-	0.45	Chapt.2
Lac., SO ₄ ²⁻	Lac.	<i>D. desulfuricans</i>	3	0.071	-	-	0.28	Chapt.2
Lac., SO ₄ ²⁻	- ^{f)}	<i>D. vulgaris</i>	3	-	0.121	ND ^{e)}	-	(13)
Lac., SO ₄ ²⁻	-	<i>Desulfotomaculum</i> <i>orientis</i>	1	-	0.040	ND	-	(13)
Lac., SO ₄ ²⁻	SO ₄ ²⁻	<i>D. vulgaris</i>	3	-	0.141	ND	-	(10)
Lac., SO ₄ ²⁻	SO ₄ ²⁻	<i>D. sapovorans</i>	3	-	0.115	ND	-	(10)
Lac., SO ₄ ²⁻	SO ₄ ²⁻	<i>D. salexigens</i>	3	-	0.125	ND	-	(10)

a) Estimated moles of ATP during the reduction of 1 mol of SO₄²⁻ to S²⁻.

b) g sulfate (g dry wt.)⁻¹ h⁻¹.

c) g lactate (g cell)⁻¹ h⁻¹.

d) g lactate (dry wt.)⁻¹ h⁻¹.

e) Not determined.

f) No limiting substrate because of batch culture experiments.

Based on this finding, maintenance coefficients reported by others should be cautiously interpreted since no distinction of biomass was made. In addition, the reported maintenance coefficients for SRB are high compared to values for aerobic microorganisms because higher maintenance energies are required under anaerobic conditions for the production of energy (ATP) from the substrate. For example, Pirt (1965) reported that the maintenance coefficients for *Aerobacter cloacae* growing in a glycerol-limited continuous culture were 0.094 and 0.473 g glycerol (g dry wt.)⁻¹ h⁻¹ under aerobic and anaerobic conditions, respectively.

Cell Yield. Observed cell yields under sulfate-limitation in these experiments are low compared to literature values for at least two reasons: (1) no yeast extract was used in the medium as is common in other SRB studies and (2) cell-associated EPS was not considered in the yield calculation. Furthermore, sulfate-limitation may result in an increase in maintenance energy requirement for *D. desulfuricans* which may significantly influence intracellular processes. Thus, maintenance energy reflects diversion of substrate away from synthesis or growth processes. Consequently, maintenance decreases the observed cell yield from substrate (Table 9). Nevertheless, intrinsic cell yields ($Y_{c/Lac}^{Intr}$) under sulfate-limiting and lactate-limiting conditions were essentially the same (Table 9).

D. desulfuricans grown on lactate and sulfate has a net ATP generation of three moles of ATP per mole of sulfate reduced (Thauer and Badzuong, 1981). About three moles of ATP are generated during electron transport coupled phosphorylation and two moles of ATP are generated in substrate level phosphorylation (Thauer and Badzuong, 1981). But two moles of ATP are consumed to activate a mole of SO_4^{2-} to adenosine

phosphosulfate (APS). The cell yield per ATP (based on three moles of ATP generation theory), $Y_{\text{ATP}}^{\text{max}}$, determined in this study is approximately 1.44 g cells (mole of ATP)⁻¹, which is one tenth of that proposed by Badziong and Thauer (11.4-14.6 g (mol ATP)⁻¹). Liu and Peck (1981) reported ATP and growth yields for *Desulfotomaculum orientis* and *Desulfovibrio vulgaris* (Table 14). *D. vulgaris* has a net ATP generation of three moles per sulfate reduced, whereas *D. orientis* has a net ATP generation of one. The cell yield per ATP, $Y_{\text{ATP}}^{\text{max}}$, in their experiments was approximately 4 g cells (mol of ATP)⁻¹. Thus, ATP yield varies significantly among species and with substrate. Clearly, the biochemistry and physiology of growth of *D. desulfuricans* with lactate and sulfate requires further investigation to rationalize the molecular and the cellular observations.

Stoichiometry. At low levels of sulfate (below K_{Sul} value), the SO_4^{2-} /lactate stoichiometric ratio decreased (Figure 14). Under these conditions, lactate is probably oxidized via pyruvate and acetate. *D. desulfuricans* possesses the pyruvic phosphoroclastic system in which, under sulfate-limitation, pyruvate is dismutated to acetyl phosphate, CO_2 , and H_2 (Peck and LeGall, 1982). There is some evidence that H_2 can be formed from lactate in small amounts if constant removal of hydrogen is occurring (Bryant et al., 1977). Thus at low levels of sulfate, *D. desulfuricans* may grow with lactate in the absence of sulfate, which is thermodynamically favorable only under very low H_2 partial pressure (Widdel, 1988). In a continuous culture system with N_2 purge, it is speculated that high levels of H_2 cannot accumulate. Contamination with other microorganisms, such as methanogenic bacteria, another possible cause for decreased SO_4^{2-} /lactate stoichiometric ratio was not observed in these experiments.

Half-Saturation Constant (K_{Sul}). The half-saturation coefficients for sulfate, K_{Sul} , at the 35°C and 43°C are 1.8 ± 0.3 and 1.0 ± 0.2 mg/L, respectively. Ingvorsen and Jorgensen (1984) reported that half-saturation coefficients for sulfate, K_{Sul} , for *Desulfovibrio vulgaris*, *Desulfovibrio sapovorans*, and *Desulfovibrio salexigens* grown in the batch culture were 0.5 mg/L, 0.7 mg/L, and 7.4 mg/L, respectively. Observed biomass yields, $Y_{o/Sul}$ for *D. vulgaris*, *D. sapovorans*, and *D. salexigens* were also determined to be 0.141, 0.115, and 0.125 g dry wt./g sulfate, respectively (Table 14). The results obtained in this study are comparable to these reported values.

Implications. The experimental results indicate that sulfate may be a promising limiting nutrient to control SRB activity if concentration in injection water can be reduced below a few mg/L. Maree and Strydom (1987) reported that biological sulfate removal using molasses as an organic source was feasible without production of H_2S by co-culturing SRB with photosynthetic sulfur bacteria which oxidize sulfide to sulfur. The process is accompanied by the precipitation of calcium carbonate and heavy metals leading to their recovery.

Effects of Nitrogen Concentration

Nitrogen Fixation. Nitrogen is needed for amino acid, purine, and pyrimidine biosynthesis. Ammonium ions are the conventional nitrogen source in culture media but *D. desulfuricans* can fix molecular nitrogen (N_2) (Riederer-Henderson, 1970; Postgate and Kent, 1985; Postgate et al., 1986). Postgate and Kent (1985) reported

that none of the *Desulfovibrio* strains tested showed acetylene reduction if NH_4Cl was present. Ammonium chloride completely repressed *nif* expression in *Desulfovibrio gigas* and addition of ammonium chloride in the range 10 to 100 μM inhibited nitrogenase activity (Postgate and Kent, 1984). Senez (1962) reported that the growth rate of *D. desulfuricans* growing on NH_4^+ as nitrogen source was more than twice as fast as on N_2 and biomass yield from N_2 was diminished significantly. Thus, nitrogen fixation by this culture ($\mu_{\text{max}} = 0.34 \text{ h}^{-1}$) in a chemostat is not significant because of a relatively high dilution rate ($D = 0.20 \text{ h}^{-1}$) and substantial amounts of NH_4^+ in the medium.

Nitrogen Requirement. The limiting C:N ratio (w/w) determined in this study for *D. desulfuricans* (C:N = 45:1-120:1) is higher than that for an aerobic mixed population (usually C:N = 10-20:1) because *D. desulfuricans* partially oxidizes lactate to acetate and CO_2 . Thus, cell production from substrate is approximately 10 times less than in aerobic systems and nitrogen requirements are 10 times less also.

EPS Production. EPS production rate increased with decreased nitrogen loading rate. Increase in EPS production may influence plugging of oil reservoirs and initial cell adsorption on surfaces. Lappan and Fogler (1990) reported that cellular polysaccharide production was a significant factor in formation damage by bacteria, especially for low permeability cores, with permeability reduction with polysaccharide production being ten times greater than without polysaccharide production.

Implications. The experimental results indicate that SRB activity may be controlled by reducing nitrogen from injection water, since the deficiency of nitrogen

results in a significant decrease in SRB activity. Nazina et al. (1979), however, reported that the mesophilic SRB isolated from oil fields have high nitrogenase activity, while the thermophilic SRB have weak nitrogenase activity. Thus, nitrogen fixation by mesophilic SRB may be considerable in an oil reservoir where the temperature range is appropriate for mesophilic SRB growth. It is of interest to determine the role of SRB in nitrogen fixation. If significant fixation occurs, removal of nitrogen from injection water may not be a reasonable means to control H_2S generation, even though growth rate and cell production will be much lower.

Effects of Sulfide Concentration

Effect of pH on Sulfide Toxicity. Sulfide inhibition of SRB probably occurs when sulfide species (H_2S , HS^- , and S^{2-}) combine with the iron of the cytochrome and other essential iron-containing compounds in the cell causing electron transport systems to cease activity (Postgate, 1984). Thus, pH of the system, which determines the distribution of sulfide species, plays a very important role in inhibition of microbial sulfate reduction. The relative distribution of H_2S and HS^- at $\text{pH} = 7.0$ is about 1:1 (pK_a of H_2S is 7.0 at 25°C). The percentage of un-ionized H_2S drops from 90% at $\text{pH} = 6.0$ to 50% at $\text{pH} = 7.0$ to 10% at $\text{pH} = 8.0$. Thus, the effects of un-ionized H_2S or ionized HS^- concentration on activity of a microbial population without pH effects can be observed by altering the culture pH in a narrow range. Toxicity of the various sulfide species to microorganisms may be different. Oleszkiewicz et al. (1989) reported that the time required to achieve 90% utilization of lactate, butyrate, acetate, and propionate by an anaerobic mixed population grown in batch serum bottles was shortened at comparable total sulfide concentrations at a pH of 7.7-7.9 as compared

to pH = 6.5-7.4. The results suggest that un-ionized H_2S is the more toxic species of sulfide to an anaerobic mixed population, presumably due to its ease of transport through the cell membrane. Hilton and Oleszkiewicz (1988) performed a series of batch experiments containing an undefined anaerobic population growing on lactate at initial pH = 6.0, 7.0, and 8.0 and at various total sulfide concentrations. They reported that sulfate reduction was inhibited in proportion to the total sulfide concentration, not the un-ionized H_2S concentration. Acidogenic and methanogenic processes were inhibited by un-ionized H_2S , more than total sulfide. Thus, at a high level of total sulfide and high pH (low concentration of H_2S), the carbon flow in the batch reactor could be diverted from sulfate reduction to methane production, as long as the H_2S concentration was below the inhibitory level to the methanogenic population.

Cell Yield, Growth, and Lactate Utilization. In continuous culture experiments reported herein, lactate utilization slightly decreased to 92 % at 280 mg/L total sulfide, whereas cell carbon production decreased from 6.0 mg/L to about 2.0 mg/L at 280 mg/L total sulfide (Figures 19 and 20). The cell yield decreased dramatically from 0.03 g cell/g lactate at 50 mg/L total sulfide to 0.005 g cell/g lactate at 600 mg/L total sulfide. Cell yield may decrease because energy is expended in countering the inhibitory effect of sulfide and, thus, is diverted from cell production (maintenance energy requirement increases). Shimada (1987) reported that 100 mg/L of H_2S inhibited the growth of a mixed SRB population in batch cultures and no significant SRB growth was observed at 500 mg/L of H_2S (the pH in this experiment was not reported). Burgess (1961) reported that microbial sulfate reduction was inhibited by 900 mg-S/L. However, batch culture results must be viewed with caution since pH

may change as sulfide accumulates, imposing another stress on the population. Also sulfide precipitates Fe, so that Fe may become the limiting substrate for growth (Postgate, 1984).

Implications. The experimental results indicate that, under preexisting high sulfide concentration in the formation, biological sulfide production is not a favorable process. Although other nutritional and physical conditions are suitable for SRB growth, SRB activity is strongly inhibited by high sulfide concentration.

Conclusions

- 1) The observed specific growth rate and cell yield for *D. desulfuricans* under sulfate-limiting conditions are lower than those obtained under lactate-limiting conditions due to an increase in the maintenance energy requirement.
- 2) The limiting C:N ratio (w/w) for *D. desulfuricans* is in the range 45:1 to 120:1. The extent of extracellular polymeric substance (EPS) production increases with increasing carbon : nitrogen ratio in the medium. Total biomass yield (cell + EPS) remains constant.
- 3) 50 % inhibition of lactate utilization by *D. desulfuricans* occurs at approximately 500 mg/L of total sulfide. EPS production increases with increasing sulfide concentration.
- 4) Sulfide inhibition of *D. desulfuricans* activity is a reversible process.
- 5) Increasing C : N ratio and increasing sulfide concentration result in decreased cell size for *D. desulfuricans*.

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CHAPTER 4

SULFIDE PRODUCT INHIBITION KINETICS
IN BATCH AND CONTINUOUS CULTURESIntroduction

Sulfate Reducing Bacteria (SRB) cause serious problems in sanitary sewer systems and industrial water systems because of production of highly toxic and corrosive hydrogen sulfide gas. For example, the corrosion of concrete sewers occurs as a result of hydrogen sulfide production from sulfate-rich sewage by the activities of SRB (Mori et al., 1992). Furthermore, in the oil industry, SRB cause serious corrosion of installations, plugging of formations, and contamination of petroleum with H_2S (souring). The environment for microbial growth varies widely throughout the petroleum formation. Sulfide concentration varies from 0 mg- H_2S /L to 1000 mg- H_2S /L in the produced water (Subcasky, 1991). Although many have studied the effects of sulfide on anaerobic wastewater treatment (e.g., methane producing bacteria) (Karhadkar et al., 1986; Koster et al., 1986; Hilton and Archer, 1987; Rinzema and Lettinga, 1988; Oleszkiewicz et al., 1989; Hilton and Oleszkiewicz, 1989; McCartney and Oleszkiewicz, 1991), no quantitative SRB product inhibition data have been reported. It is essential to understand how SRB behave under high sulfide concentration environments to predict SRB behavior in these industrial water systems.

Sulfide toxicity is strongly dependent on pH, because the chemical equilibrium

of sulfide species is pH dependent. At pH 8 most of total sulfide (TS) is in the HS⁻ form, while at pH 6 most is in the H₂S form. Molecular hydrogen sulfide (H₂S) was found to be the major toxic form of sulfide because H₂S can pass through the cell membrane (Speece, 1983; Reis et al., 1991). SRB generate sulfide that may result in product inhibition of SRB and/or toxicity to methane producing bacteria (MPB).

The overall effect of sulfide on microbial sulfate reduction is qualitatively described by some authors (Coleman, 1960; Postgate, 1984; Klemps et al., 1985; Shimada, 1987; Hilton and Oleskiewicz, 1989; Min and Zinder, 1990; McCartney and Oleszkiewicz, 1991; Reis et al., 1991; Okabe et al., 1992; Reis et al., 1992). The relevant literature is summarized in Table 15. However, no quantitative investigation of sulfide product inhibition kinetics for SRB has been reported in these studies. Postgate (1984) stated that growth of SRB is usually linear, not exponential due to sulfide product inhibition. The growth rate of SRB decreases at high sulfide concentrations. Okabe et al. (1992) reported that 50 % inhibition of lactate utilization by *D. desulfuricans* occurred at approximately 500 mg-S/L of total sulfide and that sulfide inhibition of *D. desulfuricans* activity was a reversible process. McCartney and Oleskiewicz (1991) investigated the total sulfide and molecular H₂S inhibition of SRB and MPB using batch culture techniques. They reported that the 50 % inhibition concentrations were 2.6 and 7.5 mmol/L (83.2 and 240 mg/L) total sulfide for SRB and MPB growing on lactate, acetate, and sulfate medium (pH = 7.20-7.55) in serum bottles, respectively. Also, at pH 7.80-7.95 methanogenic activity remained relatively constant in the total sulfide concentration range 100-1000 mg/L, while sulfate reduction rate decreased with increasing total sulfide. These observations indicated that the SRB were more sensitive to both total sulfide and molecular H₂S as compared

Table 15. Summary of the literature on the effects of sulfide on growth and activity.

References	Concentration	pH	B/C ^{a)}	Observation
Coleman (1960)	192-224 mg TS/L	6.5-7.0	B	Decreased growth rate and biomass yield.
Klemps et al. (1985)	64-128 mg TS/L	6.9-7.0	B	Ceased growth. After removal of H ₂ S resumed growth.
Hilton and Oleszkiewicz (1989)	400 mg TS/L	7.0	B	Reduced sulfate reduction by 50 %
Min and Zinder (1990)	64 mg TS/L	6.5	B	Ceased sulfate reduction.
McCartney and Oleszkiewicz (1991)	448 mg TS/L	7.15-7.33	B	Completely ceased sulfate reduction.
Reis et al. (1992)	550 mg H ₂ S/L	6.2-6.7	B	Ceased sulfate reduction. H ₂ S is the most inhibitory form.
Reis et al. (1992)	547 mg H ₂ S/L	6.2-6.7	B	Ceased growth. Found to be a reversible toxicity. H ₂ S is the most inhibitory form.
Okabe et al. (1992)	500 mg TS/L	7.0	C	Decreased cellular yield and cell size. Reduced lactate oxidation by 50 %

^{a)} Refers to batch or continuous (chemostat) reactor.

to the MPB. Min and Zinder (1990) reported that sulfide concentrations produced by *Desulfotomaculum thermoacetoxidans* sp. nov. growing in batch cultures rarely exceeded 2 mmol/L. Klemps et al. (1985) reported that growth of *Desulfotomaculum* species did not occur in medium containing more than 2 mmol/L of Na_2S , sulfate reduction stopped at a sulfide concentration of 4 mmol/l, and then growth proceeded after removal of H_2S by sparging the culture with N_2/CO_2 (80/20). Without removal of produced sulfide, growth was slower and stopped at lower cell densities. Hilton and Oleszkiewicz (1989) reported that SRB are more sensitive than MPB to elevated total sulfide concentrations, while both are sensitive to elevated molecular H_2S concentrations. Thus, at high total sulfide concentrations and high pH the methane producing bacteria should be able to outcompete the SRB for substrate.

Little distinction between growth and activity of SRB was made in these studies (Klemps 1985). The distinction between growth and activity is very important when the environmental conditions become extreme (high sulfide concentration) and energy consumption shifts from growth to maintenance. In many cases microbial sulfate reduction is monitored by H_2S gas production. Under extreme conditions, the growth-associated H_2S production will decrease, whereas total H_2S production will remain relatively constant because the decrease in growth is counter balanced by increased nongrowth-associated H_2S production caused by increased maintenance energy requirement (Hunik et al., 1990).

The goal of this chapter is to quantitatively describe how sulfide affects activity and growth of SRB. The distinction between growth and activity was clearly made in this study; growth was defined as biomass (Cell) production rate and activity was defined as specific substrate utilization rate (e.g., lactate and sulfate).

Theoretical Background

Microbial transformations in a lactate-limiting chemostat can be described mathematically by assuming that the two fundamental processes occurring are growth and maintenance:

$$\frac{D (S_i - S)}{X} = \frac{\mu}{Y_{c/Lac}} + m = q_s \quad (17)$$

where, D = dilution rate (h^{-1}), S_i = influent lactate concentration ($\text{M}_s \text{ L}^{-3}$), S = effluent lactate concentration ($\text{M}_s \text{ L}^{-3}$), X = cell concentration ($\text{M}_x \text{ L}^{-3}$), $Y_{c/Lac}$ = cell yield coefficient on lactate ($\text{M}_x \text{ M}_s^{-1}$), m = maintenance coefficient ($\text{M}_s \text{ M}_x^{-1} \text{ h}^{-1}$), and q_s = specific lactate utilization rate ($\text{M}_s \text{ M}_x^{-1} \text{ h}^{-1}$). At steady state, the dilution rate (D) in the chemostat is equal to the specific growth rate (μ).

Non-competitive inhibition kinetics can be described as follows (Aiba et al, 1973):

$$\mu = \frac{\mu_{\max} S K_i}{(K_{Lac} + S)(K_i + i)} \quad (18)$$

where, $\mu_{\max}$ = maximum specific growth rate (h^{-1}), i = sulfide concentration ($\text{M}_i \text{ L}^{-3}$), K_{Lac} = half-saturation coefficient for lactate ($\text{M}_s \text{ L}^{-3}$), and K_i = inhibition coefficient ($\text{M}_i \text{ L}^{-3}$). For $S \gg K_{Lac}$, Eq.(18) can be simplified as follows:

$$\mu = \frac{\mu_{\max} K_i}{K_i + i} \quad (19)$$

Eq.(19) can be linearized by plotting $1/\mu$ against i .

$$\frac{1}{\mu} = \frac{1}{\mu_{\max}} + \frac{i}{\mu_{\max} K_i} \quad (20)$$

The x-interception of a best fit line gives $-K_i$.

Maximum specific growth rates ($\mu_{\max}^{\text{inh}}$) of *D. desulfuricans* growing at a constant dilution rate ($D = 0.20 \text{ h}^{-1}$) at various sulfide concentrations can be determined by rearranging Eq.(18) as follows:

$$\mu = \frac{S}{K_{\text{Lac}} + S} \left(\frac{\mu_{\max} K_i}{K_i + i} \right) \quad (21)$$

By defining the maximum specific growth rate in presence of sulfide, $\mu_{\max}^{\text{inh}}$, as follows:

$$\mu_{\max}^{\text{inh}} = \frac{\mu_{\max} K_i}{K_i + i} \quad (22)$$

when $i=0$, $\mu_{\max}^{\text{inh}} = \mu_{\max}$. Then, at steady state

$$\mu = D = \frac{\mu_{\max}^{\text{inh}} S}{K_{\text{Lac}} + S} \quad (23)$$

or,

$$\mu_{\max}^{\text{inh}} = \frac{D (K_{\text{Lac}} + S)}{S} \quad (24)$$

Eq.(24) indicates that $\mu_{\max}^{\text{inh}}$ can be determined by measuring S , since D and K_{Lac} are known.

Experimental Materials and Methods

Microorganisms

Desulfovibrio desulfuricans (ATCC 5575) was grown in Postgate medium G, (Postgate, 1984) including Na-lactate (DL-Lactic acid, SIGMA, L-1375) and Na_2SO_4 . Trace elements and vitamins were added. The details of medium preparation and preculture of microorganisms are described in Chapter 2.

Batch Culture Experiment

Sulfide inhibition kinetics were determined in batch experiments using 500 ml erlenmeyer flasks. Flasks were equipped with butyl rubber stoppers fitted with an injection port and a gas exchange port. Four hundred mL of the pH adjusted ($\text{pH} = 7.0$) Postgate medium G containing 500 mg/L of lactate and 800 of mg/L sulfate was dispensed into each erlenmeyer flask and autoclaved at 121°C for 20 minutes. Trace elements and vitamins solutions were added after cooling. $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution was prepared separately in an air-tight bottle. A $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ crystal was rinsed with deionized water to removed oxidized surface layer, weighed, and dissolved in oxygen-free deionized distilled water under N_2 purge. After autoclaving, the pH of sulfide solution was adjusted to 7.0 in the air-tight bottle using a sterile syringe and sterile 1.0 N HCl and NaOH solutions. Then appropriate volumes of the sulfide solution were added to the autoclaved culture medium using a sterile syringe to obtain the designed

total sulfide concentrations (0, 70, 220, 350, 700 mg-S/L). After addition of sulfide solution, the pH of the culture medium was remeasured and readjusted. *D. desulfuricans* taken from an actively growing chemostat culture was inoculated into each flask and incubated at 35°C. The inoculum size from the chemostat was adjusted to obtain an initial cell count of approximately 1×10^7 cells/mL. Samples for cell count were carefully taken by a sterile syringe with N₂ purge to prevent air contamination. Cell numbers were monitored using an image analyzer (Cambridge/Olympus Quantment 10) by the epifluorescence method described by Hobbie et al. (1977).

Continuous Culture Experiment

Sulfide inhibition kinetics were also investigated in a lactate-limiting chemostat. The chemostat was equipped with a butyl rubber wall growth scraper continuously rotated by an electric motor to prevent wall growth. Constant pH (± 0.1 unit) and temperature ($\pm 0.5^\circ\text{C}$) were maintained using a pH control system with sterile 1.0 N HCl and NaOH solutions and thermoregulator, respectively. Various concentrations of sulfide solutions, with pH roughly adjusted to 7.5, were separately prepared and autoclaved, then fed to the chemostat by a peristaltic pump and speed controller (Cole-Parmer, Chicago, IL). A fine pH adjustment was conducted in the chemostat. The effect of sulfide on the activity and growth was determined at a constant dilution rate of 0.20 h^{-1} . The details of chemostat set up protocol are described in Chapter 2.

Analytical Methods

At steady state, chemostat effluent samples were obtained for the following

analyses: (1) total organic carbon (TOC); (2) soluble organic carbon (SOC); (3) total bacterial counts and cell size; (4) sulfate; (5) sulfide; (6) lactate; (7) acetate. The samples for SOC, lactate, and acetate were obtained by centrifugation (20,000 rpm for 20 minutes) at 4°C using stainless steel centrifuge tubes. The sample for sulfate analysis was first fixed with 1 % (w/v) ZnAc solution (0.3 ml of 10 % (w/v) ZnAc solution into 25 ml sample) to remove sulfide species and then filtered by Sterile Acrodisc filter (pore size 0.2 μm , Gelman Science, No. 4192). Sulfate and acetate concentrations were measured using an ion chromatograph (DIONEX). Sulfide concentration was measured as total dissolved sulfide concentration (TS) in the liquid phase. The details of the rest of chemical analytical methods are described in Chapter 2.

Results

Batch Experiments

Maximum specific growth rate in the presence of sulfide ($\mu_{\text{max}}^{\text{inh}}$) of *D. desulfuricans* was determined from the cell doubling times (t_d) at various total sulfide concentrations in batch cultures (Figure 23). Initial growth rates were determined (Table 16) by linear interpolation of the data of Figure 23 because total sulfide concentration and the pH of culture medium changed during incubation. For data interpretation initial pH, total sulfide concentration, and corresponding maximum specific growth rates ($\mu_{\text{max}}^{\text{inh}}$) are presented in Table 16. The maximum specific growth rates ($\mu_{\text{max}}^{\text{inh}}$) determined in the batch cultures never exceeded 0.20 h^{-1} at each total sulfide concentration, which is significantly lower than values for continuous culture.

The inhibition coefficient for maximum specific growth rate, K_i , was determined using the non-competitive inhibition model and found to be 251 mg-S/L (Figure 24). The maximum specific growth rates calculated at various sulfide concentrations using the non-competitive model and this value of K_i are compared with the measured maximum specific growth rates in the presence of sulfide in Figure 25. The model adequately described the sulfide effect on maximum specific growth rate in batch experiment.

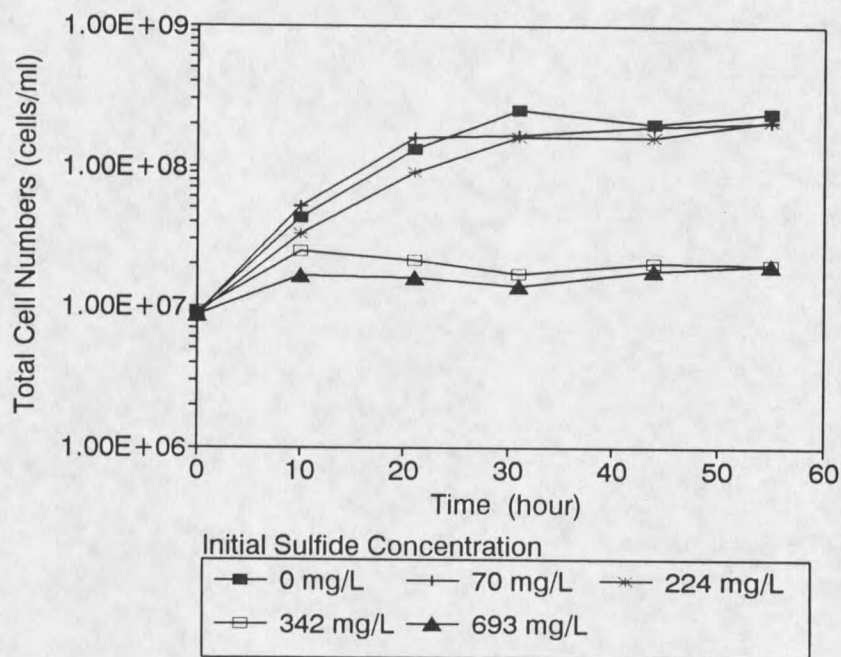


Figure 23. Effect of sulfide on growth of *D. desulfuricans* in batch cultures: Temp. = 35°C.

Table. 16 Results of sulfide effect on growth rate of *D. desulfuricans* in batch cultures.

TS (mg-S/L)	pH	t_d (h)	μ_{max}^{inh} (h^{-1})
0	6.96	4.0	0.17
70	6.95	3.5	0.20
224	7.30	5.5	0.13
342	7.00	7.0	0.10
693	6.95	6.95	0.058

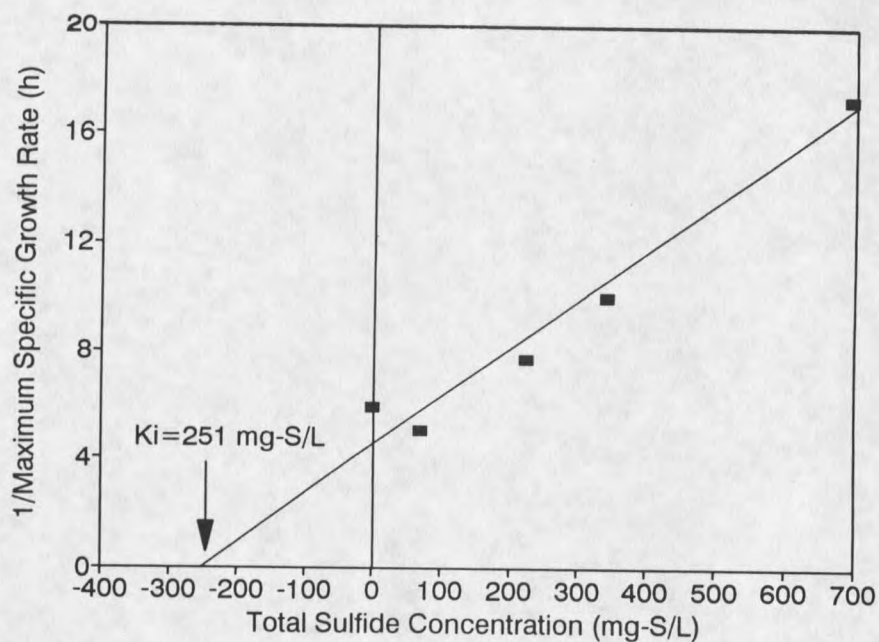


Figure 24. Determination of K_i value in the batch culture. Theoretical plot of the reciprocal of maximum specific growth rate in the presence of sulfide ($1/\mu_{\max}^{\text{inh}}$) as a function of sulfide concentration. K_i of 251 mg-TS/L was determined.

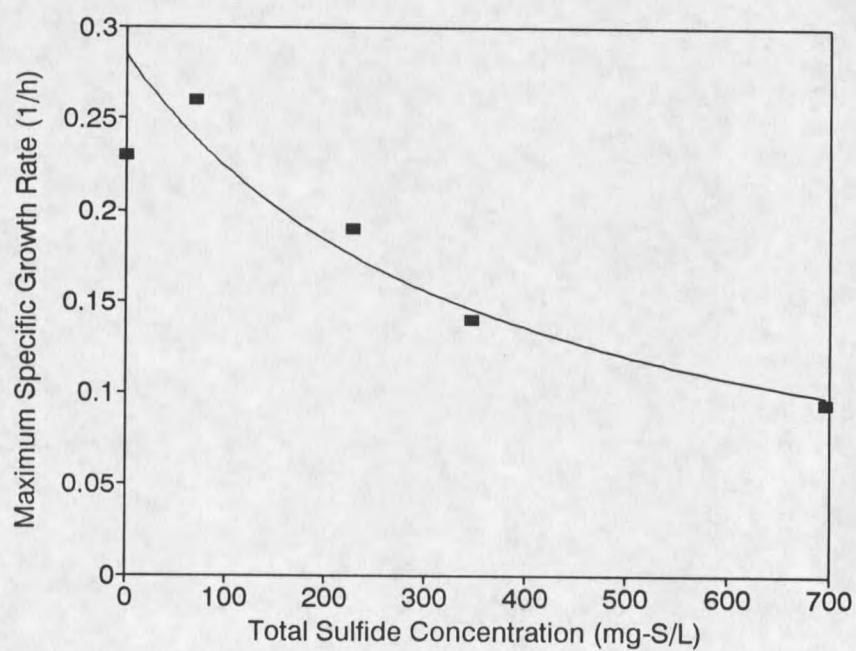
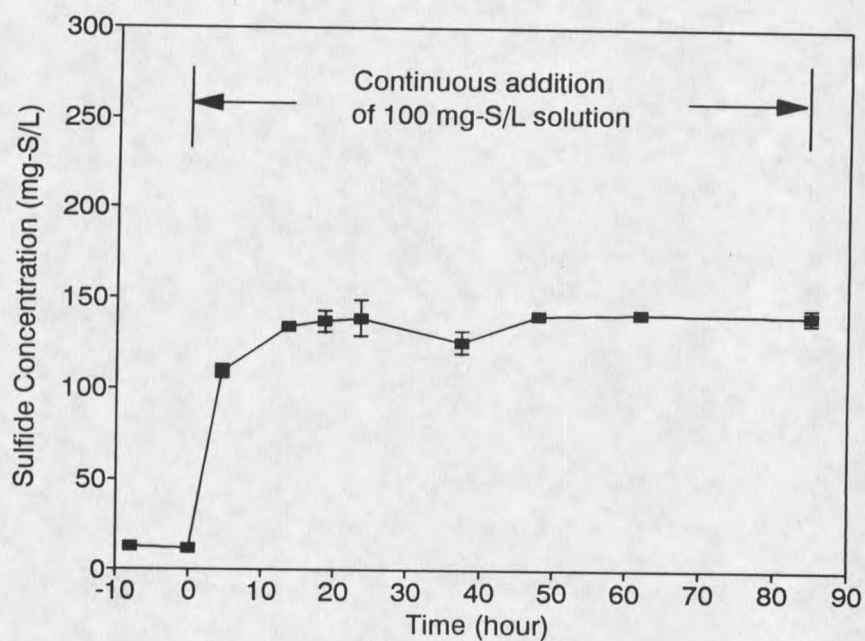


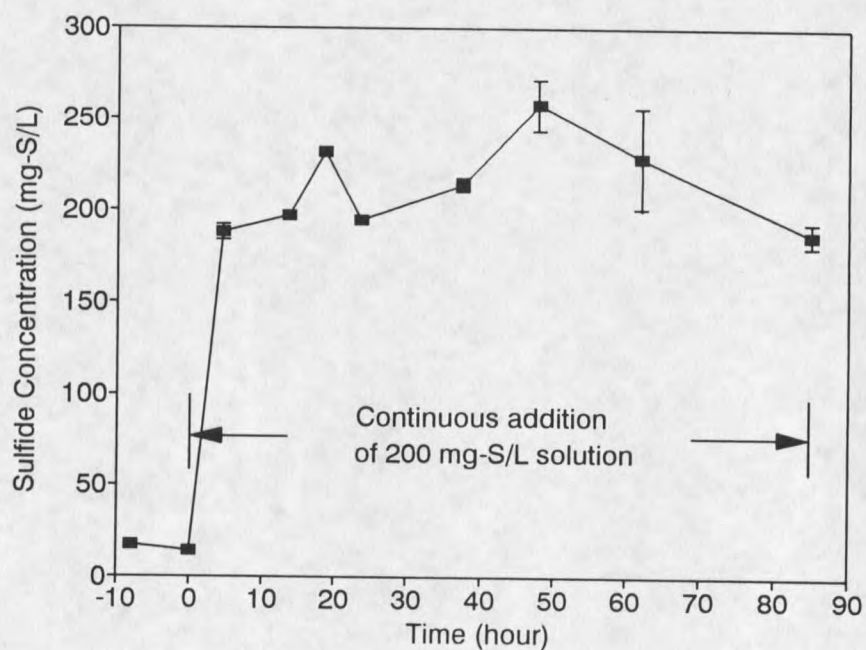
Figure 25. Effect of sulfide on maximum specific growth rate ($\mu_{\max}^{\text{inh}}$) in batch cultures. (■): experimental points. The solid line reflects the inhibition coefficient (K_i) determined by Figure 24.

Continuous Experiments

Adaption of Microorganisms to Sulfide. *D. desulfuricans* growing at a constant dilution rate (D) of 0.20 h^{-1} , at 35°C , and $\text{pH} = 7.0$ was continuously exposed to high total sulfide concentrations to examine if the bacteria could adapt to a high sulfide environment in terms of growth and activity. Sulfide solutions were fed continuously into the reactor to maintain constant dissolved total sulfide concentrations of 130 and 230 mg-S/L during the experimental period (Figures 26(a) and 26(b)). The initial sulfide concentrations in both experiments were below 20 mg-S/L. Addition of sulfide solutions were started at time zero. Cell production decreased about 25 % at 8 hours after 100 mg-S/L of TS addition, but cell production gradually recovered to the original level and reached a steady state value after about 40 hours (Figure 27 (a)). The activity expressed as lactate oxidation rate $[(S_i - S)/S_i]$ was relatively constant during the experimental period. Cell production was significantly inhibited by 200 mg/L of TS addition (Figure 27 (b)). However, cell production recovered by 20 % at 13.5 hours after sulfide addition and then approached a steady state value, which was about 55 % of the original cell production, whereas the activity remained relatively constant with a minor decrease at 13.5 hours. The culture approached steady state after about 40 hours in both cases. Therefore, all samples in later experiments were taken at about 40 hours after sulfide concentration was changed.

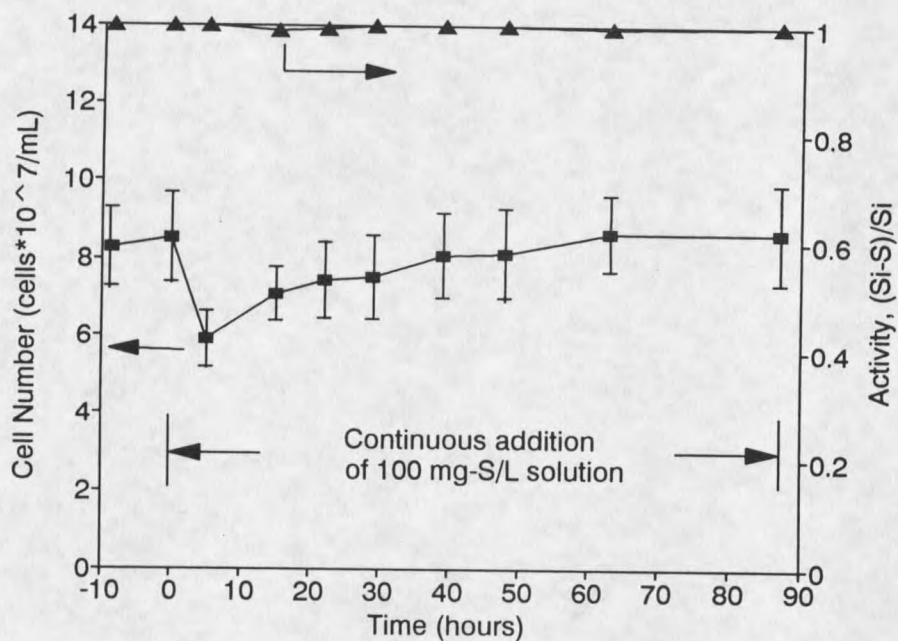


(a)

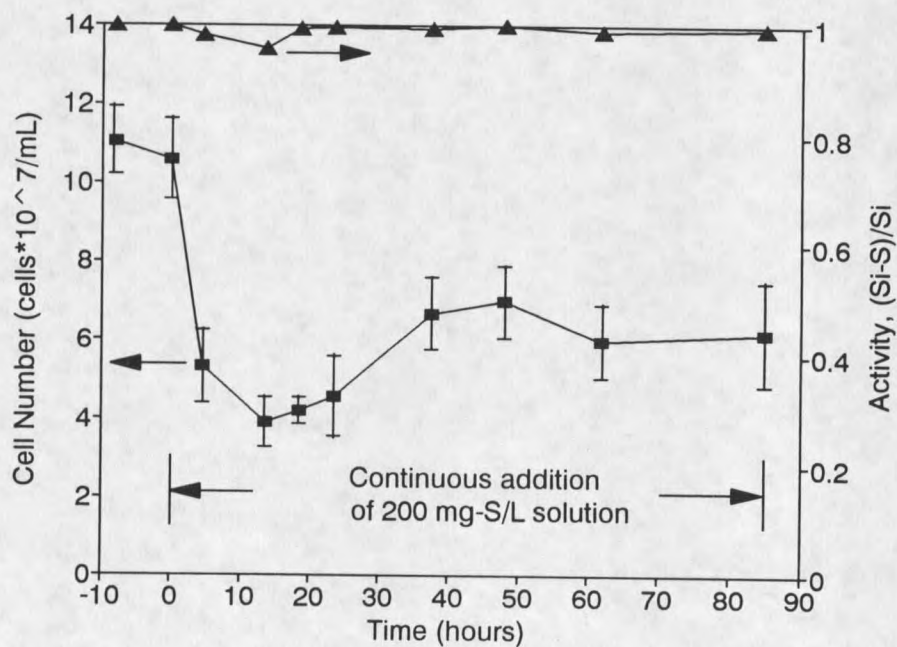


(b)

Figure 26. Change in sulfide concentration with time. (a) 100 mg-S/L and (b) 200 mg-S/L of sulfide solution were added continuously from $t=0$. Error bars represent the standard deviation of mean ($n=2$).



(a)



(b)

Figure 27. Typical response of cell production and lactate oxidation rate to exposure to (a) 130 mg-S/L and (b) 230 mg-S/L: $D = 0.20 \text{ h}^{-1}$, Temp. = 35°C , pH = 7.0.

Specific Lactate Utilization Rate and Cell Yield. *D. desulfuricans* growing at a dilution rate of 0.20 h^{-1} , at 35°C , and at $\text{pH}=7.0$ was exposed to various sulfide concentrations to determine the effect of sulfide on the activity and cell production. Lactate oxidation rate $[(S_i - S)/S_i]$ remained relatively constant with increasing TS up to 332 mg-S/L and decreased by 10 % at 437 mg-S/L (Figure 28), while cellular carbon production dramatically decreased by 50 % at about 250 mg-S/L (Figure 29). As a result of the decrease in cell production, but not lactate oxidation rate, specific lactate utilization increased from about $5.3 \text{ g lactate (g cell)}^{-1} \text{ h}^{-1}$ at 26 mg TS/L to $18.6 \text{ g lactate (g cell)}^{-1} \text{ h}^{-1}$ at 437 mg TS/L (Figure 28). Cell yield dramatically decreased from $0.036 \text{ (g cell)(g lactate)}^{-1}$ at low levels TS to $0.013 \text{ (g cell) (g lactate)}^{-1}$ at 437 mg-S/L . EPS carbon production slightly increased above 332 mg-S/L .

Determination of Inhibition Coefficient (K_i). Maximum specific growth rates ($\mu_{\text{max}}^{\text{inh}}$) of *D. desulfuricans* growing at a constant dilution rate of 0.20 h^{-1} at various sulfide concentrations were determined using Eq.(24) and K_{Lac} of 2.35 mg/L (Chapter 2). The maximum specific growth rate in the presence of sulfide ($\mu_{\text{max}}^{\text{inh}}$) decreased from 0.33 h^{-1} at low level of TS to 0.20 h^{-1} at high level of TS (Figure 30). The inhibition coefficient, K_i , was determined to be 978 mg-S/L by plotting a reciprocal $\mu_{\text{max}}^{\text{inh}}$ against TS concentration (Figure 31). This K_i was determined based on activity (lactate oxidation rate).

Stoichiometry. Stoichiometry of sulfate reduction was determined at each sulfide concentration to elucidate whether the presence of sulfide results in an

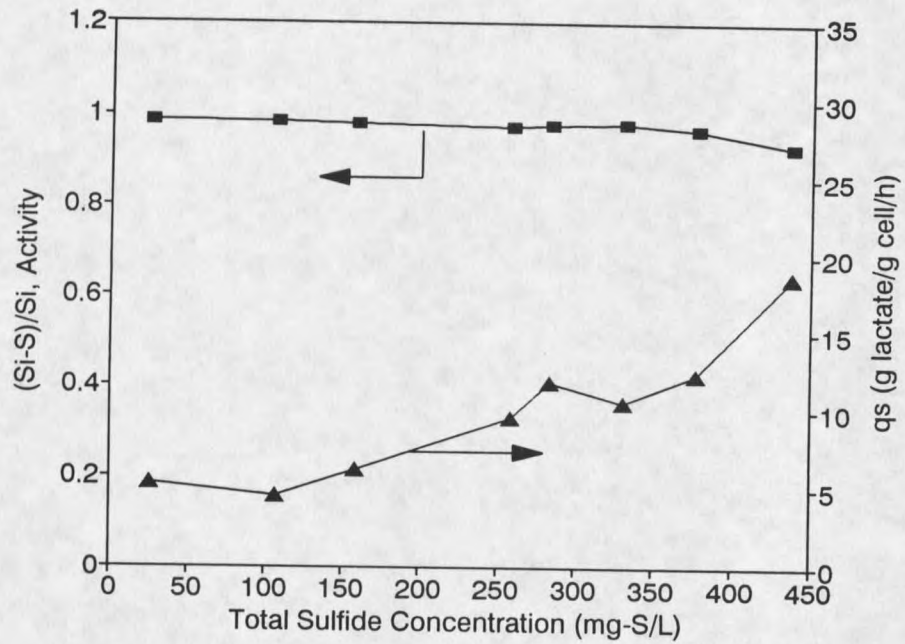


Figure 28. Effect of sulfide on lactate oxidation rate $[(S_i-S)/S_i]$ and specific lactate utilization rate (q_s): $D = 0.20 \text{ h}^{-1}$, Temp. = 35°C .

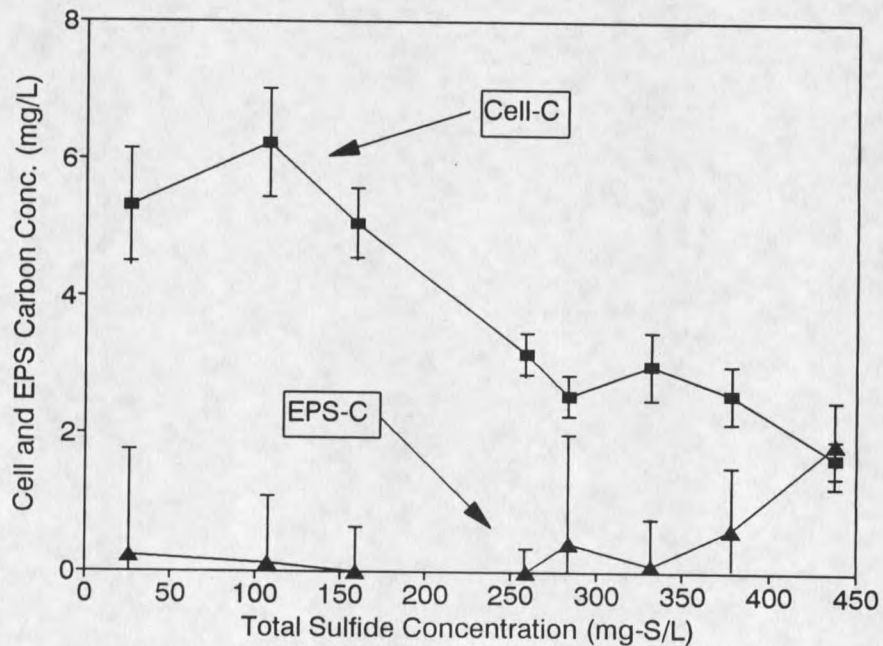


Figure 29. Change in cellular and EPS carbon concentrations with changing sulfide concentration. Error bars represent the standard deviation of mean ($n = 2$).

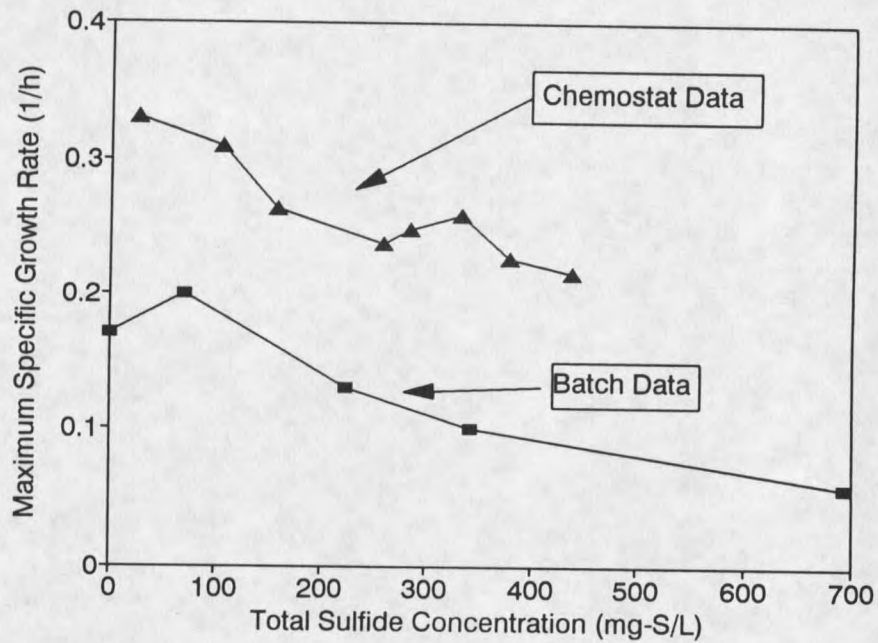


Figure 30. Effect of sulfide on maximum specific growth rate ($\mu_{\max}^{\text{inh}}$) of *D. desulfuricans* in continuous cultures: $D = 0.20 \text{ h}^{-1}$, Temp. = 35°C , pH = 7.0.

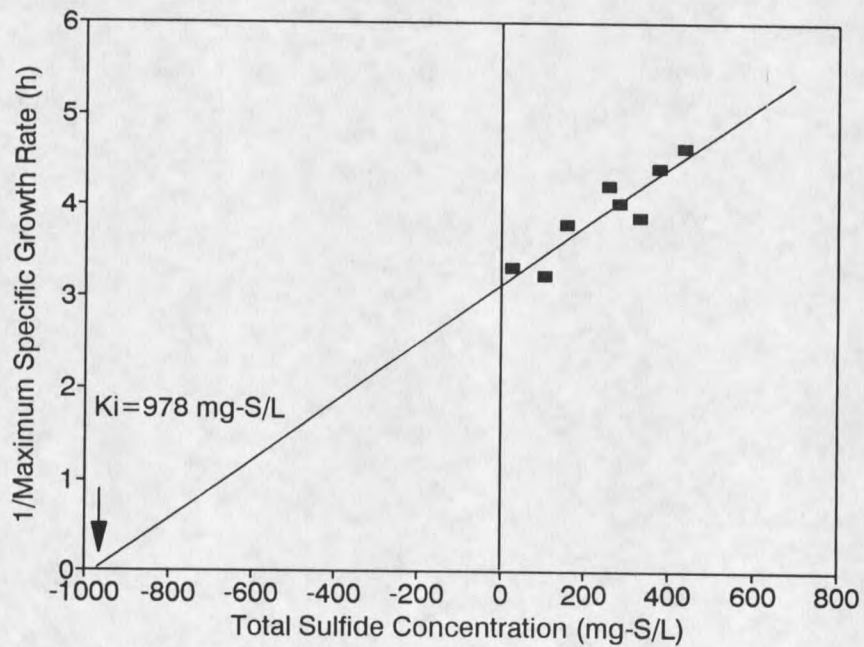


Figure 31. Determination of K_i value for $\mu_{\max}^{\text{inh}}$ from lactate oxidation rate in continuous cultures. K_i of 978 mg-S/L was determined.

alteration of the metabolic pathway(s) for sulfate reduction. The stoichiometric ratios for catabolic (energy producing) reactions such as lactate oxidized to sulfate reduced, CO₂ produced, and acetate produced were independent of sulfide concentration (Table 17). However, the stoichiometric ratio for anabolic (cell synthesis) reactions, cell/lactate, decreased with increasing total sulfide concentrations.

Table 17. Steady state stoichiometry of the continuous culture of *D. desulfuricans* exposed to various total sulfide concentrations at a constant dilution rate of 0.20 h⁻¹. (duplicate mean value $\pm$ SD)

Total Sulfide (mg-S/L)	Stoichiometric Ratio (M/M)			
	SO ₄ ²⁻ /Lac.	Ac/Lac.	CO ₂ /Lac.	Cell/Lac.
26.1 $\pm$ 1.0	0.42	0.85	1.03	0.148
108.2 $\pm$ 3.8	0.50	0.81	1.08	0.174
157.9 $\pm$ 19.0	0.49	0.84	1.05	0.128
259.1 $\pm$ 16.9	0.46	0.88	1.12	0.083
284.4 $\pm$ 7.1	0.51	0.91	1.01	0.067
332.0 $\pm$ 8.6	0.51	0.88	1.08	0.076
378.5 $\pm$ 13.0	0.48	0.83	1.09	0.065
437.5 $\pm$ 7.8	0.47	0.94	1.01	0.043

Discussion

Effects of Sulfide on Cell Yield and Specific Lactate Utilization Rate

Cell yield dramatically decreased from 0.036 (g cell)/(g lactate)⁻¹ at low levels TS to 0.013 (g cell) (g lactate)⁻¹ at 437 mg-S/L, while specific lactate utilization rate increased (Figure 28). This decrease in cell yield may have been a result of a large part of the energy source being used for maintenance and uncoupling of growth from

energy production. Cell yield is known to be dependent on μ , the maintenance coefficient (m), and the intrinsic cell yield ($Y_{c/Lac}$) and is expressed as follows (Bulthuis et al., 1989; Characklis, 1990):

$$\frac{1}{Y_{c/Lac}} = \frac{m}{D} + \frac{1}{Y_{c/Lac}^{intr}} \quad (25)$$

Thus, low cell yield would result from increase in maintenance energy (m) because of a constant dilution rate (D) and intrinsic yield ($Y_{c/Lac}^{intr}$) in the chemostat experiment (Chapter 3). The maintenance energy was calculated using the observed cell yield ($Y_{c/Lac}$) and the intrinsic cell yield ($Y_{c/Lac}^{intr} = 0.03 \text{ g cell (g lactate)}^{-1}$) (Chapter 3) (Figure 32). The calculated maintenance coefficient dramatically increased above a total sulfide concentration of about 200 mg-TS/L. A similar increase in specific substrate utilization rates has been observed in other types of bacteria. Mountfort and Asher (1979) reported that the biomass yield, the specific growth rate, and the intracellular levels of adenosine triphosphate (ATP) decreased with increasing sulfide level when *Methanosarcina barkeri* strain DM was grown in a batch system with methanol as carbon and energy source. However, specific methane production rate increased. They concluded that the decrease in biomass yield resulted from an increase in the maintenance coefficient and uncoupling of growth from energy production. Hobson and Millis (1990) reported that the maintenance energy requirements for a mixed culture growing in a two-stage chemostat with phenolics were insignificant at non-inhibitory levels of phenolics, but increased to $0.15 \text{ (g substrate)(g biomass)}^{-1} \text{ h}^{-1}$ at inhibitory levels, possibly due to the need to repair damaged cell membranes. Coleman (1960) reported that the growth of SRB isolated from the sheep rumen was inhibited when sulfide concentration reached 6-7 mmol/L and the maximum dry weight was only 50-

60 mg/L which was 50 % of bacteria dry weight grown on low level sulfide concentrations. Another contributing factor for the low cell yield is that death and lysis rates may increase at a high sulfide concentration.

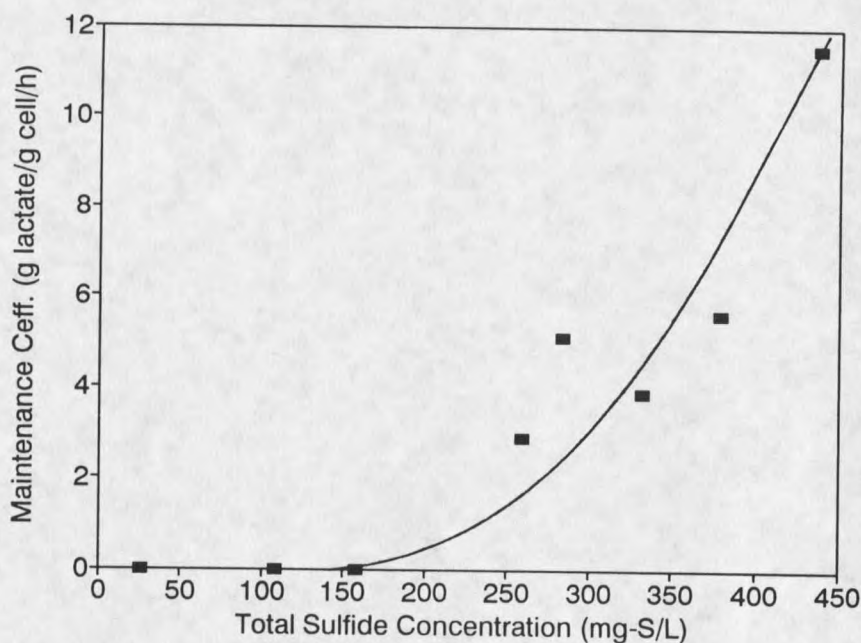


Figure 32. Effect of sulfide on maintenance coefficient (m). The maintenance coefficient was calculated based on Eq.(25) using $Y_{c/Lac}^{intr} = 0.03 \text{ g cell (g lactate)}^{-1}$ (Chapter 3).

Effect of Sulfide on Growth Rate

The inhibition coefficients, K_i , for the maximum specific growth rates in the presence of sulfide were determined to be 251 and 978 mg-S/L in the batch and the continuous experiments, respectively. The discrepancy of the values of K_i between the batch and the continuous experiment is attributed to the fact that K_i for the continuous experiment was determined based on inhibition of activity (lactate oxidation rate), whereas K_i for the batch experiment was based on inhibition of growth (cell

production). This result suggests that cell synthesis (anabolic reaction) is more sensitive to the elevating sulfide concentration than activity (catabolic reaction) is.

Furthermore, the discrepancy is probably attributed to the selection of less susceptible microorganisms to sulfide and the adaptation of *D. desulfuricans* to high sulfide levels in the continuous culture as demonstrated in Figures 27(a) and 27(b). The maximum specific growth rates in the presence of sulfide ($\mu_{\max}^{\text{inh}}$) determined in the batch culture were significantly lower than the continuous culture data at the corresponding total sulfide concentrations (Figure 30). In our previous studies, lower growth rates have been observed frequently in batch cultures as compared to the continuous data. Another contributing factor is that sulfide precipitates Fe in the batch experiment, so that Fe and other trace nutrients (not the carbon and energy source) may become the rate limiting substrate. Postgate (1984) reported that SRB growth and cell yield data in batch culture reported by others need to be interpreted cautiously because pH, sulfide concentration, and limiting substrate cannot be maintained at the same levels over many generations. If sulfide concentration changes as they grow, the pH shifts, or vice versa, another stress is imposed on the population which also requires more maintenance energy.

McCartny and Oleskiewicz (1991) reported that the 50 % sulfide inhibition concentration of SRB was 2.6 mmol/L (83.2 mg-S/L). Reis et al. (1991) reported that a SRB batch culture of the genus *Desulfovibrio* was directly inhibited by the H_2S produced and that complete inhibition was achieved at H_2S concentration of approximately 550 mg/L. Shimada (1987) reported that 100 mg/L of H_2S inhibited the growth of a mixed SRB population in batch culture and no SRB growth was observed at 500 mg/L of H_2S . As reported in Chapter 3, 50 % inhibition of lactate utilization

by a continuous culture of *D. desulfuricans* occurred at total sulfide concentration of approximately 500 mg/L. The experimental results presented herein reasonably agreed to the reported data (K_i is a concentration of sulfide which cause a decrease in growth rate or activity of 1/2 of the maximum value).

Mechanisms of Sulfide Inhibition

In non-competitive inhibition, the inhibitor binds at a site on the enzyme other than the substrate binding site, altering the conformation of the enzyme molecule so that reversible inactivation results (Lehninger, 1982). Sulfide product inhibition of *D. desulfuricans* was sufficiently described by non-competitive model in this study, in which sulfide affects only maximum specific growth rate. This evidence is indirectly supported by the fact that sulfide inhibition of *D. desulfuricans* activity was a reversible process (Chapter 3).

Sulfide toxicity is strongly dependent on pH, because molecular H_2S can pass through the cell membrane and react with the ions (Fe) of cytochrome and other essential iron-containing electron transport enzymes in the cell membrane (Postgate, 1984). Reis et al. (1991) reported the inhibitory effect of sulfide on the specific growth rate of *Desulfovibrio* and found that the H_2S form is the most inhibitory form of sulfide. It is hypothesized that if electron transport chains are blocked by FeS formation, electrons released from the oxidation of lactate may go to hydrogen ions (H^+) and generate molecular hydrogen (H_2). If electron transport blockage occurs, the stoichiometric ratios of SO_4^{2-} /lactate, CO_2 /lactate, acetate/lactate may change. However, the stoichiometric ratios for catabolic reactions in this study did not change at high levels of TS. Thus, the same amount of energy (ATP) was generated from

substrate-level-phosphorylation and electron transport system (i.e., reduction of sulfate to sulfide). However, lower cell yields were observed in this study, which may be attributed to an increase in maintenance energy and uncoupling growth from energy production. Minor changes in the anabolic products were not detected in this study because of sensitivity of analytical methods used in this study.

Effect of Wall Growth

Hill and Robinson (1975) reported that a possible wall growth in the continuous culture exerts significant effects on the biomass concentration and substrate utilization. Thus, the possible wall growth increases specific substrate utilization rate as a result of the decrease in biomass concentration. To evaluate the effect of wall growth on experimental results obtained in this study, the concentration of cell attached to the possible surfaces in the reactor system during an experimental period (about two weeks) was measured. The total numbers of attached cells were measured using a epifluorescence technique at the end of experiment and found to be 2.4×10^4 cells/cm², which is approximately 10 % of an average total suspended cell concentration for the reactor with surface area of 613 cm². Thus, the effect of wall growth is insignificant and the experimental results are reliable.

Implications in Industrial Systems

The kinetics of *D. desulfuricans* growth on lactate as a function of sulfide concentration are presented in Figure 33 based on experimentally determined rate coefficients (Chapters 2 and 3). These results suggest that under high sulfide

concentrations such as in the petroleum formation (0 - 1000 mg-H₂S/L (Subcasky, 1991)), sulfide inhibition kinetics should be considered in biological sulfide production models. Although other nutritional and physical conditions are suitable for SRB growth, SRB growth is strongly inhibited by high sulfide concentrations. Furthermore, if SRB activity is monitored as change in total SRB cell count, it may underestimate H₂S production under inhibitory levels of sulfide concentration because the specific H₂S production rate increased at higher sulfide levels even though bacterial growth rate and biomass production rate decreased with sulfide inhibition.

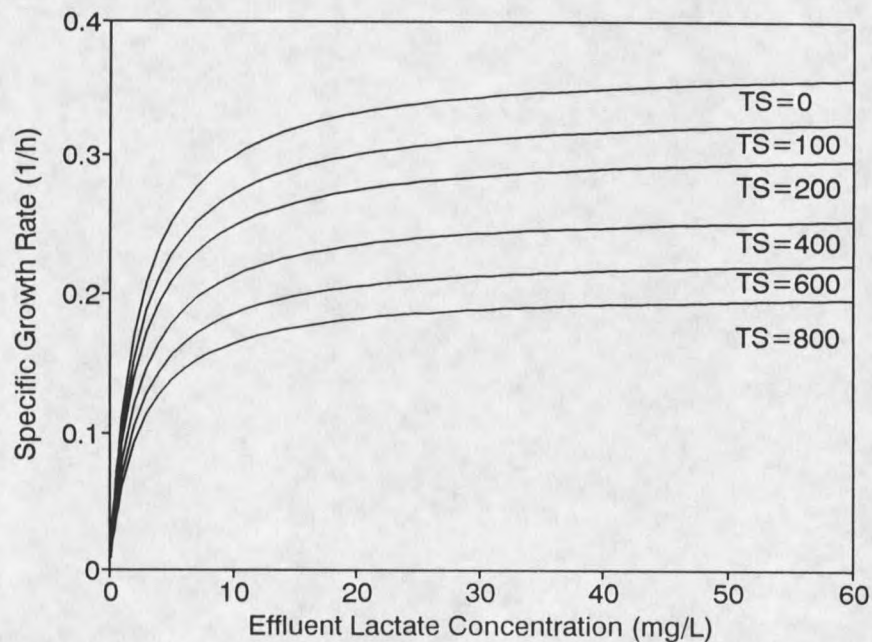


Figure 33. Results of model simulation. Inhibition coefficient (K_i) was determined from experimental data (e.g., Fig. 29): $K_i = 978$ mg-S/L, $\mu_{\max} = 0.37$ h⁻¹, $K_{\text{Lac}} = 2.35$ mg/L (Chapters 2 and 3).

Conclusions

Based on the results of this research, it can be concluded that:

1. Inhibition of activity and growth of *D. desulfuricans* by sulfide must be determined separately.
2. Cell synthesis (anabolic reaction) of *D. desulfuricans* was more sensitive to high sulfide concentrations than catabolic reaction (energy producing reaction).
3. A non-competitive inhibition model adequately described sulfide product inhibition kinetics. Inhibition coefficients for specific growth rate (K_i) were found to be 251 and 978 mg-S/L in batch and continuous culture experiments, respectively.
4. Stoichiometry for catabolic reaction did not change at high sulfide levels, which indicates that the metabolic pathway of *D. desulfuricans* did not shift in the presence of high sulfide concentration.

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CHAPTER 5

RATE AND STOICHIOMETRY OF MICROBIAL SULFATE
REDUCTION IN SUSPENDED AND BIOFILM CULTURESIntroduction

The accumulation and activity of sulfate reducing bacteria (SRB) are of critical concern in many industrial water systems. SRB cause numerous problems in petroleum production including contamination of petroleum with H_2S , injection well plugging, and corrosion. Microbial sulfate reduction in wastewater treatment systems is also a universal maintenance problem because of noxious odors, health hazards, and corrosion of facilities. The majority of SRB activity in these systems is associated with biofilms, that is, microbial cells embedded in an extracellular polymer matrix attached to a surface. Dewar (1986) reported that sessile SRB are responsible for significant industrial corrosion and water quality deterioration problems. Rosnes et al. (1990) reported that in sandstone cores, the majority of SRB activity was located in the biofilm covering the surface of the sandgrains. Electron micrographs also revealed bacterial growth on mineral surfaces and a extensive production of extracellular material, which eventually plugged the pores.

Biofilm accumulation is a complex phenomenon resulting from several processes occurring in parallel and in series. The rate and extent of these processes are influenced by numerous physical, chemical, and biological factors. Existing biofilm

accumulation models are based on stoichiometric and kinetic data derived from planktonic bacteria. However, experimental evidence of altered metabolism in biofilm cells suggests that attachment on surfaces can affect the activity and growth of cells. Many independent investigations have reported a wide variety of changes which may occur in cells after attachment on surfaces (Jannasch and Pritchard, 1972; Bright and Fletcher, 1983; Bakke et al., 1984; Doran and Bailey, 1986; Jeffr  y and Paul, 1986; Keen and Prosser, 1987; Fukui and Takii, 1990). Although it is generally assumed that cells are at an "advantage" when attached to a surface compared to being in suspension, the experimental evidence shown in the literature is inconclusive because of ambiguous definition of activity and ill-defined experimental conditions. Van Loosdrecht et al. (1990), in an extensive literature review of the influence of interfaces on microbial activity, stated that there was no conclusive evidence that adhesion directly influenced bacterial metabolism, in the sense that the bacteria undergo a structural change due to the adhesion. Observed differences between attached and free cells could, depending on conditions, all be attributed to an indirect mechanism, i.e., a mechanism by which the surroundings of cells are modified due to the presence of surfaces but not the cell itself. Over a wide range of organisms and experimental systems, cellular processes (e.g., growth) and activities (e.g., substrate assimilation, nutrient catabolism, respiration) have variously been found to increase, decrease, or remain unaffected by cell adsorption to a solid surface.

A quantitative understanding of the rate and extent of extracellular polymeric substance (EPS) formation in SRB biofilm systems appears of fundamental importance for adhesion of cells to surfaces, mass transfer resistance, formation plugging (Lappan and Fogler, 1990), and reduction of biocide efficacy (Ruseska et al., 1982; Dewar,

1986). Such an understanding is presently lacking. The rate and extent of EPS formation in SRB biofilms have not been measured.

This research was conducted to elucidate differences of growth and activity of *Desulfovibrio desulfuricans* growing in suspension and in biofilms. Comparisons of growth and activities between planktonic and biofilm (sessile) cells were performed based on following parameters: (1) specific cellular growth rate, (2) stoichiometry of sulfate reduction (e.g., SO_4^{2-} /Lactate, Acetate/Lactate, and CO_2 /Lactate), (3) cell and EPS yields on lactate, and (4) specific lactate utilization rate. The effects of temperature on zero-order volumetric substrate uptake rate constant (k_{of}) in the fully penetrated biofilm were also investigated.

This research bridges the gap between activity and growth of SRB's in suspended culture as compared to the biofilm state. Results provide fundamental information to develop a predictive model for SRB biofilm activity and accumulation in industrial systems. Furthermore, this information provides fundamental reactor design information for biological sulfate removal from sulfate-rich industrial water.

Background

Stoichiometric and Kinetic Models for SRB Biofilm

A process analysis approach is essential to account for the removal of nutrient from the bulk liquid due to cellular reproduction, EPS formation, and maintenance. Accumulation of biofilm is the net result of attachment of biomass (cellular + EPS), detachment of biomass from the biofilm, and biomass growth in the biofilm. In this model, the removal of substrate by suspended cells is assumed negligible in

comparison to substrate consumption by attached biomass because of the relatively small quantity of suspended cells in the reactor at a high dilution rate ($D \gg \mu_{\max}$). For systems with low suspended cell concentrations, the attachment rate of suspended cells is negligible (Gunawan, 1991).

Biofilm Cellular Carbon. A mass balance equation for the accumulation of cellular carbon in the biofilm can be written as follows:

$$A \frac{dx_b}{dt} = R_{xb} A - R_{dx} A \quad (26)$$

where, A = biofilm surface area (L^2), x_b = biofilm cellular carbon areal density ($M_x L^{-2}$), R_{xb} = cellular carbon reproduction rate ($M_x L^{-2} t^{-1}$), R_{dx} = cellular carbon detachment rate from the biofilm ($M_x L^{-2} t^{-1}$), and t = time (t). Biofilm specific cellular growth rate, μ_b , and biofilm specific cellular detachment rate, q_{dx} , are defined as follows:

$$R_{xb} = \mu_b x_b \quad (27)$$

$$R_{dx} = q_{dx} x_b \quad (28)$$

where μ_b = biofilm specific cellular growth rate (t^{-1}), q_{dx} = biofilm specific cellular detachment rate (t^{-1}). The biofilm cellular carbon mass balance can be rewritten as follows:

$$\frac{dx_b}{dt} = \mu_b x_b - q_{dx} x_b \quad (29)$$

At steady state, μ_b becomes equal to q_{dx} .

Biofilm EPS Carbon. A mass balance for the accumulation of EPS carbon in the

biofilm can be written as follows:

$$A \frac{dp_b}{dt} = R_{pb} A - R_{dp} A \quad (30)$$

where, p_b = biofilm EPS carbon areal density ($M_p L^{-2}$), R_{pb} = EPS carbon formation rate in the biofilm ($M_p L^{-2} t^{-1}$), R_{dp} = EPS carbon detachment rate from the biofilm ($M_p L^{-2} t^{-1}$). Defining biofilm EPS formation rate coefficients, k_b and k_b^* as described by Luedekeing and Piret (1959):

$$R_{pb} = k_b \mu_b x_b + k_b^* x_b \quad (31)$$

where, k_b = growth-associated EPS formation rate coefficient ($M_p M_x^{-1}$) and k_b^* = nongrowth-associated EPS formation rate coefficient ($M_p M_x^{-1} t^{-1}$). The biofilm EPS carbon mass balance can be rewritten as follows:

$$A \frac{dp_b}{dt} = (k_b \mu_b x_b + k_b^* x_b) A - R_{dp} A \quad (32)$$

Liquid Cellular Carbon. A mass balance equation on cellular carbon in the liquid phase of the RotoTorque™ reactor can be written as follows:

$$V \frac{dx}{dt} = F (x_i - x) + R_{dx} A + \mu x V \quad (33)$$

where, F = volumetric flow rate ($L^3 t^{-1}$), V = reactor volume (L^3), x_i = influent suspended cellular carbon concentration ($M_x L^{-3}$), x = suspended cellular carbon concentration ($M_x L^{-3}$), and μ = specific cellular growth rate (t^{-1}). For a sterile feed, $x_i = 0$ and the RotoTorque™ reactor was operated at high dilution rates ($D \gg \mu_{max}$) so that cellular growth in the liquid phase is essentially negligible. Then, Eq.(33) becomes

$$\frac{dx}{dt} = -Dx + \frac{q_{dx} x_b A}{V} \quad (34)$$

where, D = dilution rate (t^{-1}). At steady state, the cellular production in the biofilm can be determined by measuring the cellular concentration in the bulk liquid because R_{xb} is equal to R_{dx} .

Liquid EPS Carbon. A mass balance equation on EPS carbon in the liquid phase of the RotoTorque™ reactor can be written as follows:

$$V \frac{dp}{dt} = F (p_i - p) + R_{dp} A + r_p x V \quad (35)$$

where, p = suspended EPS carbon concentration ($M_p L^{-3}$), p_i = influent suspended EPS carbon concentration ($M_p L^{-3}$), r_p = specific EPS carbon formation rate ($M_p M_x^{-1} t^{-1}$). For a sterile feed, $p_i = 0$ and since the RotoTorque™ reactor was operated at $D \gg \mu_{max}$, the mass of cellular carbon in the liquid phase was small. Therefore, EPS carbon formation in the liquid phase is essentially negligible. Then, Eq.(35) becomes

$$\frac{dp}{dt} = -Dp + \frac{R_{dp} A}{V} \quad (36)$$

At steady state, the EPS carbon production in the biofilm can be determined by measuring the EPS carbon concentration in the bulk liquid because R_{pb} is equal to R_{dp} .

Substrate in the Liquid Phase. Substrate is utilized by microorganisms for three fundamental processes: (1) cellular reproduction, (2) EPS formation, and (3) maintenance. A mass balance equation on lactate in the liquid phase can be written as follows:

$$V \frac{dS}{dt} = F (S_i - S) - \frac{\mu_b x_b A}{Y_{xb/s}} - \frac{k_b \mu_b x_b A + k_b^* x_b A}{Y_{pb/s}} - m x_b A \quad (37)$$

where, S = substrate concentration ($M_s L^{-3}$), S_i = influent substrate concentration ($M_s L^{-3}$), $Y_{xb/s}$ = biofilm cellular carbon yield coefficient ($M_x M_s^{-1}$), $Y_{pb/s}$ = biofilm EPS carbon yield coefficient ($M_p M_s^{-1}$), m = maintenance coefficient ($M_s M_x^{-1} t^{-1}$).

Biofilm Kinetics. At steady state, the rate of overall process (cellular reproduction, EPS formation, and maintenance) in the biofilm is equal to surface flux (r_a) (Lamotta, 1976 b; Harremoes, 1978) and Eq.(37) can be written as follows:

$$D (S_i - S) = r_a \frac{A}{V} \quad (38)$$

The surface flux (r_a) is determined by the kinetics of internal microbial processes for a fully penetrated biofilm:

$$r_a = k_{of} L_f \quad (39)$$

In the case of a partially penetrated biofilm, the surface flux is determined by the rate of substrate transport to the biofilm:

$$r_a = k_{of} L_f^a = \sqrt{2 D_e k_{of} S} \quad (40)$$

where, L_f = biofilm thickness (L), L_f^a = active biofilm thickness (L), k_{of} = zero-order volumetric substrate uptake rate constant ($M_s L^{-3} t^{-1}$), D_e = diffusion coefficient of substrate in the biofilm ($L^2 t^{-1}$). For a condition of negligible liquid phase diffusional resistance, the substrate concentration at the surface of the biofilm is equal to the bulk liquid concentration (S).

The zero-order volumetric substrate uptake rate constant is expressed as

follows:

$$k_{of} = \left(\frac{\mu_b^{\max}}{Y_{xbfs}} + \frac{\mu_b^{\max}}{Y_{pbfs}} \frac{k_b + k_b^*}{Y_{pbfs}} + m \right) X_f \quad (41)$$

where, $\mu_b^{\max}$ = maximum biofilm cellular specific growth rate (t^{-1}) and X_f = biofilm volumetric density ($M_x L^{-3}$). Thus, k_{of} is dependent on temperature and the effect of temperature on k_{of} can be expressed as the Arrhenius equation:

$$k_{of} = A_f \exp \left(- \frac{E_a}{R T} \right) \quad (42)$$

where, A_f = Arrhenius frequency factor (t^{-1}), E_a = activation energy ($M L_2 t^{-2} mol^{-1}$), R = universal gas constant ($M L^2 t^{-2} mol^{-1} T^{-1}$), and T = temperature (T).

Measurements of x , x_b , p , p_b , and S were performed during biofilm development to determine rate and stoichiometry in the experimental system.

Experimental Materials and Methods

Experimental System

RotoTorque Reactor. Experiments were conducted in a polycarbonate RotoTorqueTM reactor system consisting of two concentric cylinders; a stationary outer cylinder and a rotating inner cylinder (Figure 34). To minimize gas transfer through the reactor, heavy wall butyl rubber tubing (Cole-Parmer, Masterflex neoprene tubing) was used for transfer of all fluids. The slow continuous nitrogen purge of the reactor maintained anaerobic conditions. Traces of oxygen in the nitrogen feed gas were removed by a reducing column containing copper wire maintained at 400°C. Twelve

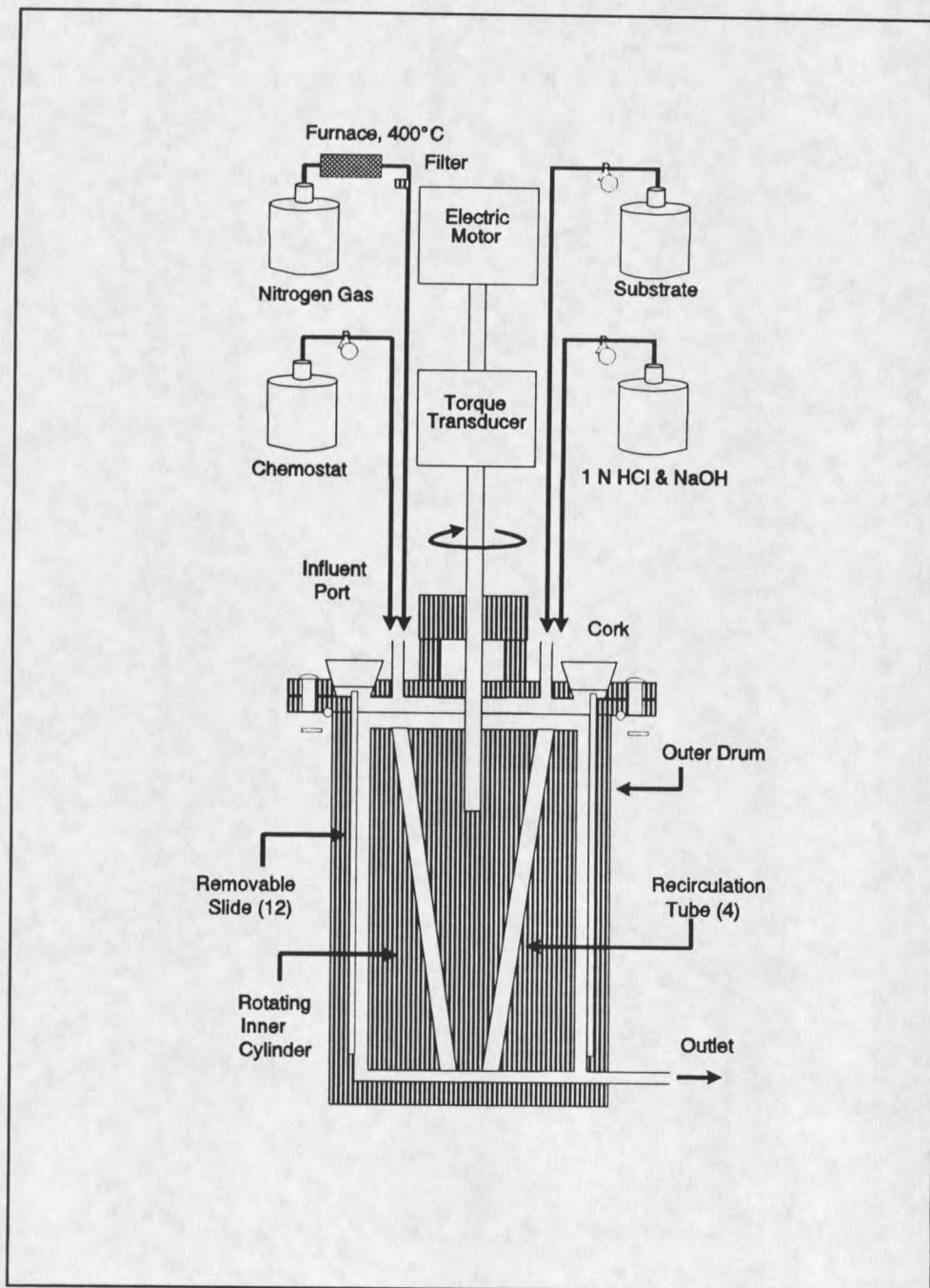


Figure 34. Diagram of RotoTorque experimental system.

removable polycarbonate slides installed on the inside wall of the outer cylinder were used to sample the biofilm. The reactor liquid phase was completely mixed by virtue of draft tubes bored through the inside cylinder. The inside cylinder rotated at approximately at 200 rpm (105 cm s^{-1}). At this speed, effect of the external mass transfer resistance on substrate utilization rate is insignificant (Lamotta, 1976 a; Trulear and Characklis, 1980). The pH and temperature were maintained at 7.0 and 35°C using automatic pH controller (Cole-Parmer) with sterile 1 N NaOH and HCl solutions and a temperature controlled water bath, respectively. The RotoTorqueTM reactor provided a wetted surface area to volume ratio, $A/V = 250 \text{ m}^{-1}$.

Medium and Biofilm Growth Conditions. *Desulfovibrio desulfuricans* (ATCC 5575) was grown in Postgate medium G (Postgate, 1984), containing 1.24 g/L Na-lactate (50 % solution) and 1.0 g/L Na_2SO_4 . The pH was adjusted to 7.0 with NaOH. RotoTorqueTM experiments were started by feeding microorganisms from a steady state chemostat culture of *D. desulfuricans* (chemostat flow rate = 120 mL h^{-1}) and fresh medium (flow rate = 120 mL h^{-1}) into the RotoTorqueTM reactor for a period of about 90 hours to provide initial attachment and colonization. The details of chemostat system and operation procedures were described in Chapter 2. The dilution rate in the RotoTorqueTM reactor for the initiation period was maintained at about 0.41 h^{-1} . After the initiation period, the chemostat effluent into the RotoTorqueTM reactor was stopped and the flow rate of nutrient medium was increased to obtain appropriate dilution rates. This time was defined as time zero.

Experimental Design. A summary of experiments, systems, parameter

variables, and the intent of the experiment is presented in Table 18. In particular, experiment A (chemostat) was performed under conditions which were previously shown not to promote EPS synthesis (Chapter 2). The results of the LPS assay technique as calibrated in experiment A were applied in experiment B to compare with alternative methods for distinguishing between cellular and EPS carbon. In experiment C, to ensure that biofilms are not substrate-limited, a relatively high substrate loading rate was applied and effluent lactate and sulfate concentrations were monitored with time. In experiment D, sulfate is a limiting substrate and various concentrations of sulfate were fed with lactate concentration of 2000 mg/L to determine the sulfate flux to biofilms.

Table 18. Experimental design

Experiment	System	Conditions	Purpose
A	Chemostat	$0.05 \leq D \leq 0.24$ Temp. = 35°C.	Determination of LPS/Cell-C
B	RotoTorque	$D = 2.2 \text{ h}^{-1}$ Temp. = 35°C.	Apply/Compare LPS method to EPI ^{a)}
C-1 ^{b)}	RotoTorque	$D = 0.7 \text{ h}^{-1}$	Determination of biofilm kinetics and Stoichiometry to compare with planktonic data
-2	RotoTorque	$D = 1.7 \text{ h}^{-1}$	
-3	RotoTorque	$D = 2.2 \text{ h}^{-1}$	
-4	RotoTorque	$D = 3.5 \text{ h}^{-1}$	
D-1	RotoTorque	Temp. = 15°C. ^{c)}	Determination of temperature effect on sulfate flux into biofilms
-2	RotoTorque	Temp. = 20°C. ^{c)}	
-3	RotoTorque	Temp. = 25°C. ^{c)}	
-4	RotoTorque	Temp. = 35°C. ^{d)}	
-5	RotoTorque	Temp. = 35°C. ^{c)}	

^{a)} Epifluorescence method

^{b)} Biofilm kinetic data were not obtained.

^{c)} $D = 3.25 \text{ h}^{-1}$.

^{d)} $D = 2.31 \text{ h}^{-1}$.

Analytical Methods

Chemical Analyses. Effluent samples were subjected to the following analyses: (1) lactate, (2) sulfate, (3) acetate, (4) total organic carbon (TOC), (5) soluble organic carbon (SOC), (6) Lipopolysaccharide (LPS), (7) total bacterial counts and cell size, and (8) total soluble sulfide. The samples for SOC, lactate, sulfate, and acetate analyses were prepared by centrifugation at 20,000 rpm, 4°C for 0.25 hours. Cellular carbon concentration was determined by both LPS and epifluorescence methods. EPS carbon concentration was determined by subtracting cellular carbon from total biomass carbon (effluent TOC- effluent SOC). The details of all but the LPS method are described in Chapter 2.

Lipopolysaccharide (LPS) Determination. The method for discriminating between cell and EPS biomass using an epifluorescence technique (Hobbie et al., 1977) requires conversion of cell volume to cell carbon (Chapter 2). This technique is tedious and subject to errors due to cell size fluctuations in bacterial populations (Watson et al., 1977). A lipopolysaccharide (LPS) assay has been reported which might be more reliable for this purpose (Watson et al., 1977). The LPS content is more closely correlated with bacterial cell mass than with cell number, and may also provide a more accurate measurement of cellular carbon (Watson et al., 1977). Thus, LPS assay was studied to quantify cellular carbon concentration.

To prevent contamination and false reaction, all glassware was rendered pyrogen free by baking at 200°C for at least four hours. Pyrogen-free distilled water

(Associates of Cape Cod, Woods Hole, Mass.) was used for all analysis. The disposable plastic pipette tips were found to be pyrogen-free. Disposable borosilicate glass assay tubes (10 mm $\times$ 75 mm) (Fisher Scientific) were used for dilutions and reaction tubes. Lipopolysaccharide of *D. desulfuricans* was determined spectrophotometrically. Limulus ameobocyte lysate for spectrophotometric method (Associates of Cape Cod, Inc., code #100-K5) was obtained freeze-dried in 5 ml/vials and was reconstituted immediately before use by the addition of 5 ml of pyrogen-free water and stored on ice during use. *Escherichia coli* endotoxin (Associates of Cape Cod, Inc., code # 800-1) was used as the standard. The *E. coli* endotoxin standard was reconstituted with pyrogen-free distilled water to a stock solution concentration of 100 ng/mL. Serial dilutions of the stock solution were made to obtain standard solution ranging 1 pg/mL to 20 pg/mL. A 0.8 mL sample of standard and 0.2 mL of lysate were mixed on a Vortex shaker and then incubated at 37°C in a circulated water bath for exactly 30 minutes. After the incubation period, the tubes were removed from the water bath, mixed thoroughly, and poured into microcuvettes (Markson Science, Inc., Phoenix, Arizona) having a 1-cm light path. The absorbance at 360 nm was immediately determined on a spectrophotometer (Varian DMS 90).

Biofilm Thickness. The biofilm thickness was determined with an optical microscope using the method of Bakke and Olsson (1986). A removable polycarbonate slide was withdrawn from the RotoTorque™ reactor and placed on the microscope stage. The 40 $\times$ objective (40 $\times$ 10 = 400 total magnification) was lowered until the biofilm surface was in focus and the fine adjustment dial setting of the stage micrometer was recorded. The objective lens was then lowered further until the inert

polycarbonate slide surface was in focus. The biofilm thickness was calculated by multiplying the difference in fine adjustment setting by a conversion factor of 1.33 to account for refractive index differences. Biofilm thickness was reported as the mean of 8 measurements along the slide. The standard error of each thickness measurement was calculated from the standard deviation of the eight measurements of biofilm on a single slide.

Biofilm Carbon Areal Density. The biofilm carbon areal density was determined as TOC concentration per biofilm surface area (g m^{-3}). The biofilm was rinsed twice with deionized water, scraped into 50 mL of deionized water from a known surface area, and homogenized using a tissue homogenizer (Tekmar, Cincinnati, OH). The homogenized biofilm sample was acidified using concentrated phosphoric acid and the total organic carbon concentration was measured by an ultraviolet-promoted persulfate oxidation followed by infrared detection (Dohrmann DC-80 total organic carbon analyzer).

Biofilm Specific Cellular Growth Rate. The biofilm specific cellular growth rate (μ_b) and biofilm specific cellular detachment rate (q_{dx}) were determined using unsteady state biofilm accumulation models. The mass balance equation on biofilm cellular carbon, x_b , (Eq.(29)) and on liquid cellular carbon, x , (Eq.(34)) can be expressed in difference forms as follows:

$$x_{b2} = x_{b1} + \left(\mu_b \frac{(x_{b1} + x_{b2})}{2} - q_{dx} \frac{(x_{b1} + x_{b2})}{2} \right) \delta t \quad (43)$$

Therefore,

$$\mu_b - q_{dx} = \frac{2}{(x_{bl} + x_{b2})} \frac{(x_{b2} - x_{bl})}{\delta t} \quad (44)$$

Similarly, Eq.(34) can be expressed as follows:

$$x_2 = x_1 + \left(-D \frac{(x_1 + x_2)}{2} + q_{dx} \frac{(x_{bl} + x_{b2})}{2} \frac{A}{V} \right) \delta t \quad (45)$$

Then,

$$q_{dx} = \frac{2}{(x_{bl} + x_{b2})} \frac{V}{A} \left(\frac{(x_2 - x_1)}{\delta t} + D \frac{(x_1 + x_2)}{2} \right) \quad (46)$$

All quantities on the right side of Eq.(46) are measurable. Once q_{dx} is determined, μ_b can be calculated using Eq.(44). The progression of biomass carbon concentration and cell number in the liquid and biofilm phases must be measured to determine μ_b and q_{dx} . The meaningful comparison of the activity and growth of *D. desulfuricans* cells in suspension and in biofilm can be made for only the condition of no substrate limitation. Therefore, relatively high substrate concentrations (lactate and sulfate) and dilution rates were used to obtain high substrate loading rates. The effluent lactate and sulfate concentrations were monitored to ensure that biofilms were not substrate-limited.

Results

Determination of EPS Carbon by LPS Test

To determine if the LPS assay could be used to estimate bacterial cellular carbon concentration, the cellular and EPS carbon concentrations were determined by the LPS and epifluorescence techniques (Hobbie et al., 1977) and compared. The

average LPS and cellular carbon contents of *D. desulfuricans* cells were determined by measuring LPS concentration, cellular carbon concentration, and cell numbers in chemostat cultures (Exp. A, Table 18). Previous chemostat data indicated that *D. desulfuricans* growing in the chemostat did not produce significant EPS (Chapters 2 and 3). Furthermore, a EPS staining (Allison and Sutherland, 1984) was conducted to visualize EPS production and proved that *D. desulfuricans* growing in the chemostat did not produce significant EPS. Thus, the bacterial cellular carbon was determined as the difference between the effluent TOC and SOC. The LPS and cellular carbon content and cell numbers are presented in Table 19. Cellular carbon and LPS contents per cell were fairly uniform throughout measurement. The average cell-C/LPS ratio remained relatively the same. The average concentration of LPS per cell was found to be 41.0 ± 2.31 fg/cell. The average cell-C/LPS ratio was found to be 2.50 ± 0.26 .

Cellular carbon and EPS carbon concentrations in the biofilm phase (Exp. B, Table 18) were determined by both LPS and epifluorescence techniques using the experimentally determined conversion factors (Chapter 2) including cell-C/LPS = 2.50 ± 0.26 as described above (Figures 35(a) and 35(b)). Calculations of cellular and EPS carbon concentrations in the LPS method were performed as follows:

$$\text{Cellular carbon concentration} = \text{LPS concentration} \times 2.50 \pm 0.26$$

$$\text{Biomass carbon concentration} = \text{Eff. TOC} - \text{Eff. SOC}$$

$$\text{EPS carbon concentration} = \text{Biomass carbon concentration} - \text{Cellular carbon concentration}$$

There was no significant difference between the LPS and epifluorescence methods in determining cellular and EPS carbon concentrations at the 99 % confidence level (Figures 35(a) and 35(b)).

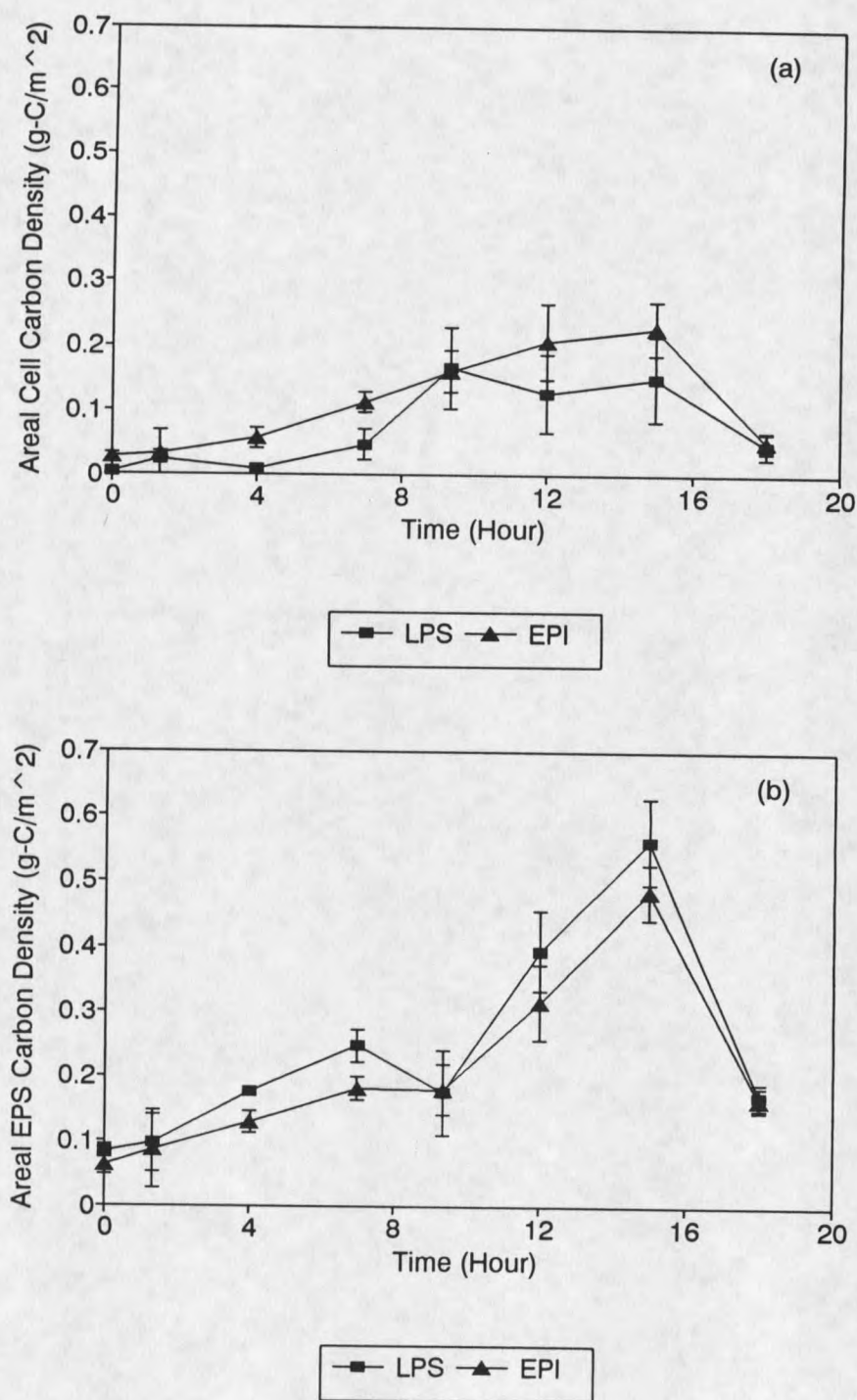


Figure 35. Typical progression curves of (a) biofilm cellular carbon and (b) biofilm EPS carbon areal densities with time determined by the LPS assay and the epifluorescence method. Influent lactate and sulfate concentrations were 600 mg/L and 700 mg/L, respectively. $D = 2.2 \text{ h}^{-1}$, $\text{pH} = 7.0$, $\text{Temp.} = 35^\circ\text{C}$. (Exp. C-3, Table 18).

Table 19. LPS and cellular concentrations in *D. desulfuricans* cells from chemostat cultures at various dilution rates (AVG $\pm$ SE).

D (h ⁻¹)	Cell Number (cells $\times 10^7$ /mL)	Cellular-C (fg ^a /cell)	LPS (fg/mL)	Cellular-C/LPS (fg/fg)
0.05	5.62 $\pm$ 0.22	72.6	45.7	1.58
0.08	4.72 $\pm$ 0.23	100.6	49.0	2.05
0.10	5.53 $\pm$ 0.63	95.5	32.9	2.90
0.12	5.29 $\pm$ 0.43	95.5	36.6	2.61
0.12	3.56 $\pm$ 0.49	130.6	34.1	3.83
0.12	4.12 $\pm$ 0.43	105.6	41.2	2.56
0.24	4.45 $\pm$ 0.22	94.4	47.5	2.00
AVG.		99.2 $\pm$ 6.0	41.0 $\pm$ 2.3	2.50 $\pm$ 0.26

^a fg = femtogram = 10^{-15} gram

Biofilm Growth

Typical time progressions of the average biofilm thickness and sulfide production in experiment C (Table 18) are given in Figure 36. An exponential increase in the biofilm thickness was observed through the first 23.5 hours, after which biofilm sloughing event occurred. Thereafter, the biofilm thickness fluctuated and reached a steady state thickness of about 40 μ m. Sulfide concentration in liquid phase increased exponentially with increasing biofilm thickness and reached a steady state sulfide concentration of about 80 mg/L after about 30 hours. The thickness of monopopulation *D. desulfuricans* biofilms in this study rarely exceeded a thickness of 70 μ m, even though a relatively high lactate carbon loading rate of 6.6 g C m⁻² h⁻¹

($D = 3.5 \text{ h}^{-1}$)(Exp. C-4, Table.18) was applied.

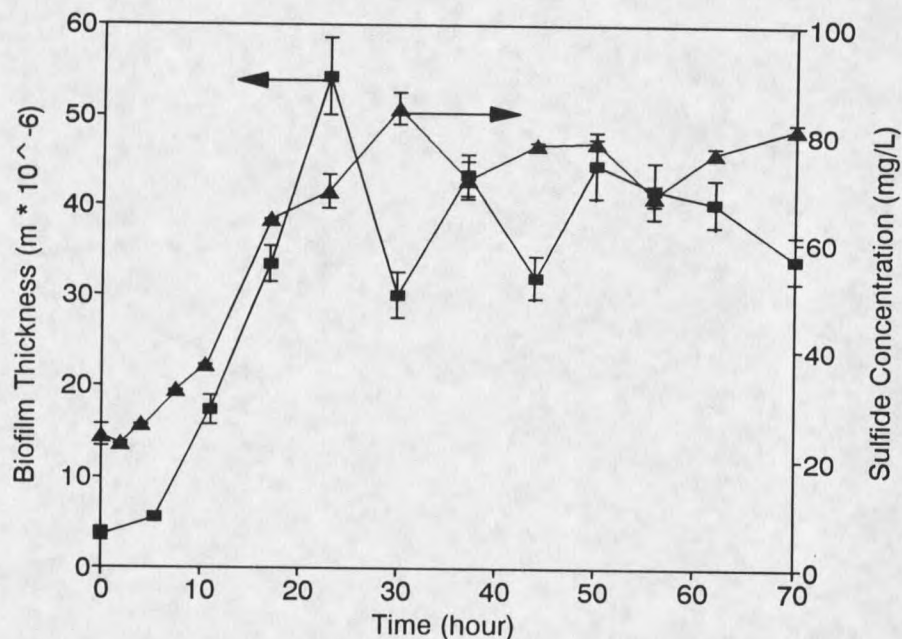


Figure 36. Typical biofilm growth and sulfide production. Influent lactate and sulfate concentrations were 600 and 700 mg/L, respectively. Dilution rate (D) = 1.7 h^{-1} , Temp. = 35°C , pH = 7.0. (Exp. C-2, Table 18).

Cellular Reproduction and EPS Formation

Cellular reproduction and EPS formation were determined in a RotoTorque™ reactor at various dilution rates (Exp. C, Table 18). Biofilm cellular and EPS carbon areal densities in the biofilm phase increased with time due to the processes of cellular reproduction and EPS formation (Figure 37). Cellular and EPS carbon areal densities approached steady state values after 30 hours. The EPS carbon content in biofilm biomass carbon was about 70 % at steady state.

The biofilm carbon areal density was measured at various dilution rates. The biofilm carbon areal density increased in proportion to the biofilm thickness (Figure 38).

The average biofilm density was determined to be approximately 15.4 kg-C/m³.

Biofilm Specific Cellular Growth and Detachment Rates

Biofilm specific cellular growth and detachment rates were determined from unsteady state biofilm experiments (Exp. C, Table 18) using mass balance equations (Eqs. (44) and (46)) (Figures 39(a) and 39(b)). Biofilm specific cellular growth rate was approximately the maximum specific growth rate for suspended *D. desulfuricans* ($\mu_{\max} = 0.37 \text{ h}^{-1}$) determined in a chemostat experiment at the initial biofilm growth phase (Chapter 2), thereafter decreased and approached a steady state value of about 0.1 h^{-1} after about 30 hours in three experiments (Figure 39(a)). Biofilm specific cellular detachment rate (q_{dx}) was approximately 0.2 h^{-1} at the initial biofilm growth phase, thereafter decreased similarly to cellular growth rate (μ_b) and approached a steady state value of about 0.1 h^{-1} (Figure 39(b)). After about 30 hours, the biofilm specific cellular growth rates were essentially the same as the detachment rates. This evidence indicated that the biofilm accumulation reached steady state. The biofilm specific cellular and detachment rates were determined at three different dilution rates. The three independent experimental results were very consistent.

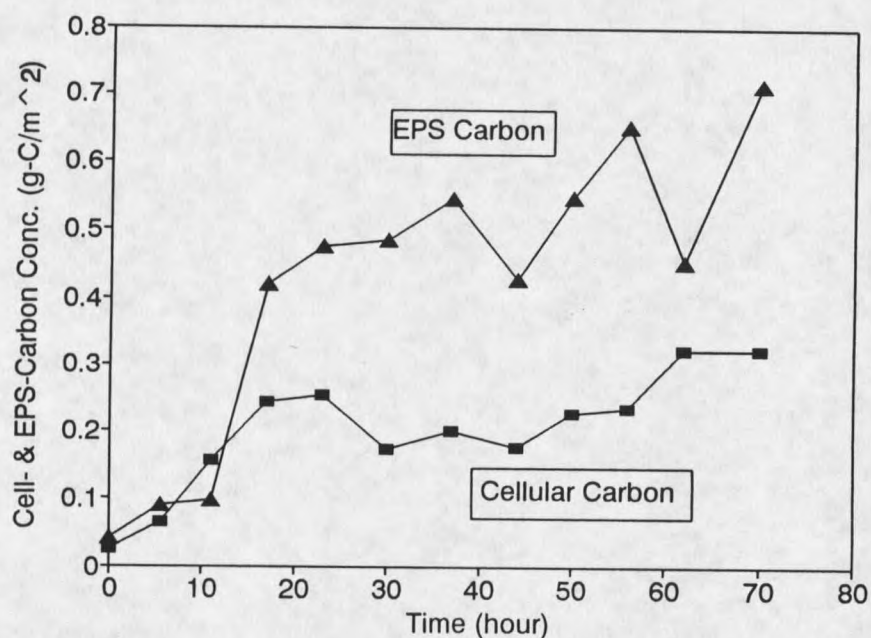


Figure 37. Accumulation of biofilm cellular carbon and EPS carbon at a dilution rate (D) of 1.7 h^{-1} . Influent lactate and sulfate concentrations were 600 mg/L and 700 mg/L , respectively. $\text{pH} = 7.0$, $\text{Temp.} = 35^\circ\text{C}$. (Exp.C, Table 18).

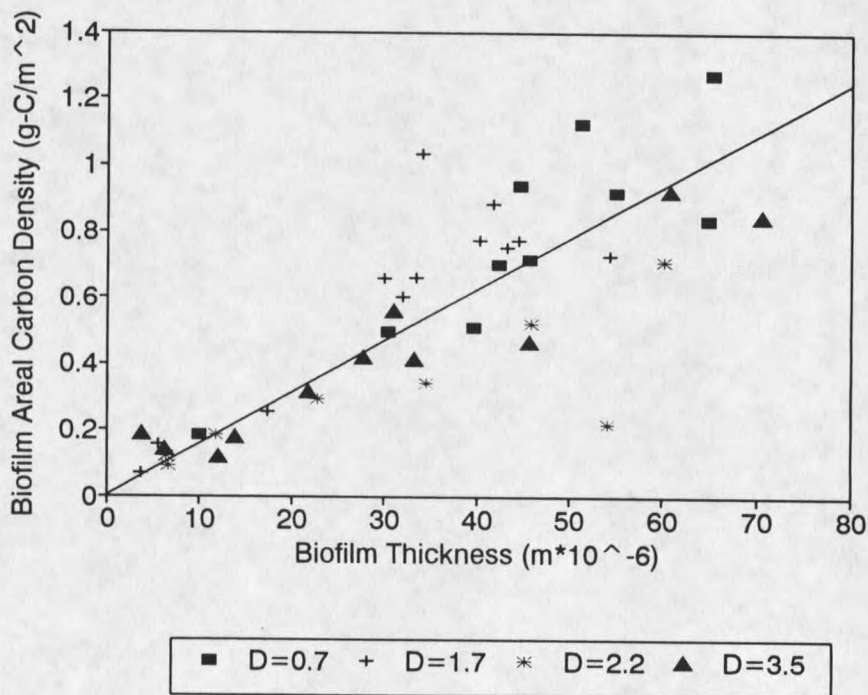
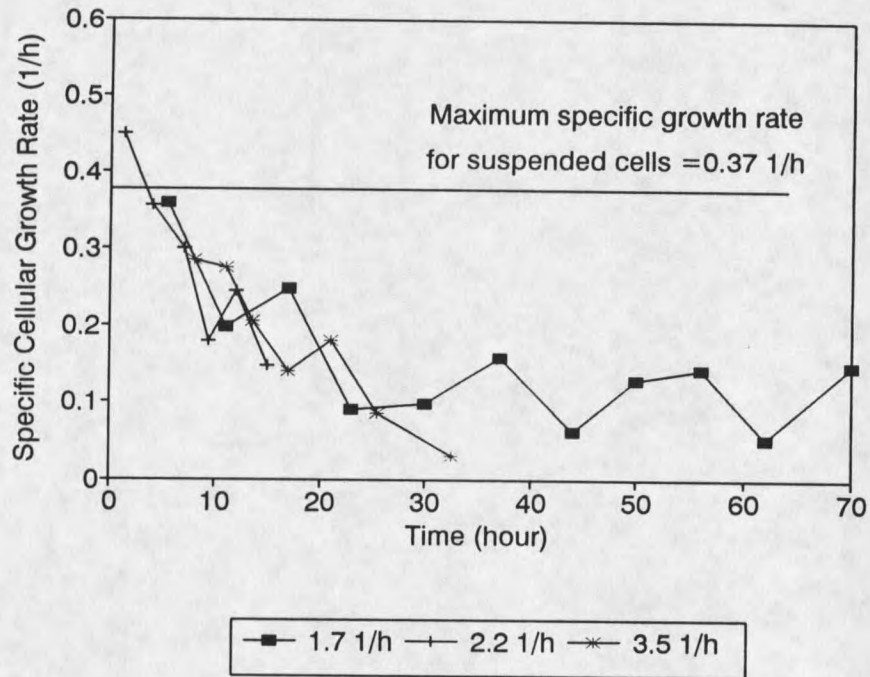
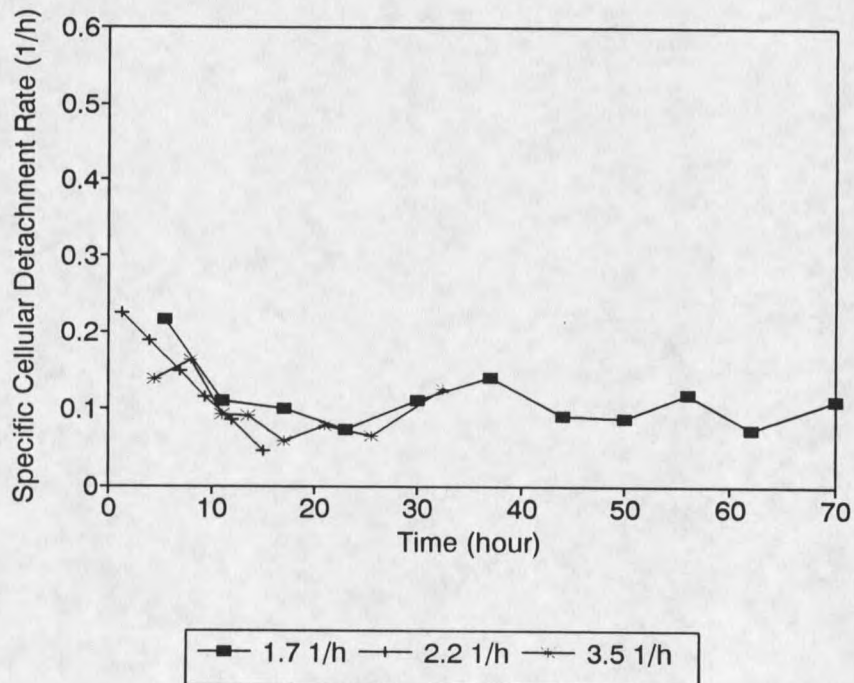


Figure 38. Change in biofilm areal carbon density with increasing biofilm thickness. The biofilm areal carbon density was a linear function of the biofilm thickness ($r^2 = 0.67$). $\text{pH} = 7.0$, $\text{Temp.} = 35^\circ\text{C}$. (Exp. C, Table 18).



(a)



(b)

Figure 39. Change in (a) biofilm specific cellular growth rate (μ_b) and (b) biofilm specific detachment rate (q_{dx}) during a biofilm growth experiment. μ_b at steady state was essentially equal to q_{dx} . pH = 7.0, Temp. = 35°C (Exp. C, Table 18).

EPS Formation Rate Coefficients

The biofilm growth- and nongrowth-associated EPS formation rate coefficients, k_b and k_b^* , were evaluated by linearizing Eq.(31) as follows:

$$\frac{R_{pb}}{x_b} = k_b \mu_b + k_b^* \quad (47)$$

Figure 40 is a plot of R_{pb}/x_b vs. μ_b . This figure suggests that the rate constants k_b and k_b^* are independent of the dilution rate. The growth-associated EPS formation rate coefficient (k_b) of 1.46 ± 0.28 g EPS-C (g cell-C)⁻¹ and nongrowth-associated EPS formation rate coefficient (k_b^*) of 0.00 ± 0.11 g EPS-C (g cell-C)⁻¹ h⁻¹ were determined.

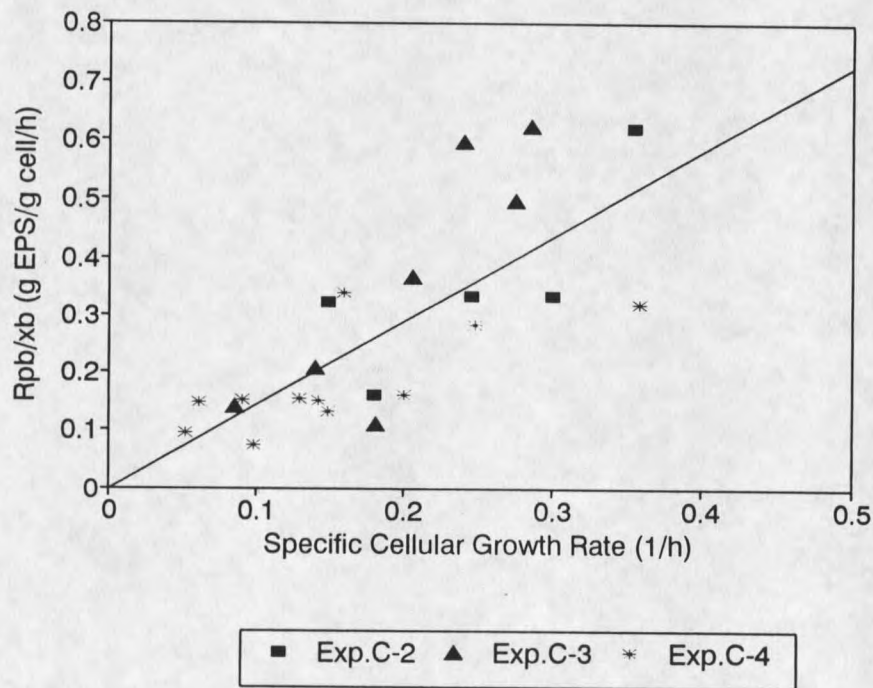


Figure 40. Determination of biofilm growth-associated EPS formation rate coefficient (k_b) and nongrowth-associated EPS formation rate coefficient (k_b^*). The rate constants k_b and k_b^* are independent on the dilution rate.

Specific Substrate Utilization Rate

Equation (37) predicts a linear relationship between the steady state biofilm specific substrate utilization rate (q_s) and the biofilm specific cellular growth rate (μ_b):

$$\frac{V}{A} \frac{D (S_i - S)}{x_b} = \mu_b \left(\frac{1}{Y_{xb/s}} + \frac{k_b}{Y_{pb/s}} \right) + \left(\frac{k_b^*}{Y_{pb/s}} + m \right) \quad (48)$$

Biofilm data were compared to chemostat data in Figure 41. A linear regression was not appropriate due to changes in cell metabolism (e.g., μ_b and $Y_{xb/s}$) over the course of the study. Biofilm specific substrate utilization rates (q_s) exceeded those from the chemostat at low biofilm specific cellular growth rate (μ_b) determined at pseudo steady

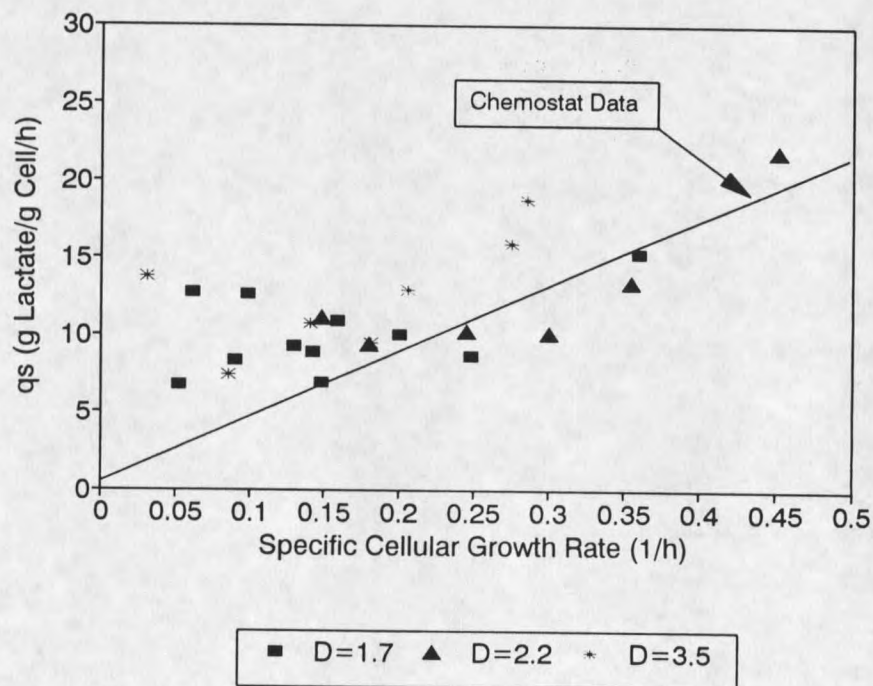


Figure 41. Relationship between specific cellular growth rate and specific lactate utilization rate (q_s) in chemostat and biofilm experiments (Exp. D, Table 18).

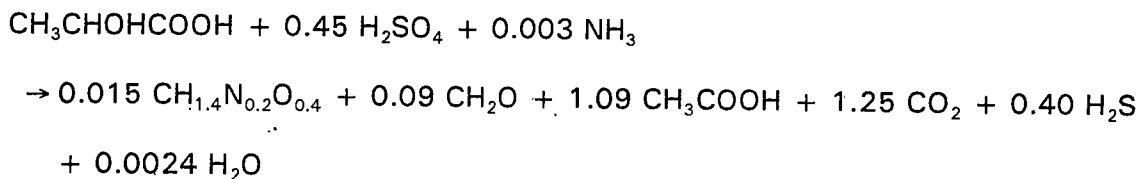
state (thicker biofilms), whereas it was essentially the same as the chemostat data at higher μ_b determined at unsteady state (thin biofilms).

Stoichiometry

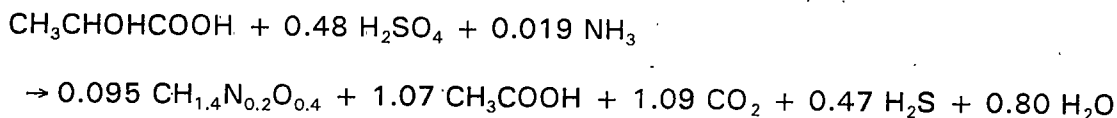
Stoichiometry of microbial sulfate reduction by the biofilm was determined and compared to that in the chemostat (Table 20). Empirical formulas $\text{CH}_{1.4}\text{N}_{0.2}\text{O}_{0.4}$ and CH_2O were used for bacterial cells and EPS, respectively (Characklis, 1990). All stoichiometric coefficients except for H_2O and NH_3 were determined based on experimental data. Stoichiometric coefficients for NH_3 were estimated from bacterial cell production. Extensive EPS production was observed in the biofilm system. The stoichiometric ratios of $\text{SO}_4^{2-}/\text{Lactate}$, $\text{Acetate}/\text{Lactate}$, and $\text{CO}_2/\text{Lactate}$ were essentially the same as those of suspended cells.

Table 20. Stoichiometry of microbial sulfate reduction by biofilm and planktonic *D. desulfuricans*.

BIOFILM



PLANKTONIC^{a)}



^{a)} From Chapter 2.

Cellular and EPS Yield Coefficients

The biofilm cellular and EPS carbon yield coefficients, $Y_{xb/s}$ and $Y_{pb/s}$, were determined at steady state (Table 21). Since at steady state, the biofilm specific growth rate is the same as the biofilm specific detachment rate ($\mu_b = q_{dx}$) as demonstrated in Figures 39(a) and 39(b), cellular carbon and EPS carbon concentrations in liquid phase and lactate consumption were measured at steady state. One sixth of cellular carbon yield (0.002 ± 0.0001 (g cell-C)(g lactate)⁻¹) was observed in the biofilm as compared to planktonic cells (0.013 ± 0.003 (g cell-C)(g lactate)⁻¹), while EPS carbon yield coefficient of 0.012 ± 0.004 (g EPS-C)(g lactate)⁻¹ was determined in the biofilm.

Table 21. Biofilm cellular carbon and EPS carbon yield coefficients at steady state.

	Cellular Carbon Yield, $Y_{xb/s}$ (g cell-C)(g lactate) ⁻¹	EPS carbon Yield, $Y_{pb/s}$ (g EPS-C)(g lactate) ⁻¹
Biofilm	0.002 ± 0.0001	0.012 ± 0.004
Planktonic ^{a)}	0.013 ± 0.003	NS ^{b)}

^{a)} From Chapter 3.

^{b)} Not significant.

Biofilm Kinetics

The sulfate flux (r_s) at different sulfate concentrations was measured at various temperatures (Exp. D, Table 18) (Figure 42). The experiments were conducted with a

lactate feed concentration of 2000 mg/L to ensure that lactate did not limit the sulfate reduction rate. The biofilm thicknesses and biomass areal carbon densities were measured at the beginning and the end of each experiment and the average biofilm thickness was used to calculate the zero-order volumetric substrate uptake rate constants (k_{of}) to minimize the effect of biofilm growth during the experiment, which normally lasted 7 to 9 hours (Table 22). The sulfate flux increased for each biofilm until the sulfate concentration exceeded a certain value (Figure 42). Beyond these values (e.g., 55 mg/L at 35°C) the biofilms were fully penetrated with sulfate. The zero-order volumetric substrate uptake rate constant (k_{of}) was relatively constant in the region 25°C-35°C, but dramatically decreased below 25°C (Figure 43). The activation energy for k_{of} in this study was 78 kJ/mol below 25°C.

Table 22. The sulfate flux (r_a) into *D. desulfuricans* biofilms and zero order volumetric rate constant (k_{of}) at various temperatures.

Temp. (°C)	r_a (g m ⁻² h ⁻¹)	L_f (μm)	k_{of} (g m ⁻³ h ⁻¹)
35	2.45	46.6 ± 11.5	5.25 × 10 ⁴
35	2.12	52.3 ± 8.9	4.05 × 10 ⁴
25	2.24	53.1 ± 1.3	4.21 × 10 ⁴
20	1.44	52.2 ± 4.8	2.76 × 10 ⁴
15	0.78	45.1 ± 0.4	1.72 × 10 ⁴

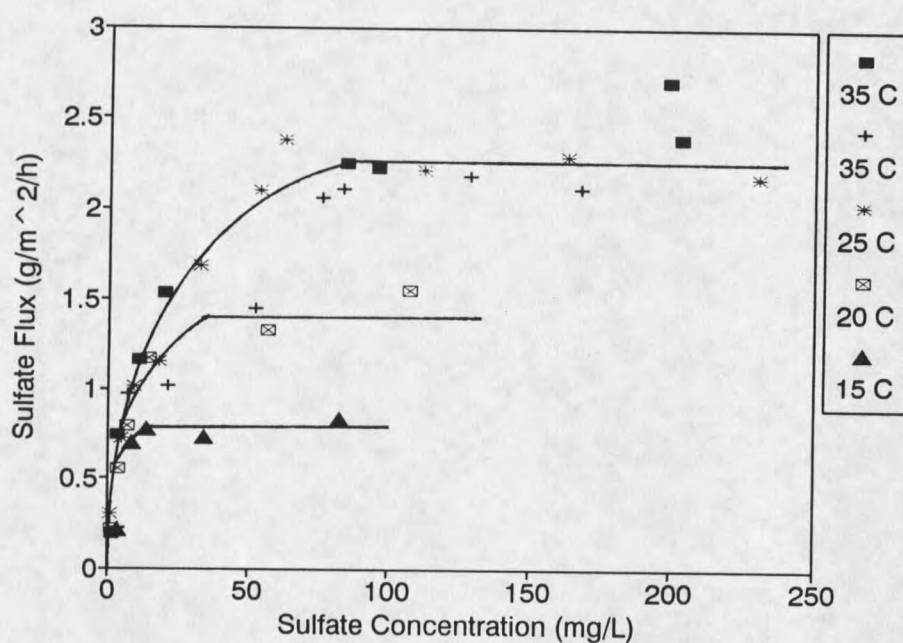


Figure 42. Sulfate flux (r_s) at various concentrations of sulfate and at various temperatures (15, 20, 25, and 35°C). The given biofilm thicknesses are average thickness.

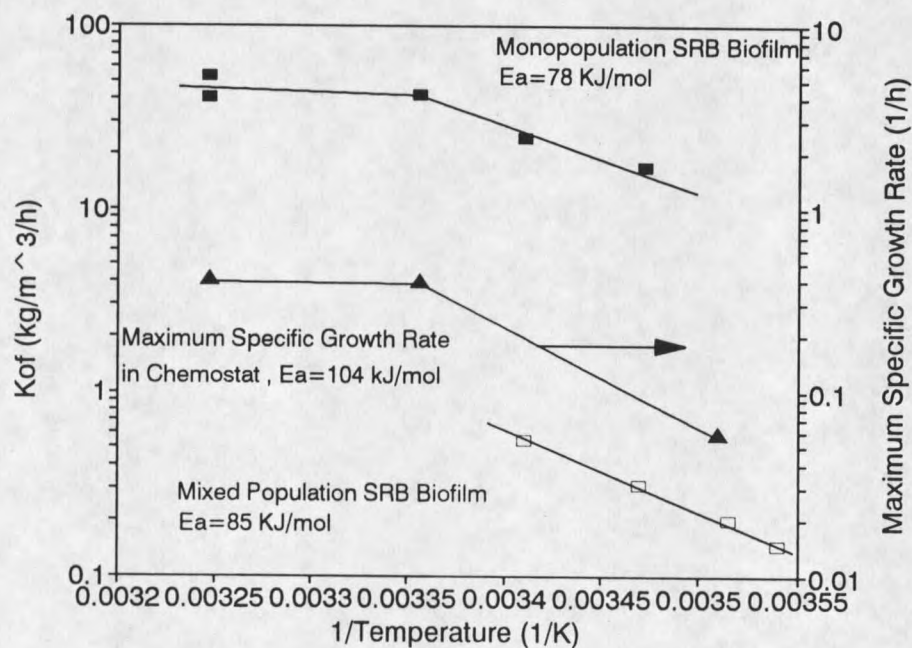


Figure 43. Temperature dependence of zero-order volumetric substrate uptake rate constant (k_{of}). k_{of} was relatively constant from 25°C–35°C, but dramatically decreased below 25°C. The activity energy for k_{of} in this study was 78 kJ/mol below 25°C.

Discussion

Effectiveness Factor

It is critical to investigate whether or not the biofilms studied were substrate diffusion limited. The literature suggested that liquid phase diffusional resistance became negligible for rotational speeds greater than 175 rpm (93.5 cm s^{-1}) since surface flux (r_a) reaches maximum values (Lamotta, 1976 a; Trulear and Characklis, 1980). However, this does not indicate that there is no liquid phase diffusional resistance in these systems. The effect of substrate transport on the biofilm activity can be evaluated by calculating effectiveness factor (η) including the effect of liquid phase diffusional resistance for each RotoTorqueTM experiment. The effectiveness factor is a ratio of the actual flux to the maximum steady state flux that would occur if the entire biofilm were fully penetrated at the bulk concentration of S and is defined as follows:

$$\eta = \frac{\text{Actual Flux}}{\text{Maximum Steady State Flux}} = \frac{\frac{D (S_i - S) V}{A}}{k_{of}^{Lac} L_f} \quad (49)$$

The effectiveness factor was determined based on lactate flux because lactate was a limiting substrate in experiment C (Table 18). The measurements of the actual lactate flux and the maximum steady state lactate flux were determined independently from different experiments. The zero-order volumetric lactate uptake rate constant (k_{of}^{Lac}) was determined by multiplying k_{of} for sulfate (Table 22), which was determined in

experiment D, and the stoichiometric ratio of lactate to sulfate (lactate/sulfate = 2.2)(Table 20). The actual lactate flux was determined using the measured lactate concentrations in the influent and the bulk liquid in experiment C. Figure 44 is a plot of the progression of effectiveness factor for *D. desulfuricans* biofilm at three different lactate loading rates. The effectiveness factor is always greater than 0.85, indicating the biofilms were fully penetrated with lactate. The effectiveness factors are greater than 1.0 in the initial biofilm accumulation period for three individual experiments. The reasons for this are (1) the specific growth rates of biofilm cells were high in the initial biofilm accumulation period as compared to the steady state values (Figure 39(a)) and (2) thin biofilm thickness measurements were associated with relatively large standard errors.

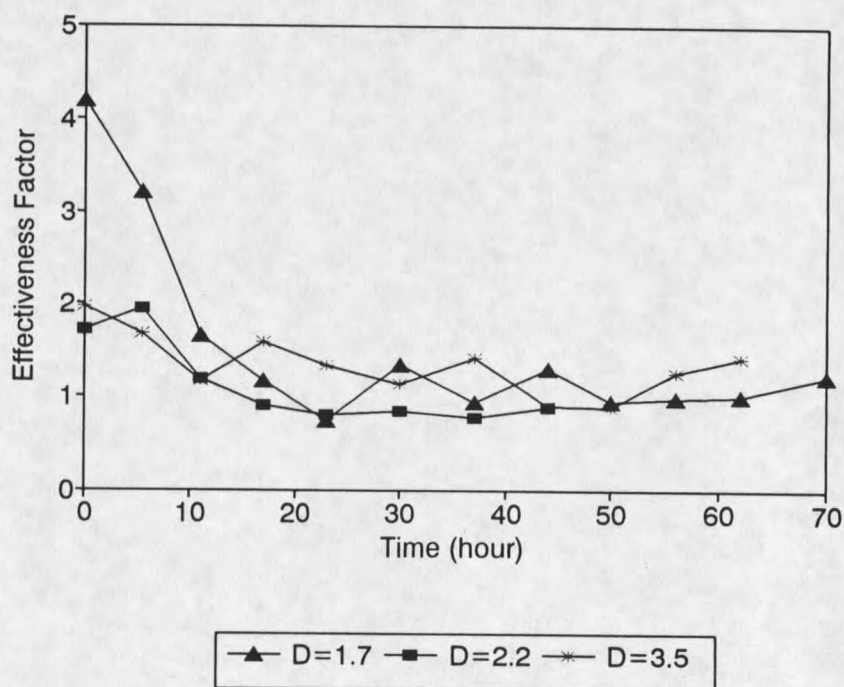


Figure 44. Progression of effectiveness factor for *D. desulfuricans* biofilm at three lactate loading rates (Exp. C, Table 18). The effectiveness factor is a ratio of the actual flux to the maximum steady state flux.

Kinetic Coefficients

Rate coefficients derived from the chemostat did not adequately describe the growth of *D. desulfuricans* cells in biofilms. Comparison in kinetic and stoichiometric coefficients between planktonic and biofilm *D. desulfuricans* cells is summarized in Table 23.

Table 23. Summary of comparison in stoichiometric ratios and kinetic coefficients between planktonic and biofilm *D. desulfuricans*.

Parameter	Unit	Planktonic Cells ^{a)}	Biofilm Cells
$\mu_{\max}, \mu_b^{\max}$	h^{-1}	0.37 ± 0.01	0.37 ± 0.04
$Y_{c/\text{Lac}}, Y_{xb/s}$	$(\text{g cell-C})(\text{g lactate})^{-1}$	0.012 ± 0.004	0.002 ± 0.0001
$Y_{p/\text{Lac}}, Y_{pb/s}$	$(\text{g EPS-C})(\text{g lactate})^{-1}$	NS ^{b)}	0.013 ± 0.003
k_b	$(\text{g EPS-C})(\text{g cell-C})^{-1}$	ND ^{c)}	1.46 ± 0.28
k_b^*	$(\text{g EPS-C})(\text{g cell-C})^{-1} \text{h}^{-1}$	ND	0.00 ± 0.11

Stoichiometric Ratios (M/M)

$\text{SO}_4^{2-}/\text{Lactate}$	0.48 ± 0.07	0.45 ± 0.03
Acetate/Lactate	$1.07^{\text{d)}$	1.09 ± 0.01
$\text{CO}_2/\text{Lactate}$	1.09 ± 0.10	1.25 ± 0.17

^{a)} From Chapters 2 and 3.

^{b)} Not significant.

^{c)} Not determined.

^{d)} This value was calculated from carbon mass balance.

In the initial biofilm growth phase, biofilm specific cellular growth rate (μ_b) was $0.37 \pm 0.04 \text{ h}^{-1}$ (Table 23), which is about the same as the maximum specific growth rate for planktonic cells ($\mu_{\max} = 0.37 \pm 0.01 \text{ h}^{-1}$) (Chapter 2) (Figure 39(a)). However, μ_b then decreased to about 0.1 h^{-1} and remained at that level, even though the biofilm was not limited by nutrient transport (e.g., lactate and sulfate). μ_b and q_{dx} were determined based on two independent parameters such as cell numbers and biomass carbon, and the results agreed reasonably. Thus, the accuracy of the measurement of μ_b and q_{dx} is reasonable. Since the activity and growth of *D. desulfuricans* were not limited by nutrient availability, other explanations must be sought.

A possible reason for the decrease in biofilm specific cellular rate in the biofilm may be due to a direct change in cell metabolism itself (e.g., an increase in maintenance energy requirement). Another explanation could be metabolic product inhibition by sulfide and acetate (Postgate, 1984; Reis et al., 1990; Reis et al., 1992). Reis et al., (1992) reported that the hydrogen sulfide and acetic acid produced are responsible for inhibition of SRB growth. The inhibition coefficient (K_i) for acetic acid was found to be 57 mg/L and a hydrogen sulfide concentration of 547 mg/L completely inhibited the growth of SRB. Okabe et al. (1992) reported that 50 % inhibition of the activity of *D. desulfuricans* in a chemostat was observed at a total sulfide concentration of approximately 500 mg-S/L at pH 7. However, typical concentration ranges of sulfide and acetate in the bulk liquid in this study were about 50-100 mg-S/L and 200-400 mg/L (25-50 mg H_2S /L and 1.0-2.0 mg (acetic acid)/L), respectively. Thus, effect of these metabolic products on the biofilm specific growth rate (μ_b) may be insignificant even when it is taken into account that the concentrations of these products in the biofilm are greater than the bulk concentrations

due to diffusive resistance to transport of products and change in pH (pH slightly decreases due to removal of alkalinity by sulfate reduction). Biofilm cells could be exposed to moderate sulfide concentrations (even not inhibitory levels) longer due to a longer cell residence time in the biofilm systems, which may be an alternative reason for the decrease in the biofilm specific growth rate. It is apparent that further researches must be conducted to elucidate effects of metabolic product concentrations and exposure time on the biofilm specific growth rate.

As a result of low growth rate and high detachment rate, the biofilm thickness decreased after a brief peak at about 20 hours (Figure 36). The steady state biofilm kinetics at $D = 1.7 \text{ h}^{-1}$ was achieved after about 30 hours, at which μ_b of 0.1 h^{-1} was the same as the detachment rate (q_{dx}) (Figures 39(a) and 39(b)). This evidence confirms the steady state biofilm growth ($\mu_b = q_{dx}$).

Comparable growth rates in suspended and biofilm systems are not always found in the literature. Keen and Prosser (1987) reported that the growth rate of *Nitrobacter* cells increased 25% by attaching on the surface. They reasoned that the increased growth rate was due, most likely, to formation of an extracellular slime layer, which helps to create a suitable microenvironment. Conversely, Fukui and Takii (1990) reported that free-living *D. desulfuricans* cells produced colonies more rapidly than particle-associated ones. This result led to the conclusion that the activity of free-living cells was much higher than that of aggregating ones. Bakke et al. (1984) reported that μ_b is not significantly different than specific cellular growth rate (μ) in planktonic state growth for *P. aeruginosa* and can be determined from liquid phase substrate concentration based on the Monod equation only when biofilm diffusional resistance is negligible. This implies that stoichiometry and kinetic coefficients for *P. aeruginosa*

derived in suspended continuous cultures can be used to describe steady-state biofilm processes. Stewart and Robertson (1988) reported that the comparison of the growth kinetics of immobilized and free-living cells revealed no further physiological differences between cells. They also concluded that a simple one-dimensional reaction-diffusion model, which incorporated data on the product inhibited growth kinetics of free-living cells collected in a product-limited chemostat, satisfactorily predicted product inhibition of immobilized cell growth. Jeffrey and Paul (1986) reported that the specific growth rate decreased when they attached onto the surfaces because of loss of cell surface area available for substrate uptake due to attachment. Ellwood et al. (1982) observed that, at least initially, the population increase of the attached cells on glass slides in a continuous culture of *Pseudomonas* sp. at a dilution rate of 0.06 h^{-1} was faster than the growth of suspended cells.

Activity (Specific Substrate Utilization Rate)

The maintenance coefficient (m)(specific lactate utilization rate at $\mu_b = 0$) in the biofilm was estimated using only steady state data in Figure 41 and found to be approximately $7.0 \text{ (g lactate)(g cell)}^{-1}\text{h}^{-1}$, which is very high as compared to the growth in suspension ($m = 0.45 \pm 1.08 \text{ (g lactate)(g cell)}^{-1}\text{h}^{-1}$) (Chapter 3). The higher maintenance coefficient in the biofilm is not attributed to sulfide product inhibition because sulfide concentration was far below the inhibitory level (the maintenance coefficient dramatically increased above 200 mg TS/L (Figure 32 in Chapter 4). The decreases in specific cellular growth rate and cellular yield support the considerably high maintenance coefficient. Growth yield is known to be dependent on growth rate (μ), maintenance coefficient (m), and the intrinsic growth yield corrected for

maintenance energy ($Y_{xb/s}^{Int}$) and is expressed:

$$\frac{1}{Y_{xb/s}} = \frac{m}{\mu_b} + \frac{1}{Y_{xb/s}^{Int}} \quad (50)$$

Thus, low growth yields ($Y_{xb/s}$) would result from low growth rate and high maintenance coefficient. q_s is expressed by rearranging Eq.(50):

$$\frac{\mu_b}{Y_{xb/s}} = \frac{\mu_b}{Y_{xb/s}^{Int}} + m = q_s \quad (51)$$

This equation indicates that the decrease in μ_b and the increase in q_s contributed to the decreased $Y_{xb/s}$. In Figure 41, the specific lactate utilization rates (q_s) in the biofilm were essentially the same as the planktonic data at higher μ_b (thin biofilm), whereas they were about 2-3 times higher than the planktonic data at lower μ_b (thicker biofilm). This result may suggest that uncoupling between growth and energy production occurred as indicated by an increase in maintenance energy requirement. Attachment to surfaces may lead to changes in cell physiology, which results in uncoupling between growth and energy production and the higher maintenance energy requirement.

Doran and Bailey (1986) reported that specific ethanol production rate by the immobilized *Saccharomyces cerevisiae* was 40-50 % greater than for the suspended cell performance. The immobilized cells consumed glucose twice as fast as the suspended cells, but their growth rate was reduced by 45 % and their yield of biomass was one-third of the value for the suspended cells. Fletcher (1986) compared the specific glucose utilization by free-living and attached *Pseudomonas fluorescens* and drew the conclusion that glucose assimilation by attached cells exceeded that by free-living cells

by a factor of between 2 and 5 or more, and respiration of glucose by attached cells was greater than that by free-living cells. Bright and Fletcher (1983) reported that the maximum specific substrate uptake rate ($V_{\max}$) for surface-associated and free-living bacteria were similar and the half-saturation coefficient of surface-associated cells was greater than free-living cells. According to Bright and Fletcher, there are two possible explanations: (1) change in environment of the cell (i.e., mass transport limitation), or (2) a real difference in assimilation process. Bakke et al. (1984) reported that there was no significant difference between specific glucose utilization rate for *P. aeruginosa* in biofilm and in suspended culture at 95 % confidence.

The importance of the biomass separation into two components, cell and EPS mass, must be emphasized for successful comparison of microbial activities (Bakke et al., 1984). The entire biofilm biomass should not be used in the determination of the specific substrate removal rate because biofilm contains a large amount of EPS. In this study, cellular carbon and EPS carbon concentrations were determined by two different methods, the LPS method and the epifluorescence method. The results agreed reasonably well, which indicates that these concentrations reported herein are reliable (Figures 35(a) and 35(b)). The LPS method could be a useful method to determine bacterial cellular mass in laboratory experiments.

Stoichiometry

Stoichiometry for anabolic reactions (cell synthesis) in the biofilm was different from in suspension (Table 23). A significantly greater amount of EPS was formed in biofilms, whereas no significant EPS production was observed in suspension (Chapters 2 and 3). The significant EPS production in the biofilm is not due to sulfide product

inhibition, because the significant EPS production was observed only above sulfide concentration of 400 mg-S/L (Figure 29 in Chapter 4). The rate and extent of EPS formation was dependent upon the cellular specific growth rate and on the quantity of *D. desulfuricans* present (Figure 40). However, the stoichiometric ratios for the catabolic reactions such as acetate/lactate, CO_2 /lactate, and SO_4^{2-} /lactate were essentially identical to those in suspension (Table 23). This result suggests that *D. desulfuricans* cells produce energy from the same metabolic pathway, but anabolism shifts from cellular synthesis to EPS synthesis. Doran and Bailey (1986) reported that the immobilized yeast stored a large quantity of reserve carbohydrates and contained more polysaccharide than did suspended cells. Their results indirectly support the change in anabolic reactions by attachment to surfaces.

Cellular and EPS Yield Coefficients

The cellular carbon yield coefficient, $Y_{\text{xb/s}}$, was low in biofilms compared to the $Y_{\text{c/Lac}}$ in chemostat cultures (Table 21). Van Loosdrecht et al. (1990) reported the growth of *Escherichia coli* in a glucose-peptone medium with and without glass beads. In the presence of glass surfaces, especially at low nutrient concentrations, a much lower cell yield was obtained. This result is comparable to the results reported by Jannasch and Pritchard (1972). Significantly high EPS yield was observed in biofilm systems. This result indicates that the substrate (lactate) is used for EPS production and possible maintenance energy. Thus this energy loss results in a low cellular yield.

Biofilms contain a high proportion of EPS, which is postulated to play an important role in maintaining the structural integrity of the biofilm matrix. The relative EPS content of the biofilm changed with biofilm development (Bryers, 1980). Because

biofilm production is the combined effect of cellular reproduction and EPS production, variations in EPS production affect the magnitude of cellular yield. The EPS carbon content of the steady state biofilm in this study was approximately 70 % of total biofilm biomass carbon. Characklis (1981) reported that EPS materials occupied up to 90 % of total biofilm volume in some biofilms.

Biofilm Kinetics (k_{of})

The specific lactate utilization rate (q_s) was determined using the obtained k_{of} values, biofilm thickness (L_f), and biofilm areal cellular carbon density (x_b) determined in experiment D (Table 18) to compare with the q_s values determined in experiment C (Table 18). The value of q_s in experiment D was in the range 5.8-10.0 (g lactate)/(g cell)⁻¹ h⁻¹, which is in reasonable agreement with the values of q_s obtained in experiment C. This result suggests that measurement of k_{of} is reliable.

The k_{of} values determined in this study were compared to those obtained from mixed population SRB biofilms by Nielsen (1987) (Figure 43). The k_{of} values determined in this study are higher than those determined by Nielsen by a factor of about 100. The reason for this is probably that the density of SRB in this study is higher than ones in his study. Nielsen reported that the densities of anaerobic mixed population biofilms were determined to be 10-15 kg C m⁻³ (Nielsen, 1987) and 30-40 kg C m⁻³ (Nielsen and Hvitved-Jacobsen, 1988). Thus, the biofilm density observed (15.4 kg-C m⁻³) in this study is comparable to his data. However, his biofilm carbon densities do not directly indicate SRB population density because of mixed population biofilms.

The k_{of} value was relatively constant in the temperature range 25-35°C, but dramatically decreased below 25°C (Figure 43). The activation energy was determined

to be 78 kJ mole^{-1} below 25°C , which is essentially the same as the one for the mixed population SRB biofilm reported by Nielsen (1987). The temperature dependence of sulfate reduction in the biofilm was compared to the one of maximum specific growth rate of *D. desulfuricans* in suspension, in which the activation energy of $\mu_{\max}$ was 104 kJ mole^{-1} (Chapter 2). The magnitude of temperature dependence of $\mu_{\max}$ in suspension is very similar to the one of k_{of} in the biofilm. The data suggest that the effect of temperature on microbial sulfate reduction in biofilms is not different for mixed population or monopopulation SRB biofilms. The temperature effect can be predicted in this temperature range by the data derived in suspension.

Biofilm Thickness

Aerobic biofilm reactions are often limited by the availability of oxygen, the exogenous electron acceptor. Therefore, the biofilm thickness may be determined by the availability of oxygen. For the SRB biofilms, however, the exogenous electron acceptor and donor usually do not determine the activity and the biofilm thickness because the concentrations of electron acceptor and donor can be controlled. The biofilm thickness observed in this study never exceeded $70 \mu\text{m}$ even though sulfate and lactate are not limiting. This is probably an ecological and physiological nature of monopopulation SRB biofilm or a result of metabolic products inhibition.

Conclusions

Based on the results of this research, it can be concluded that:

1. Maximum biofilm specific cellular growth rate ($\mu_b^{\max} = 0.37 \pm 0.04$) was

essentially the same as $\mu_{\max} = 0.37 \pm 0.01 \text{ h}^{-1}$ for planktonic cells.

2. The decreases in specific growth rate (μ_b) and cellular yield ($Y_{x/b/s}$) in the biofilm might be attributed to uncoupling between growth and energy production and the high maintenance energy requirement.
3. Stoichiometry for the catabolic reactions is the same for biofilm and planktonic cells.
4. Stoichiometry for cellular synthesis reactions is different in biofilm than in suspension.
5. Rate and stoichiometric data determined from planktonic cells must be used cautiously in developing a model to predict biofilm growth and activity.

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CHAPTER 6

SUMMARY

General Conclusions

In this research, the effects of temperature, limiting nutrients (phosphorous, nitrogen, and sulfate), and sulfide inhibition on rate and stoichiometry of microbial sulfate reduction by *Desulfovibrio desulfuricans* were investigated. In addition, the comparison of the activities and growth of *D. desulfuricans* in suspension and in biofilm was performed. The following major conclusions could be drawn from the preceding chapters:

1. Rate coefficients of microbial sulfate reduction ($\mu_{\max}$ and K_s) are dependent on temperature. However, stoichiometry is independent of temperature.
2. Steady state experimental results adequately predicted the transient response of *D. desulfuricans* activity and growth to temperature change.
3. The stoichiometric limiting C:P and C:N ratios for *D. desulfuricans* are in the range of 400:1-800:1 and 45:1-120:1, respectively.
4. The extent of extracellular polymeric substance (EPS) formation increased with increasing C:P and C:N ratios in the medium.
5. A non-competitive inhibition model adequately described sulfide product inhibition kinetics for *D. desulfuricans*.
6. The catabolic reactions were not inhibited by high sulfide concentrations,

whereas the anabolic reactions (cell synthesis) were strongly influenced by elevating sulfide concentration.

7. Uncoupling of catabolism and anabolism was observed at high sulfide concentrations, resulting in high activity despite low growth rate.
8. The maximum specific cellular growth rate in biofilms was essentially the same as in suspension.
9. In the biofilm, the cellular yield and specific cellular growth rate decreased due to the high maintenance energy and uncoupling growth from energy production.
10. The stoichiometry for the catabolic reactions was the same for biofilm and planktonic cells, whereas the stoichiometry for anabolic reactions was different in biofilms than in suspension.

Implications

Control Strategies based on Nutrient Requirement

As all SRB require a source of N, P, and S to support and maintain growth, reduction of one or all nutrients to below limiting levels may provide a means to control SRB activity. The laboratory experimental results for nutrient requirements indicate that the removal of sulfate from industrial water has the greatest potential to control SRB activity. However, phosphorous and nitrogen requirements for SRB growth were so low (below a few mg/L) that it is difficult to achieve such low levels by the existing biological and/or chemical treatments. In the petroleum reservoir, for example, formation waters contain sufficient quantities of measurable ammonium ions to support balanced growth on the available carbon. Ammonium ion levels of as high as

250 mg/L have been measured (Herbert et al., 1985). With the North Sea oil fields operated by Shell Expro, ammonium ions levels up to 87 mg/L were measured in formation waters (Herbert et al. 1985). The major contribution to the nutrient status in the oil reservoir is likely to be the formation water itself. Thus, removal of nitrogen from the injection water may not be a feasible means to control SRB activity in the oil field. In addition, scale and corrosion inhibitor chemicals ironically may enrich the system in C, N, and P.

Sulfate is sometimes absent in some formation water *prior to* introduction of seawater. Therefore, sulfate removal from injection water may have a potential to control SRB activity and growth in seawater injection oil secondary recovery plants. However, problems related to sulfate removal must be solved including corrosion of treatment facilities by sulfide and treatment of huge amounts of sulfide produced from sulfate reduction.

Summary

The experimental results suggest that microbial kinetic behavior in biofilms may differ significantly from the behavior in the bulk liquid. Thus, the kinetic data of planktonic cells must be used cautiously to predict cell behavior in biofilms. When the nutritional requirements of SRB and the effects of the physico-chemical factors on SRB growth and activity are understood and quantified, a more rational image of SRB behavior in environments such as oil reservoirs during waterflooding can be obtained. This approach will eventually lead to the development of a comprehensive model that will permit more accurate prediction of SRB behavior. It will also permit means of

controlling SRB growth and activity in industrial water systems where SRB cause many problems.

Recommendations for Further Research

Recommendations toward future research are outlined in the following paragraphs.

(1) In the biofilm, growth rate and cellular yields of *D. desulfuricans* were substantially below those in suspension. This evidence suggests that in SRB biofilm systems, sulfide product inhibition of biofilm SRB cells may occur as a result of diffusive transport limitation of sulfide and acetic acid. In addition to depressing the overall reaction rate and growth kinetic parameters, product inhibition could lead to increased rates of cell death, lysis, and degradation, and loss of viability. Therefore, it is necessary to investigate quantitative effects of metabolic products (sulfide and acetic acid) on SRB biofilm activity and growth.

(2) Kinetic and stoichiometric data reported herein were obtained from a pure population culture under well controlled laboratory experiments. Real environments are more complex and dynamic systems in which many interactions between SRB and other components (the substratum, other bacterial species, and metabolic products) are occurring. Thus, further investigations must be conducted with mixed populations including SRB obtained from representative industrial sites. Comparison with pure culture data will provide information critical to further model development and calibration.

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NOMENCLATURE

A	Reactor surface area (L^2)
A_f	Arrhenius frequency factor (t^{-1})
D	Dilution rate (t^{-1})
D_e	Effective diffusion coefficient in the biofilm ($M_s L^{-2}$)
E_a	Activation energy ($M L^2 t^{-2} mol^{-1}$)
F	Volumetric flow rate ($L^3 t^{-1}$)
i	Inhibitor concentration ($M_i L^{-3}$)
k_b	Growth-associated EPS formation rate coefficient ($M_p M_x$)
k_b^*	Nongrowth-associated EPS formation rate coefficient ($M_p M_x^{-1} t^{-1}$)
K_i	Inhibition coefficient ($M_s L^{-3}$)
k_{of}	Zero-order volumetric substrate uptake rate constant ($M_s L^{-3} t^{-1}$)
k_{of}^{Lac}	Zero-order volumetric lactate uptake rate constant ($M_s L^{-3} t^{-1}$)
K_{Ace}	Half-saturation coefficient for acetate ($M_s L^{-3}$)
K_{Lac}	Half-saturation coefficient for lactate ($M_s L^{-3}$)
K_{Pro}	Half-saturation coefficient for propionate ($M_s L^{-3}$)
K_s	Half-saturation coefficient ($M_s L^{-3}$)
K_{Sul}	Half-saturation coefficient for sulfate ($M_s L^{-3}$)
L_f	Biofilm thickness (L)
L_f^a	Active biofilm thickness (L)
m	Maintenance coefficient ($M_s M_x^{-1} t^{-1}$)
p	Suspended EPS carbon concentration ($M_p L^{-3}$)
P	Suspended EPS concentration ($M_p L^{-3}$)
p_b	Biofilm EPS carbon areal density ($M_p L^{-2}$)

P_i	Influent suspended EPS carbon concentration ($M_p \text{ L}^{-3}$)
P_i	Influent EPS concentration ($M_p \text{ L}^{-3}$)
q_{dx}	Biofilm specific cellular detachment rate (t^{-1})
q_s	Specific substrate utilization rate ($M_s M_x^{-1} t^{-1}$)
R	Universal gas constant ($M \text{ L}^2 t^{-2} \text{ mol}^{-1} T^{-1}$)
r_a	Surface flux ($M_s \text{ L}^{-2} t^{-1}$)
R_{dp}	EPS carbon detachment rate from the biofilm ($M_p \text{ L}^{-2} t^{-1}$)
R_{dx}	Cellular carbon detachment rate from the biofilm ($M_x \text{ L}^{-2} t^{-1}$)
R_p	Specific EPS formation rate ($M_p M_x^{-1} t^{-1}$)
r_p	Specific EPS carbon formation rate ($M_p M_x^{-1} t^{-1}$)
R_{pb}	EPS carbon formation rate in the biofilm ($M_p \text{ L}^{-2} t^{-1}$)
R_{xb}	Cellular carbon reproduction rate ($M_x \text{ L}^{-2} t^{-1}$)
S	Limiting substrate concentration ($M_s \text{ L}^{-3}$)
S_i	Influent limiting substrate concentration ($M_s \text{ L}^{-3}$)
SS	Suspended solids concentration ($M_{ss} \text{ L}^{-3}$)
t	Time (t)
V	Reactor volume (L^3)
V_{max}	Maximum specific substrate uptake rate ($M_s M_x^{-1} t^{-1}$)
x	Suspended cellular carbon concentration ($M_x \text{ L}^{-3}$)
X	Cell concentration ($M_x \text{ L}^{-3}$)
x_b	Biofilm cellular carbon areal density ($M_x \text{ L}^{-2}$)
x_i	Influent suspended cellular carbon concentration ($M_x \text{ L}^{-3}$)
X_i	Influent cell concentration ($M_x \text{ L}^{-3}$)
X_f	Biofilm volumetric density ($M_x \text{ L}^{-3}$)

Y	Yield coefficient ($M M_s^{-1}$)
Y_{ATP}^{max}	Cell yield on ATP ($M_x \text{ mol}^{-1}$)
Y_b	Biomass yield coefficient ($M_b M_s^{-1}$)
Y_c	Cell yield coefficient ($M_x M_s^{-1}$)
$Y_{c/Lac}$	Cell yield coefficient on lactate ($M_x M_s^{-1}$)
$Y_{c/Lac}^{Intr}$	Intrinsic cell yield coefficient on lactate ($M_x M_s^{-1}$)
$Y_{c/Pro}$	Cell yield coefficient on propionate ($M_c M_s^{-1}$)
$Y_{c/Sul}$	Cell yield on sulfate ($M_x M_s^{-1}$)
Y_o	Overall yield coefficient ($M_{ss} M_s^{-1}$)
$Y_{o/Ace}$	Overall yield coefficient on acetate ($M_{ss} M_s^{-1}$)
$Y_{o/Lac}$	Overall yield coefficient on lactate ($M_{ss} M_s^{-1}$)
$Y_{o/Sul}$	Overall yield coefficient on sulfate ($M_{ss} M_s^{-1}$)
$Y_{o/Pro}$	Yield coefficient on propionate ($M_{ss} M_s^{-1}$)
Y_p	EPS yield coefficient ($M_p M_s^{-1}$)
$Y_{pb/s}$	Biofilm EPS carbon yield coefficient ($M_p M_s^{-1}$)
$Y_{p/Lac}$	EPS yield coefficient on lactate ($M_p M_s^{-1}$)
$Y_{xb/s}$	Biofilm cellular carbon yield coefficient ($M_x M_s^{-1}$)
$Y_{xb/s}^{Intr}$	Intrinsic biomass yield coefficient ($M_x M_s^{-1}$)
μ	Specific cellular growth rate (t^{-1})
μ_b	Biofilm specific cellular growth rate (t^{-1})
μ_b^{max}	Maximum biofilm specific cellular growth rate (t^{-1})
μ_{max}	Maximum specific cellular growth rate (t^{-1})
μ_{max}^{inh}	Maximum specific growth rate in the presence of sulfide (t^{-1})

APPENDICES

APPENDIX A

Determination of Cellular Carbon Concentration
by Epifluorescence Method and Sampling Procedure

Estimation of Cellular Carbon Concentration

Cells were homogenized and preserved in 2 % formaldehyde solution at 4°C in a refrigerator. The cells were stained with acridine orange to allow direct counting on 0.22 μm polycarbonate membrane black filters (Poretics Corporation, Livermore, CA) by epifluorescence microscopy using an image analyzer. Average cell size and cell number were determined. Average cell volumes were estimated using the average cell size. Cells were represented as short cylinders with a half-sphere on each end, the half-sphere having a radius equal to half of the cell width. Average cell width was relatively constant ($w = 0.5 \mu\text{m}$) in this study.

To convert cell volume to cellular carbon, the following factors were used: wet specific gravity ($1.07 \times 10^{-6} \text{ g wet cell m}^{-3}$) (Doetsch and Cook, 1973), dry cell mass to wet cell mass ratio ($0.22 \text{ g dry cell [g wet cell]}^{-1}$) (Luria, 1960), and cellular carbon content ($0.465 \text{ g cellular carbon [g dry cell]}^{-1}$) (Postgate, 1984).

EPS carbon concentrations were calculated by subtracting cellular carbon concentration from total biomass carbon concentration (effluent TOC - effluent SOC).

Sampling Procedure

Samples for sulfide and sulfate analyses were taken directly from reactors using sterile syringes to minimize contamination of samples with oxygen. The sample liquid (about 25 mL of volume) was transferred directly from the syringe to 3 mL of 10 % ZnAc solution in glass scintillation vials to fix sulfide compounds. The mixture of

sample liquid and ZnAc solution was filtered with a 0.22 μm cellulose nitrate filter for sulfate analysis. Analyses for sulfide and sulfate were conducted immediately after sample preparation. Samples for the rest of analyses except for cell number and cell size were taken from reactor effluent, centrifuged, and preserved in a refrigerator until analyses. The details of sample preparation procedure were described in each Chapter.

APPENDIX B

Raw Data of Temperature Effect Experiments

Temperature Effects**Table 24.** Influent Analyses of a lactate-limiting chemostat at 12°C (mg/L).

D(h ⁻¹)	TOC	Lac.	SO ₄ ²⁻
0.015	75.84	190.2	321.8
	75.94	189.3	325.5
	76.50	-	325.5
0.020	Influent solution was the same as D=0.015 h ⁻¹ .		
0.027	84.26	207.8	256.4
	83.99	208.8	254.6
	83.68	-	252.7
0.032	Influent solution was the same as D=0.015 h ⁻¹ .		
0.034	Influent solution was the same as D=0.015 h ⁻¹ .		
0.046	Influent solution was the same as D=0.015 h ⁻¹ .		
0.052	Influent solution was the same as D=0.015 h ⁻¹ .		
0.057	Influent solution was the same as D=0.015 h ⁻¹ .		

Table 25. Effluent Analyses of a lactate-limiting chemostat at 12°C (mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.015 h⁻¹</u>					
50.00	48.60	1.54	230.9	24.2	117.2
50.09	48.19	1.59	234.6	24.4	133.5
49.64	48.15	-	230.9	-	-
<u>D=0.020 h⁻¹</u>					
50.74	49.55	1.39	292.7	-	-
51.22	49.58	1.46	290.9	-	-
50.88	49.66	-	294.6	-	-
<u>D=0.027 h⁻¹</u>					
59.61	56.10	2.59	172.7	-	-
58.61	55.89	2.73	163.6	-	-
59.37	54.95	-	163.6	-	-
<u>D=0.032 h⁻¹</u>					
50.75	46.66	2.22	232.7	27.5	133.2
50.15	46.67	2.37	234.6	27.7	147.0
50.60	46.85	-	238.2	-	-
<u>D=0.034 h⁻¹</u>					
53.69	51.69	3.74	238.2	19.5	-
53.59	51.76	3.72	240.0	19.8	-
53.29	51.40	-	240.0	-	-
<u>D=0.046 h⁻¹</u>					
51.50	49.57	22.46	232.7	20.6	-
50.43	49.58	22.41	234.6	20.7	-
50.63	49.13	-	236.4	-	-
<u>D=0.052 h⁻¹</u>					
54.79	54.16	24.39	252.7	23.6	88.4
56.30	54.79	23.90	256.4	23.7	92.2
56.27	54.22	-	254.6	-	-
<u>D=0.057 h⁻¹</u>					
61.93	61.30	94.88	270.9	-	92.2
62.27	61.69	95.00	272.7	-	92.2
62.80	61.43	-	274.6	-	-

Table 26. Microbial Analyses of a lactate-limiting chemostat at 12°C.

D (h ⁻¹)	Cell # (10 ⁷ /mL)	Size (μm ²)	Cell-C (mg/L)	EPS-C (mg/L)	SS (mg/L)
0.015	-	-	1.40	-	12.0
	-	-	1.49	-	20.5
	-	-	1.94	-	9.5
0.020	-	-	1.19	-	17.0
	-	-	1.64	-	18.5
	-	-	1.22	-	26.0
0.027	3.124	0.716	3.51	-	18.0
	4.028	0.733	2.72	-	25.5
	3.206	0.803	4.42	-	23.0
0.032	-	-	4.09	-	-
	-	-	3.48	-	-
	-	-	3.75	-	-
0.034	-	-	2.00	-	10.0
	-	-	1.83	-	18.5
	-	-	1.89	-	20.0
0.046	-	-	1.93	-	6.0
	-	-	0.85	-	5.0
	-	-	1.50	-	16.0
0.052	-	-	0.63	-	34.0
	-	-	1.51	-	29.5
	-	-	2.05	-	57.5
0.057	-	-	0.63	-	-
	-	-	0.58	-	-
	-	-	1.37	-	-

Table 27. Influent Analyses of a lactate-limiting chemostat at 25°C (Run 1) (mg/L).

D(h ⁻¹)	TOC	Lac.	SO ₄ ²⁻
0.050	70.62	173.7	307.3
	70.19	168.0	309.1
	70.92	-	298.2
0.100	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.130	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.180	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.210	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.253	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.310	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.360	75.26	187.3	316.4
	75.77	185.4	318.2
	74.16	-	321.8
0.375	Influent solution was the same as D = 0.360 h ⁻¹ .		
0.400	Influent solution was the same as D = 0.360 h ⁻¹ .		

Table 28. Effluent Analyses of a lactate-limiting chemostat at 25°C (Run 1) (mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.050 h⁻¹</u>					
46.30	43.98	0.20	200.0	11.3	95.4
45.26	43.71	2.61	198.2	11.4	98.9
45.35	43.37	-	196.4	-	-
<u>D=0.100 h⁻¹</u>					
48.65	45.83	0.20	223.6	12.2	67.4
47.54	45.37	2.37	210.9	12.3	67.4
48.05	46.07	-	216.4	12.2	-
<u>D=0.130 h⁻¹</u>					
48.17	45.91	0.05	229.1	17.0	96.3
48.20	45.72	1.78	218.2	17.1	85.8
47.03	44.27	-	220.0	17.2	-
48.57	44.54	-	-	-	-
<u>D=0.180 h⁻¹</u>					
47.27	45.32	0.27	220.0	30.7	73.5
47.81	45.19	3.41	214.6	30.7	78.8
47.43	45.06	-	218.2	30.7	-
<u>D=0.210 h⁻¹</u>					
47.48	45.22	0.63	227.3	31.9	87.5
47.03	44.31	1.90	227.3	32.1	98.0
47.61	45.46	-	230.9	32.1	-
47.96	45.26	-	-	-	-
<u>D=0.253 h⁻¹</u>					
48.66	45.77	1.98	218.2	30.3	-
48.16	45.69	2.44	220.0	30.6	-
48.07	46.18	-	223.6	30.5	-
<u>D=0.310 h⁻¹</u>					
47.11	44.60	1.46	225.5	30.4	90.3
47.43	43.87	1.46	232.7	30.9	104.2
46.46	45.08	-	220.0	30.9	-
46.98	44.58	-	-	-	-
<u>D=0.360 h⁻¹</u>					
47.17	43.03	2.73	223.6	21.7	82.3
48.36	43.62	2.24	229.1	22.0	77.0
47.56	43.17	-	225.5	22.1	-
<u>D=0.375 h⁻¹</u>					
-	-	73.41	-	-	-
-	-	67.93	-	-	-
<u>D=0.400 h⁻¹</u>					
60.41	59.89	97.07	272.7	17.4	61.3
61.15	60.30	95.61	276.4	17.6	70.0
60.55	60.03	-	287.3	17.6	-

Table 29. Microbial Analyses of a lactate-limiting chemostat at 25°C (Run 1).

D (h ⁻¹)	Cell # (10 ⁷ /mL)	Size (μm ²)	Cell-C (mg/L)	EPS-C (mg/L)	SS (mg/L)
0.050	1.480	0.795	2.32	-	22.0
	1.151	0.712	1.55	-	-
	0.921	0.738	1.98	-	-
0.100	0.493	0.962	2.82	-	4.50
	0.411	0.725	2.17	-	12.5
	0.576	0.894	1.98	-	16.0
0.130	1.480	0.842	2.26	-	38.0
	0.987	0.748	2.48	-	88.0
	0.740	1.179	2.76	-	-
	-	-	4.03	-	-
0.180	1.233	0.769	1.95	-	50.5
	1.233	0.787	2.62	-	27.0
	0.904	0.597	2.37	-	50.0
0.210	1.151	1.090	2.26	-	22.0
	0.904	0.744	2.72	-	20.0
	1.315	0.732	2.15	-	23.3
	-	-	2.70	-	-
0.253	-	-	2.89	-	29.0
	-	-	2.39	-	22.0
	-	-	1.89	-	15.3
0.310	0.904	0.680	2.51	-	16.3
	1.891	0.738	3.56	-	14.7
	2.220	0.681	1.38	-	-
			2.40	-	-
0.360	0.904	0.764	4.74	-	47.5
	0.411	0.711	4.39	-	37.5
	1.069	0.602	4.14	-	28.5
0.400	0.493	0.816	0.85	-	37.5
	0.329	1.033	0.65	-	89.0
	0.329	0.663	0.52	-	34.5

Table 30. Influent Analyses of a lactate-limiting chemostat at 25°C (Run 2) (mg/L).

D(h ⁻¹)	TOC	Lac.	SO ₄ ²⁻
0.050	74.45	188.1	289.1
	75.73	183.7	292.7
	73.57	-	294.6
0.120	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.133	81.06	213.7	310.9
	81.07	211.7	312.7
	81.30	-	310.9
0.180	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.267	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.330	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.346	Influent solution was the same as D = 0.050 h ⁻¹ .		
0.387	Influent solution was the same as D = 0.133 h ⁻¹ .		

Table 31. Effluent Analyses of a lactate-limiting chemostat at 25°C (Run 2) (mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.050 h⁻¹</u>					
49.89	47.99	1.20	212.7	10.1	123.0
49.97	47.98	1.12	212.7	10.0	105.7
49.86	48.00	-	214.6	-	107.6
<u>D=0.120 h⁻¹</u>					
47.61	45.49	3.15	210.9	33.3	121.1
48.44	45.83	2.88	212.7	33.4	123.0
47.93	46.04	-	214.6	-	123.0
<u>D=0.133 h⁻¹</u>					
52.06	50.39	2.88	212.7	-	145.4
52.37	50.57	2.71	210.9	-	128.8
51.97	50.34	-	214.6	-	167.4
<u>D=0.180 h⁻¹</u>					
49.35	47.37	16.85	220.0	24.3	94.4
49.55	47.38	17.05	218.2	24.6	90.4
49.50	47.40	-	218.2	-	-
<u>D=0.267 h⁻¹</u>					
51.56	48.74	21.29	205.5	32.2	116.4
52.08	48.45	21.15	207.3	33.5	117.3
51.94	48.80	-	209.1	-	105.9
<u>D=0.330 h⁻¹</u>					
56.82	55.74	41.10	214.6	-	94.5
56.62	55.44	40.49	212.7	-	90.1
57.48	55.58	-	216.4	-	-
<u>D=0.346 h⁻¹</u>					
52.34	52.27	45.49	230.9	-	96.9
52.64	52.63	46.31	227.3	-	93.4
52.66	52.23	-	229.1	-	92.5
<u>D=0.387 h⁻¹</u>					
69.66	68.28	123.17	265.5	-	56.4
69.99	68.23	124.15	267.3	-	63.9
69.53	68.43	-	267.3	-	70.0

Table 32. Microbial Analyses of a lactate-limiting chemostat at 25°C (Run 2).

D (h ⁻¹)	Cell # (10 ⁷ /mL)	Size (μm ²)	Cell-C (mg/L)	EPS-C (mg/L)	SS (mg/L)
0.050	-	-	1.84	-	-
	-	-	1.99	-	-
	-	-	1.86	-	-
0.120	-	-	2.12	-	-
	-	-	2.61	-	-
	-	-	1.89	-	-
0.133	-	-	1.67	-	-
	-	-	1.80	-	-
	-	-	1.67	-	-
0.180	-	-	1.98	-	-
	-	-	2.17	-	-
	-	-	2.10	-	-
0.267	-	-	2.82	-	-
	-	-	3.63	-	-
	-	-	3.14	-	-
0.333	-	-	1.08	-	-
	-	-	1.18	-	-
	-	-	1.90	-	-
0.346	-	-	0.07	-	-
	-	-	0.01	-	-
	-	-	0.43	-	-
0.387	-	-	1.38	-	-
	-	-	1.76	-	-
	-	-	1.10	-	-

Table 33. Influent Analyses of a lactate-limiting chemostat at 35°C (Run 1) (mg/L).

D(h ⁻¹)	TOC	Lac.	SO ₄ ²⁻
0.088	77.19	213.6	2500.0
	76.85	-	-
	77.55	-	-
0.170	77.52	200.0	2500.0
	77.82	199.8	2523.3
	78.29	200.5	2523.3
0.240	76.49	194.6	2360.5
	75.37	194.9	2337.2
	75.33	196.0	2430.2
0.320	78.00	202.9	2314.0
	76.79	203.9	2267.4
	75.57	206.7	2244.2
	78.99	204.9	-
0.450	76.64	197.0	2372.1
	75.71	198.7	2453.5
	76.29	197.4	2441.9
0.547	78.37	201.2	2325.6
	77.30	202.5	2418.6
	76.79	201.8	2511.6

Table 34. Effluent Analyses of a lactate-limiting chemostat at 35°C (Run 1) (mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.088 h⁻¹</u>					
48.83	45.77	1.72	2383.7	-	115.9
48.99	45.81	1.00	2395.4	-	135.4
48.97	45.88	1.00	2372.1	-	136.6
<u>D=0.170 h⁻¹</u>					
51.57	48.54	2.07	2500.0	-	107.2
51.53	48.15	4.48	2523.3	-	106.4
51.75	48.39	3.44	2523.3	-	117.5
<u>D=0.240 h⁻¹</u>					
51.62	46.71	2.00	2267.4	-	116.2
51.42	46.36	3.00	2267.4	-	101.6
51.05	46.61	3.40	2267.4	-	131.9

Table 34. Effluent Analyses of a lactate-limiting chemostat at 35°C (Run 1)(mg/L)
(Continued).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.320 h⁻¹</u>					
50.09	45.93	6.20	2430.2	-	125.8
49.19	46.23	10.33	2430.2	-	121.9
49.44	45.96	-	2441.9	-	117.5
<u>D=0.450 h⁻¹</u>					
49.99	45.94	4.10	2348.8	-	118.0
49.70	46.01	4.10	2348.8	-	119.9
49.18	45.49	3.79	2383.7	-	120.3
<u>D=0.547 h⁻¹</u>					
50.72	46.08	9.99	2290.7	-	126.6
50.40	45.97	9.99	2267.4	-	135.7
50.74	46.28	9.30	2395.4	-	128.2

Table 35. Microbial Analyses of a lactate-limiting chemostat at 35°C (Run 1).

D (h ⁻¹)	Cell # (10 ⁷ /mL)	Size (μm ²)	Cell-C (mg/L)	EPS-C (mg/L)	SS (mg/L)
0.088	3.940	1.090	2.23	0.84	14.4
	4.575	1.879	4.56	0.00	14.8
	3.782	2.179	4.39	0.00	12.4
0.170	4.776	1.224	3.05	0.33	14.4
	4.378	1.379	3.16	0.20	14.0
	5.470	1.822	5.28	0.00	16.0
0.240	4.062	2.505	5.44	0.00	14.0
	5.208	1.738	4.79	0.25	13.6
	5.939	1.501	4.69	0.00	17.2
0.320	6.955	0.967	3.46	0.70	18.4
	7.025	0.984	3.56	0.00	15.6
	6.428	1.071	3.56	0.00	14.8
0.450	3.309	2.341	4.13	0.00	9.5
	4.987	2.092	5.55	0.00	9.5
	4.908	0.778	1.93	0.00	15.0
0.547	3.538	0.892	1.78	2.87	16.0
	3.538	0.839	1.51	2.92	12.0
	4.464	1.894	4.48	0.16	12.0

Table 36. Influent Analyses of a lactate-limiting chemostat at 35 °C (Run 2) (mg/L).

D(h ⁻¹)	TOC	Lac.	SO ₄ ²⁻
0.050	86.12	212.2	315.5
	86.37	212.5	317.3
	86.11	-	318.2
0.108	Influent solution was the same as D=0.050 h ⁻¹ .		
0.180	Influent solution was the same as D=0.050 h ⁻¹ .		
0.263	Influent solution was the same as D=0.050 h ⁻¹ .		
0.310	80.42	200.6	360.0
	81.04	198.8	361.8
	81.34	-	358.2
0.360	Influent solution was the same as D=0.310 h ⁻¹ .		

Table 37. Effluent Analyses of a lactate-limiting chemostat at 35 °C (Run 2)(mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.050 h⁻¹</u>					
58.39	54.55	0.49	242.7	-	-
58.84	55.24	3.90	240.0	-	-
59.38	55.90	-	240.9	-	-
<u>D=0.108 h⁻¹</u>					
56.86	52.78	0.37	226.4	13.0	-
56.89	53.77	4.15	228.8	12.3	-
56.93	53.88	-	231.8	12.6	-
<u>D=0.180 h⁻¹</u>					
56.90	53.97	1.32	231.8	34.5	-
55.64	53.35	1.63	233.6	34.4	-
55.46	52.49	-	234.6	26.0	-
<u>D=0.260 h⁻¹</u>					
56.38	54.50	3.49	205.5	26.8	-
56.48	54.48	6.51	201.8	26.0	-
56.99	54.84	-	212.7	25.3	-

Table 37. Effluent Analyses of a lactate-limiting chemostat at 35°C (Run 2) (mg/L) (Continued).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.310 h⁻¹</u>					
55.53	54.03	44.15	263.6	31.4	-
54.68	53.96	44.63	270.9	30.6	-
54.88	53.96	-	263.6	29.9	-
<u>D=0.360 h⁻¹</u>					
61.40	59.42	61.22	292.7	26.0	-
60.62	59.36	56.34	290.9	24.1	-
61.09	59.36	-	289.1	-	-

Table 38. Microbial Analyses of a lactate-limiting chemostat at 35°C (Run 2).

D (h ⁻¹)	Cell # (10 ⁷ /mL)	Size (μm ²)	Cell-C (mg/L)	EPS-C (mg/L)	SS (mg/L)
0.050	3.823	0.444	3.84	-	14.0
	3.946	0.453	3.60	-	15.5
	4.398	0.481	3.48	-	20.0
0.108	6.002	0.566	4.08	-	23.5
	8.961	0.562	3.12	-	20.0
	5.508	0.585	3.05	-	22.5
	8.468	0.532	-	-	-
0.180	4.357	0.597	2.93	-	15.5
	4.522	0.693	2.29	-	15.0
	3.371	0.594	2.97	-	18.5
0.263	4.686	0.712	1.88	-	16.0
	5.015	0.760	2.00	-	22.0
	4.028	0.687	2.15	-	23.0
0.310	3.289	0.702	1.50	-	14.5
	3.289	0.677	0.72	-	15.0
	3.864	0.723	0.92	-	13.8
0.360	2.138	0.782	1.98	-	18.0
	3.124	0.769	1.20	-	16.5
	2.466	0.831	1.73	-	14.5

Table 39. Influent Analyses of a lactate-limiting chemostat at 43°C (mg/L).

D(h ⁻¹)	TOC	lac.	SO ₄ ²⁻
0.086	78.24	206.7	2360.5
	77.75	205.6	2360.5
	77.78	209.4	2360.5
0.252	76.29	194.3	2325.6
	76.65	202.5	2290.7
	76.56	-	2302.3
0.525	76.58	210.1	2337.2
	75.44	213.9	2314.0
	77.38	217.7	2255.8
	76.73	-	-
	77.23	-	-
	77.77	-	-

Table 40. Effluent Analyses of a lactate-limiting chemostat at 43°C (mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.086 h⁻¹</u>					
52.93	48.56	0.34	2302.3	30.3	101.9
52.74	48.58	2.07	2267.4	30.6	106.2
52.75	48.32	2.41	2290.7	32.0	104.9
<u>D=0.252 h⁻¹</u>					
50.31	47.28	5.51	2197.7	32.7	126.4
50.40	47.31	9.64	2209.3	-	102.8
49.78	47.26	10.3	2220.9	-	105.2
50.23	-	8.61	-	-	-
51.20	-	-	-	-	-
50.16	-	-	-	-	-
49.95	-	-	-	-	-
<u>D=0.525 h⁻¹</u>					
76.18	75.81	210.1	2290.7	-	4.6
76.18	76.12	212.9	2325.6	-	0.0
76.55	76.49	215.3	2279.1	-	9.6

Table 41. Microbial Analyses of a lactate-limiting chemostat at 43°C.

D (h ⁻¹)	Cell # (10 ⁷ /mL)	Size (μm ²)	Cell-C (mg/L)	EPS-C (mg/L)	SS (mg/L)
0.086	3.976	1.541	3.23	1.09	16.0
	3.958	1.585	3.31	0.85	16.0
	3.888	1.630	3.34	1.09	16.0
0.252	2.445	2.798	3.22	0.0	12.0
	2.742	1.988	2.89	0.20	16.0
	2.818	2.428	3.65	0.0	16.0
0.525	2.269	1.230	1.46	0.0	0.0
	1.858	1.523	1.48	0.0	0.14
	1.973	1.234	1.27	0.0	0.14

Table 42. Influent Analyses of a lactate-limiting chemostat at 48°C (mg/L).

D(h ⁻¹)	TOC	lac.	SO ₄ ²⁻
0.027	81.36	187.3	330.9
	81.08	185.4	329.1
	80.74	-	-
0.036	80.16	198.2	292.7
	80.25	-	292.7
	81.11	-	294.6
0.060	Influent solution was the same as D=0.036 h ⁻¹ .		
0.074	Influent solution was the same as D=0.027 h ⁻¹ .		
0.082	Influent solution was the same as D=0.027 h ⁻¹ .		
0.083	88.23	207.8	270.9
	87.02	208.8	272.7
	86.94	-	269.1
0.093	Influent solution was the same as D=0.083 h ⁻¹ .		
0.100	91.28	223.4	270.9
	91.58	229.3	272.7
	91.97	-	269.1
0.110	Influent solution was the same as D=0.036 h ⁻¹ .		

Table 43. Effluent Analyses of a lactate-limiting chemostat at 48°C (mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.027 h⁻¹</u>					
59.56	56.16	0.71	258.2	-	-
59.31	56.14	0.76	261.8	-	-
59.37	56.39	-	258.2	-	-
<u>D=0.035 h⁻¹</u>					
55.29	53.16	1.50	190.9	-	-
55.42	52.84	2.32	189.1	-	-
54.69	52.67	-	192.7	-	-
<u>D=0.060 h⁻¹</u>					
60.33	58.20	4.88	194.6	-	-
61.13	58.22	5.41	196.4	-	-
60.82	57.92	-	200.0	-	-
<u>D=0.074 h⁻¹</u>					
61.13	59.14	24.78	252.7	17.1	-
61.68	59.34	24.76	254.6	17.3	-
60.37	59.84	-	250.9	-	-
<u>D=0.082 h⁻¹</u>					
64.46	62.05	73.90	227.3	-	-
63.43	62.40	-	227.3	-	-
63.35	62.27	-	225.5	-	-
<u>D=0.083 h⁻¹</u>					
63.83	61.20	13.39	174.6	24.23	124.9
63.51	61.00	13.41	178.2	24.08	128.7
63.45	61.38	-	180.0	-	-
<u>D=0.093 h⁻¹</u>					
64.54	61.92	20.46	190.9	30.55	-
64.75	61.92	20.29	192.7	31.18	-
64.41	62.57	-	194.6	31.25	-
<u>D=0.100 h⁻¹</u>					
70.49	66.74	47.56	196.4	27.28	112.2
69.52	67.32	49.51	198.2	27.62	122.6
68.49	67.48	-	194.6	-	108.7
<u>D=0.110 h⁻¹</u>					
73.36	72.59	130.98	250.9	-	-
73.34	72.32	-	249.1	-	-
-	-	-	252.7	-	-

Table 44. Microbial Analyses of a lactate-limiting chemostat at 48°C.

D (h ⁻¹)	Cell # (10 ⁷ /mL)	Size (μm ²)	Cell-C (mg/L)	EPS-C (mg/L)	SS (mg/L)
0.027	5.590	0.708	3.40	-	-
	7.564	0.689	3.17	-	-
	6.413	0.690	2.77	-	-
0.035	3.453	0.727	2.13	-	20.5
	2.713	0.716	2.58	-	21.5
	3.946	0.702	2.02	-	18.0
0.060	1.973	0.609	2.13	-	10.0
	1.315	0.733	1.81	-	26.0
		0.611	1.61	-	30.0
0.074	2.631	0.857	1.99	-	103.0
	2.302	1.066	2.34	-	88.0
	1.973	0.844	0.53	-	86.5
0.082	1.069	0.629	2.41	-	18.5
	0.822	0.779	1.03	-	20.0
	0.658	0.771	1.08	-	17.5
0.083	1.510	0.690	2.63	-	78.0
	0.822	0.612	2.51	-	88.5
	0.904	0.614	2.07	-	55.0
	1.644	0.867	-	-	-
0.093	2.877	0.881	2.62	-	-
	4.042	0.818	2.94	-	-
	3.151	0.734	1.84	-	-
0.100	2.124	0.811	3.01	-	-
	2.261	0.814	2.20	-	-
	2.261	0.845	1.75	-	-
0.110	0.822	0.679	0.77	-	-
	0.863	0.558	1.04	-	-
	0.781	0.631	-	-	-

Transient Response to Step Changes in Temperature

Table 45. Influent analyses of a chemostat operated at continuously varying temperature (35-53°C) and at $D=0.1 \text{ h}^{-1}$ (mg/L).

Time(h)	TOC	lac.	SO ₄ ²⁻	Temp.(°C)
0.0	80.42	198.8	360.0	35
	81.04	200.6	361.8	
	81.34	-	358.2	
4.5	Influent solution was the same as T=0			43
8.5	Influent solution was the same as T=0			53
13.0	Influent solution was the same as T=0			53
23.5	Influent solution was the same as T=0			53
71.5	80.16	198.2	292.7	48
	80.25	-	292.7	
	81.11	-	294.6	

Table 46. Effluent Analyses of a chemostat operated at continuously varying temperature (35-53°C) and at $D=0.1 \text{ h}^{-1}$ (mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Temp.(°C)
<u>T=0.0 h</u>					
54.20	50.42	2.71	256.4	23.8	35
51.76	50.26	4.10	254.6	24.5	
51.83	50.17	-	-	-	
<u>T=4.5 h</u>					
55.46	52.03	1.22	245.5	26.0	43
54.48	51.62	5.00	243.6	-	
53.57	-	-	-	-	
<u>T=8.5 h</u>					
59.30	57.51	40.6	260.0	7.7	53
59.41	56.61	40.8	260.0	8.4	
58.92	57.01	-	-	-	
<u>T=13.0 h</u>					
69.63	68.15	70.5	296.4	3.5	53
69.56	68.15	69.5	284.6	-	
69.77	67.75	-	-	-	

Table 46. Effluent Analyses of a chemostat operated at continuously varying temperature (35-53°C) and at $D=0.1 \text{ h}^{-1}$ (mg/L) (Continued).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Temp.(°C)
<u>T = 23.5 h</u>					
73.03	69.69	166.6	294.6	5.5	53
72.60	68.91	167.1	292.7	-	
71.54	69.01	-	-	-	
<u>T = 71.5 h</u>					
73.36	72.59	131.0	250.9	-	48
73.36	72.32	-	249.09	-	
-	-	-	252.73	-	

Table 47. Microbial Analyses of a chemostat operated at continuously varying temperature (35-53°C) and at $D=0.1 \text{ h}^{-1}$.

D (h^{-1})	Temp. (°C)	Cell # ($10^7/\text{mL}$)	Size (μm^2)	Cell-C (mg/L)	SS (mg/L)
0.0	35	4.111	0.591	3.78	15.5
		4.686	0.642	1.50	13.7
		5.179	0.646	1.66	12.0
4.5	43	2.055	0.629	3.43	18.5
		1.973	0.623	2.86	17.0
		1.151	0.501	-	15.7
8.5	53	1.768	0.595	1.79	26.0
		1.891	0.605	2.80	39.5
		1.439	0.755	1.91	21.3
13.0	53	1.343	0.651	1.48	33.5
		1.096	0.734	1.41	28.0
		1.398	0.689	2.02	30.0
23.5	53	0.548	0.709	3.34	-
		0.493	0.690	3.69	-
		0.521	0.596	2.53	-
71.5	48	0.822	0.679	0.77	40.0
		0.863	0.558	1.04	-
		0.781	0.631	-	-

APPENDIX C

Raw Data of Batch Experiments

Table 48. Chemical analyses of a batch experiment at 35°C.

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>T=0.0 h</u>					
162.0	158.8	392.2	696.4	0.0	-
159.1	157.0	-	698.2	0.0	-
160.4	156.4	-	-	-	-
<u>T=10.0 h</u>					
160.4	158.7	385.4	-	-	-
158.5	-	-	-	-	-
159.6	-	-	-	-	-
<u>T=32.0 h</u>					
158.3	154.8	362.0	685.5	10.3	-
157.6	153.8	-	687.3	12.2	-
157.0	152.8	-	692.7	12.1	-
<u>T=36.0 h</u>					
147.5	145.4	337.6	663.6	24.0	-
147.2	144.6	-	667.3	24.4	-
147.5	144.1	-	-	27.2	-
<u>T=44.0 h</u>					
106.6	104.8	59.8	556.4	76.0	-
107.5	104.2	60.5	-	80.0	-
107.0	103.7	-	-	74.0	-
<u>T=49.0 h</u>					
99.04	94.25	3.27	545.5	92.0	-
98.65	93.72	-	540.0	90.0	-
99.40	93.20	-	-	-	-
<u>T=58.0 h</u>					
99.59	96.83	1.44	505.5	96.0	-
98.53	96.19	-	507.3	96.0	-
97.50	96.18	-	-	-	-
<u>T=70.0 h</u>					
103.2	97.92	3.49	503.6	76.0	-
104.3	96.63	-	507.3	78.0	-
100.3	96.11	-	-	77.0	-
<u>T=93.0 h</u>					
99.06	96.94	2.66	536.4	56.0	-
97.80	96.52	-	545.5	56.8	-
97.60	96.27	-	-	58.4	-

Table 49. Microbial Analyses of a batch experiment at 35°C.

D (h ⁻¹)	Cell # (10 ⁷ /mL)	Size (μm ²)	Cell-C (mg/L)	SS (mg/L)	pH
0.0	0.045	0.917	3.2	16.0	7.00
	0.027	0.884	2.1	6.0	
	0.027	0.750	4.0	-	
10.0	0.493	0.823	1.7	-	6.98
	0.288	0.921	1.9	-	
	0.493	0.726	0.8	-	
32.0	2.138	0.637	4.2	10.5	6.98
	1.315	0.562	3.5	12.7	
	1.480	0.508	3.8	-	
36.0	1.398	0.536	2.1	-	6.97
	1.315	0.535	2.6	-	
	2.384	0.688	3.4	-	
44.0	6.906	0.699	1.8	-	6.96
	6.906	0.736	3.3	-	
	6.002	0.526	3.3	-	
49.0	5.015	0.618	4.8	30.5	7.05
	5.755	0.580	4.9	41.0	
	5.508	0.579	6.2	-	
58.0	7.892	0.676	2.76	43.0	6.85
	10.36	0.515	2.34	41.6	
	5.262	0.642	1.32	-	
	7.975	0.615	-	-	
70.0	10.36	0.787	5.28	20.5	6.95
	8.961	0.749	7.67	43.0	
	6.577	0.825	4.19	-	
	10.28	0.804	-	-	
93.0	8.632	0.719	2.12	31.0	7.24
	10.36	0.650	1.28	27.3	
	9.783	0.710	1.33	-	

APPENDIX D

Raw Data of Nutrient Requirement Experiments

Sulfate-limiting Experiment**Table 50.** Influent Analyses of a sulfate-limiting chemostat at 35 °C (mg/L).

D(h ⁻¹)	TOC	lac.	SO ₄ ²⁻
0.027	148.0	369.5	136.0
	149.9	353.7	131.1
	148.8	-	-
0.057	Influent solution was the same as D = 0.027 h ⁻¹		
0.100	142.0	350.0	123.1
	142.1	363.4	122.5
	142.4	-	-
0.173	Influent solution was the same as D = 0.100 h ⁻¹		
0.230	146.7	382.9	135.6
	146.8	402.4	139.4
	147.3	-	-
0.280	Influent solution was the same as D = 0.230 h ⁻¹		
0.250	147.5	365.9	128.0
	147.6	362.2	130.5
	147.2	-	-
0.290	150.7	385.4	129.8
	151.4	387.8	131.1
	151.3	-	-
0.320	Influent solution was the same as D = 0.290 h ⁻¹		

Table 51. Effluent Analyses of a sulfate-limiting chemostat at 35 °C (mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>D=0.027 h⁻¹</u>					
106.7	105.6	35.1	0.9	22.5	253.3
106.9	104.1	35.4	1.1	22.6	211.1
104.2	-	-	-	-	242.2
104.0	-	-	-	-	-
<u>D=0.057 h⁻¹</u>					
111.7	108.6	61.0	0.45	26.2	211.1
111.9	108.4	57.3	0.55	26.6	208.9
112.2	108.9	-	-	-	262.2
<u>D=0.100 h⁻¹</u>					
106.9	102.8	70.0	1.1	31.6	162.2
106.4	102.2	73.7	0.9	-	162.2
106.8	102.4	-	-	-	197.8
<u>D=0.173 h⁻¹</u>					
105.9	102.2	82.9	1.9	37.8	168.9
104.9	101.2	80.7	2.1	38.0	162.2
104.8	100.8	-	-	-	-
<u>D=0.230 h⁻¹</u>					
105.8	102.2	78.0	4.4	-	202.2
104.9	101.1	80.5	4.8	-	206.7
104.9	101.6	-	-	-	162.2
<u>D=0.250 h⁻¹</u>					
107.9	104.2	93.7	4.1	29.2	168.9
107.6	103.6	91.5	4.5	29.6	186.7
108.1	104.7	-	-	-	173.3
<u>D=0.280 h⁻¹</u>					
116.4	113.5	179.8	38.2	40.5	137.8
117.0	113.7	176.8	39.3	41.5	126.7
116.9	114.1	-	-	-	195.6
<u>D=0.280 h⁻¹</u>					
-	-	-	24.8	-	133.3
-	-	-	-	-	111.1
-	-	-	-	-	122.2
<u>D=0.320 h⁻¹</u>					
135.1	134.3	264.6	74.0	14.6	66.7
135.1	134.8	262.2	75.5	14.5	88.7
135.8	134.9	-	-	-	64.4

Table 52. Influent Analyses of a sulfate-limiting chemostat at 43°C (mg/L).

D(h ⁻¹)	TOC	lac.	SO ₄ ²⁻
0.009	148.6 148.2	372.0 378.0	133.3 131.8
0.046	156.0 154.8	389.0 393.9	143.2 138.9
0.054	Influent solution was the same as D = 0.009 h ⁻¹ .		
0.113	Influent solution was the same as D = 0.046 h ⁻¹ .		
0.220	150.9 150.8	384.1 387.8	139.5 133.5
0.290	Influent solution was the same as D = 0.220 h ⁻¹ .		
0.340	Influent solution was the same as D = 0.046 h ⁻¹ .		

Table 53. Effluent Analyses of a sulfate-limiting chemostat at 43°C (mg/L).

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻
<u>D = 0.009 h⁻¹</u>				
103.5	101.5	7.1	2.6	35.7
102.6	100.7	7.3	2.0	36.0
<u>D = 0.046 h⁻¹</u>				
104.5	102.1	13.9	0.27	-
105.1	102.1	14.4	0.45	-
<u>D = 0.054 h⁻¹</u>				
106.6	104.0	47.1	0.4	48.7
105.9	103.6	48.8	0.4	49.1
<u>D = 0.113 h⁻¹</u>				
114.4	111.0	70.0	2.1	35.5
115.0	111.5	71.0	3.5	35.9
<u>D = 0.220 h⁻¹</u>				
113.8	108.9	87.3	8.8	31.7
113.5	109.0	88.3	9.2	31.7
<u>D = 0.290 h⁻¹</u>				
111.8	108.0	121.2	10.4	29.9
111.2	109.0	120.7	11.8	30.2
<u>D = 0.340 h⁻¹</u>				
131.9	129.4	259.5	76.5	25.7
132.2	130.0	263.4	81.2	25.9

Phosphorous-Limiting Experiment**Table 54.** Influent Analyses of a phosphorous-limiting chemostat at 35°C (mg/L).

Phosphorous	TOC	Lac.	SO ₄ ²⁻
48.375	158.2	401.3	2569.8
48.563	158.5	408.1	2564.0
48.469	158.1	397.8	2546.5
4.613	163.8	454.6	2570.0
4.613	163.2	454.6	2593.0
4.570	164.2	403.0	2558.1
0.396	169.9	382.3	2529.1
0.384	169.3	427.1	2529.1
0.394	170.3	423.7	2540.5
0.036	155.8	375.4	2581.4
0.034	156.7	390.9	2517.4
0.034	155.8	382.3	2523.3

Table 55. Effluent Analyses of a phosphorous-limiting chemostat at 35°C (mg/L). (Mean ± SD)

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac
<u>P=48.47±0.08</u>					
99.2	95.3	0.0	2337.2	69.8	244.6
99.3	94.9	0.0	2343.0	-	240.1
99.2	95.5	0.0	2343.0	-	248.2
<u>P=4.60±0.02</u>					
107.1	102.7	2.4	2337.2	72.7	257.9
106.2	102.3	4.8	2360.5	72.1	246.6
105.8	101.4	3.8	2325.6	72.2	251.9
<u>P=0.39±0.01</u>					
111.8	107.5	4.1	2302.3	59.9	262.4
112.0	106.7	5.2	2296.5	60.0	258.8
112.1	106.8	5.9	2290.0	59.9	258.5
<u>P=0.03±0.00</u>					
124.4	120.9	115.7	2401.2	55.8	161.4
124.3	121.0	115.7	2395.4	-	167.1
124.1	121.4	108.5	2418.6	-	156.6

Table 56. Microbial Analyses of a phosphorous-limiting chemostat at 35°C.
(Mean $\pm$ SD)

P (mg/L)	size (μm^3)	Cell # ($10^7/\text{mL}$)	SS (mg/L)	EPS-C (mg/L)	Cell-C (mg/L)
48.47	1.03	10.47	27.3	0.0	4.41
± 0.08	1.19	9.75	30.2	0.0	3.69
	1.16	9.97	27.9	0.0	3.90
4.60	1.61	5.59	26.0	0.0	4.75
± 0.02	1.38	5.26	26.0	0.61	3.79
	1.32	4.96	35.0	0.48	3.90
0.39	2.04	4.11	29.6	0.83	4.47
± 0.01	2.32	3.87	27.2	0.52	4.78
	1.54	2.87	27.6	1.98	2.32
0.03	1.10	2.13	9.9	2.29	1.22
± 0.00	0.94	2.35	10.2	2.17	1.14
	0.82	1.94	10.0	1.89	0.81

Nitrogen-Limiting Experiment**Table 57.** Influent Analyses of a nitrogen-limiting chemostat at 35°C (mg/L).

Ammonium-N	TOC	Lac.	SO ₄ ²⁻
66.69	144.6	373.2	645.5
65.04	144.6	380.5	645.5
6.28	142.7	352.4	640.0
6.50	142.9	354.9	649.1
3.33	147.5	387.8	669.1
3.31	148.6	391.5	680.0
1.25	144.1	372.0	610.9
1.18	144.2	379.3	612.7
0.65	145.5	369.5	640.0
0.63	145.9	374.4	636.4

Table 58. Effluent Analyses of a nitrogen-limiting chemostat at 35°C (mg/L). (Mean $\pm$ SD)

TOC	SOC	Lac.	SO ₄ ²⁻	S ²⁻	Ac.
<u>N = 65.59 $\pm$ 0.55</u>					
96.04	90.61	2.56	450.9	48.8	250.0
96.17	90.38	3.27	461.8	48.6	246.2
97.51	90.95	2.68	454.5	-	223.3
97.09	90.74	1.34	447.3	-	222.2
<u>N = 6.39 $\pm$ 0.11</u>					
96.28	90.71	1.24	443.6	49.8	188.7
96.20	90.50	1.85	450.9	49.8	176.3
96.46	90.98	0.07	480.0	53.1	228.0
96.52	91.32	0.54	447.3	40.6	240.6
<u>N = 3.32 $\pm$ 0.01</u>					
97.75	92.38	1.15	483.6	54.6	225.0
97.92	92.37	1.49	480.0	54.9	217.5
98.05	93.69	1.68	463.6	-	248.7
97.77	93.15	1.05	483.6	-	260.5
<u>N = 1.22 $\pm$ 0.04</u>					
108.6	105.5	42.4	460.0	56.4	92.9
108.3	104.7	43.7	440.0	57.1	82.4
110.6	106.5	68.1	436.4	46.2	164.1
109.8	106.8	70.5	443.6	46.7	163.8
<u>N = 0.64 $\pm$ 0.01</u>					
129.1	127.2	211.2	549.1	18.7	114.5
129.4	127.6	213.2	556.4	19.0	102.6
128.5	126.6	235.6	509.1	20.2	92.3
128.1	126.7	239.5	510.9	20.4	87.8

Table 59. Microbial Analyses of a nitrogen-limiting chemostat at 35°C. (Mean $\pm$ SD)

N (mg/L)	size (μm^3)	Cell # (10 ⁷ /mL)	EPS-C (mg/L)	Cell-C (mg/L)
<u>65.59</u>	0.78	12.83	0.09	5.52
± 0.55	0.86	13.32	0.11	6.34
<u>6.39</u>	0.67	12.83	0.92	4.71
± 0.11	0.75	11.84	0.48	4.86
<u>3.32</u>	0.58	9.70	2.38	3.08
± 0.01	0.56	9.04	1.73	2.76
<u>1.22</u>	0.71	4.93	1.49	1.91
± 0.04	0.65	5.34	1.70	1.90
<u>0.64</u>	0.61	3.72	0.66	1.24
± 0.01	0.59	2.99	0.69	0.96

APPENDIX E

Raw Data of Sulfide Product Inhibition Experiments

Sulfide Product Inhibition Experiment (Run 1)**Table 60.** Influent Analyses of a chemostat exposed to various sulfide concentrations at 35°C and pH = 7.0 (Run 1) (mg/L).

TS	TOC	Lac.	SO ₄ ²⁻
48.8	144.6	385.4	645.5
48.6	144.6	380.5	645.5
148.4	150.2	426.8	-
149.3	148.9	408.5	-
151.7	-	-	-
151.5	-	-	-
272.1	145.9	396.3	-
273.4	147.0	391.5	-
281.9	-	-	-
283.1	-	-	-
632.8	152.6	406.1	-
648.4	154.8	393.9	-
554.7	-	-	-
549.2	-	-	-

Table 61. Effluent Analyses of a chemostat exposed to various sulfide concentrations at 35°C and pH=7.0 (mg/L) (Run 1). (Mean $\pm$ SD)

TS	TOC	SOC	Lac.	Ac.
48.7 $\pm$ 0.1	96.04 96.17 97.51 97.09	90.61 90.38 90.95 90.74	2.56 3.27 2.68 1.34	223.3 188.6 227.2 239.5
150.2 $\pm$ 1.4	103.4 103.4 104.7 102.0	97.07 96.09 98.01 98.09	7.32 7.20 7.56 7.63	216.5 210.3 214.6 218.4
277.6 $\pm$ 4.9	101.4 101.5 102.3 101.9	98.50 99.15 100.0 100.1	27.6 27.9 35.0 37.0	221.1 212.3 156.4 156.6
596.3 $\pm$ 44.7	140.8 140.7 142.8 143.2	140.9 140.5 142.8 142.9	290.2 281.7 256.1 261.0	84.9 97.4 55.4 58.4

Table 62. Microbial Analyses of a chemostat exposed to various sulfide concentrations at 35°C and pH=7.0 (Run 1). (Mean $\pm$ SD)

TS (mg/L)	size (μm^3)	Cell # ($10^7/\text{mL}$)	EPS-C (mg/L)	Cell-C (mg/L)
<u>48.7 $\pm$ 0.1</u>	0.78 0.86	12.83 13.32	0.09 0.11	5.52 6.34
<u>150.2 $\pm$ 1.4</u>	0.74 0.60	13.11 15.33	1.50 0.26	5.32 5.04
<u>277.6 $\pm$ 4.9</u>	0.72 0.67	4.33 5.43	0.93 0.10	1.70 2.00
<u>596.3 $\pm$ 44.7</u>	0.69 0.71	0.72 0.72	0.00 0.00	0.27 0.28

Recovery from 600 mg-S/L Treatment**Table 63.** Recovery of cell numbers from 600 mg-S/L treatment. (Mean $\pm$ SD)

Cell # (cells/mL)		
Time (h)	Control	Sample treated by 600 mg-S/L
0	$3.35 \pm 0.86 \times 10^5$	7.24×10^5
10	$4.25 \pm 0.97 \times 10^6$	-
15	-	$1.15 \pm 0.56 \times 10^6$
32	$1.64 \pm 0.36 \times 10^7$	-
36.5	$1.70 \pm 0.49 \times 10^7$	-
39	-	$2.30 \pm 0.37 \times 10^6$
44	$6.61 \pm 0.43 \times 10^7$	-
49	$5.43 \pm 0.31 \times 10^7$	-
58	$7.87 \pm 1.80 \times 10^7$	-
63	-	$2.69 \pm 0.47 \times 10^6$
70	$9.05 \pm 1.53 \times 10^7$	-
87	-	$4.93 \pm 0.35 \times 10^6$
93	$9.59 \pm 0.72 \times 10^7$	-
113	-	$1.91 \pm 0.15 \times 10^7$
140	-	$6.20 \pm 0.63 \times 10^7$
165	-	$11.4 \pm 1.85 \times 10^7$
210	-	$10.6 \pm 1.26 \times 10^7$

Sulfide Product Inhibition Experiment (Run 2)**Table 64.** Influent Analyses of a chemostat exposed to various sulfide concentrations at 35°C and pH = 7.0 (Run 2) (mg/L).

TS	TOC	Lac.	SO ₄ ²⁻
25.3	113.6	274.4	482.7
25.5	113.8	272.1	485.4
27.8	-	-	-
25.6	-	-	-
104.2	113.6	274.4	479.7
113.8	113.8	272.1	478.7
109.6	-	-	-
105.2	-	-	-
159.9	123.7	302.5	485.4
187.5	124.6	305.6	505.4
147.4	-	-	-
136.7	-	-	-
276.0	119.8	289.7	525.6
242.2	121.2	293.6	532.6
277.3	118.7	287.2	508.5
291.5	120.1	291.0	505.6
323.4	122.5	299.6	480.8
340.6	123.4	302.7	500.5
373.4	124.8	305.3	489.9
382.8	125.7	308.4	510.0
445.3	126.4	309.0	495.9
429.7	127.3	312.2	516.2

Table 65. Effluent Analyses of a chemostat exposed to various sulfide concentrations at 35°C and pH=7.0 (mg/L) (Run 2). (Mean $\pm$ SD)

TS	TOC	SOC	Lac.	SO ₄ ²⁻	Ac
26.1	76.37	71.24	3.8	365.6	153.8
± 1.0	76.56	72.79	3.4	362.4	155.3
	77.63	70.40	-	-	-
	75.45	70.17	-	-	-
108.2	74.60	68.75	4.2	338.5	133.3
± 3.8	73.96	67.95	4.4	330.2	136.0
	75.24	68.43	-	-	-
	74.94	68.24	-	-	-
157.9	82.86	78.39	5.8	339.6	167.0
± 19.0	83.37	77.95	6.3	347.9	168.0
	-	78.24	-	-	-
	-	77.63	-	-	-
259.1	77.75	75.83	7.5	375.0	165.0
± 16.9	78.01	75.52	5.9	404.2	165.3
	77.63	75.27	-	-	-
	77.90	74.95	-	-	-
284.4	81.11	78.60	7.4	354.2	172.3
± 7.1	81.75	78.09	7.8	350.0	171.5
	-	79.00	-	-	-
	-	78.01	-	-	-
332.0	80.67	77.82	5.7	352.1	173.6
± 8.6	80.07	77.33	5.6	320.8	173.8
	81.17	77.77	-	-	-
	80.79	77.42	-	-	-
378.5	83.21	78.28	9.8	349.2	160.4
± 13.0	81.59	79.04	9.8	347.2	167.3
	82.42	79.72	-	-	-
	81.89	79.34	-	-	-
437.5	86.03	83.41	21.8	375.0	181.1
± 7.8	87.85	84.34	22.5	360.4	181.0
	87.27	84.73	-	-	-
	87.80	84.49	-	-	-

Table 66. Microbial Analyses of a chemostat exposed to various sulfide concentrations at 35°C and pH=7.0 (Run 2). (Mean $\pm$ SD)

TS (mg/L)	size (μm^3)	Cell # ($10^7/\text{mL}$)	EPS-C (mg/L)	Cell-C (mg/L)
26.1 $\pm$ 1.0	0.85 $\pm$ 0.27	11.37 $\pm$ 1.75	0.24 $\pm$ 1.52	5.33 $\pm$ 0.82
108.2 $\pm$ 3.8	0.77 $\pm$ 0.25	14.73 $\pm$ 1.86	0.11 $\pm$ 0.97	6.23 $\pm$ 0.79
157.9 $\pm$ 19.0	0.73 $\pm$ 0.24	12.66 $\pm$ 1.26	0.00 $\pm$ 0.64	5.07 $\pm$ 0.50
259.1 $\pm$ 16.9	0.61 $\pm$ 0.22	9.50 $\pm$ 0.90	0.00 $\pm$ 0.34	3.16 $\pm$ 0.30
284.4 $\pm$ 7.1	0.62 $\pm$ 0.22	7.52 $\pm$ 0.87	0.41 $\pm$ 1.58	2.54 $\pm$ 0.29
332.0 $\pm$ 8.6	0.57 $\pm$ 0.28	9.64 $\pm$ 1.58	0.10 $\pm$ 0.66	2.98 $\pm$ 0.49
378.5 $\pm$ 13.0	0.61 $\pm$ 0.21	7.73 $\pm$ 1.25	0.61 $\pm$ 0.91	2.57 $\pm$ 0.42
437.5 $\pm$ 7.8	0.54 $\pm$ 0.19	5.63 $\pm$ 0.92	1.85 $\pm$ 0.63	1.65 $\pm$ 0.27

Adaption to High Sulfide Concentrations

Table 67. Transient response of *D. desulfuricans* culture to 120 mg-S/L sulfide at 35°C, pH=7.0, and D=0.20 h⁻¹. (Mean ± SD)

Time (h)	TS (mg-S/L)	Cell # (10 ⁷ /mL)	Lactate	
			In	Eff
-8.5	12.6	8.29 ± 1.00	441.8	0.23
	12.8		449.1	0.00
0	12.6	8.54 ± 1.14	441.8	0.00
	11.9		449.1	0.00
5	112.9	5.91 ± 0.74	441.8	0.00
	105.5		449.1	0.00
15	134.4	7.10 ± 0.70	441.8	5.14
	133.7		449.1	3.77
22	142.5	7.46 ± 0.98	441.8	2.73
	131.7		449.1	2.95
29	129.0	7.52 ± 1.08	441.8	0.00
	148.5		449.1	0.00
39	119.6	8.08 ± 1.08	480.0	0.00
	131.7		490.9	0.00
48	141.1	8.15 ± 1.14	480.0	0.00
	139.1		490.9	0.00
63	141.8	8.66 ± 0.97	480.0	2.27
	141.1		490.9	1.95
87	145.2	8.65 ± 1.27	480.0	0.00
	136.4		490.9	0.00

Table 68. Transient response of *D. desulfuricans* culture to 230 mg-S/L sulfide at 35°C, pH=7.0, and $D=0.20\text{ h}^{-1}$. (Mean $\pm$ SD)

Time (h)	TS (mg-S/L)	Cell # (10^7 /mL)	Lactate	
			In	Eff
-8	16.8	11.07 ± 0.85	427.3	0.00
	17.6		412.7	0.00
0	14.4	10.60 ± 1.02	427.3	0.00
	13.8		412.7	0.00
4.5	192.3	5.32 ± 0.94	427.3	7.73
	183.9		412.7	7.36
13.5	198.3	3.90 ± 0.63	427.3	17.6
	195.6		412.7	17.9
18.5	232.5	4.20 ± 0.33	427.3	3.27
	231.2		412.7	2.36
23.5	195.6	4.56 ± 1.02	427.3	1.55
	193.5		412.7	2.45
37.5	217.7	6.68 ± 0.91	427.3	2.73
	210.3		412.7	2.41
48	243.3	6.98 ± 0.92	427.3	0.00
	271.5		412.7	0.00
62	200.9	5.98 ± 0.94	427.3	4.00
	256.0		412.7	5.00
85	180.1	6.17 ± 1.31	427.3	3.82
	193.5		412.7	0.91

APPENDIX F

Raw Data of Biofilm Experiments

Sulfate Flux (r_s)**Table 69.** Determination of sulfate flux at 15°C, pH=7.0, and $D=3.25 \text{ h}^{-1}$. Biofilm thickness was 45-46 μm .

$\text{SO}_4^{2-}{}_{\text{in}}$	$\text{SO}_4^{2-}{}_{\text{out}}$	AVG. $D \times (\text{SO}_4^{2-}{}_{\text{in}} - \text{SO}_4^{2-}{}_{\text{out}})$
18.3	3.8	2.21
21.1	3.5	
59.5	9.1	7.24
63.2	8.4	
70.0	13.6	8.08
75.2	14.2	
88.6	35.1	7.58
90.0	33.3	
142.7	83.4	8.67
148.6	81.8	

Table 70. Determination of sulfate flux at 20°C, pH=7.0, and $D=3.25 \text{ h}^{-1}$. Biofilm thickness was 45-59 μm .

$\text{SO}_4^{2-}{}_{\text{in}}$	$\text{SO}_4^{2-}{}_{\text{out}}$	AVG. $D \times (\text{SO}_4^{2-}{}_{\text{in}} - \text{SO}_4^{2-}{}_{\text{out}})$
17.2	0.7	2.26
18.6	2.3	
45.8	3.5	5.75
45.5	4.2	
66.9	6.8	8.23
66.4	6.9	
98.7	14.7	12.20
107.5	14.1	
167.3	55.6	13.85
147.0	57.3	
225.9	109.1	16.19
224.1	105.5	

Table 71. Determination of sulfate flux at 25°C, pH=7.0, and $D=3.25 \text{ h}^{-1}$. Biofilm thickness was 51-55 μm .

$\text{SO}_4^{2-} \text{ in}$	$\text{SO}_4^{2-} \text{ out}$	AVG. $D \times (\text{SO}_4^{2-} \text{ in} - \text{SO}_4^{2-} \text{ out})$
23.7	1.0	3.17
24.5	1.1	
58.0	5.1	7.50
61.0	4.9	
85.5	9.4	10.53
86.5	9.5	
105.0	18.9	12.02
106.0	17.3	
163.2	32.4	17.58
157.7	32.9	
214.5	54.2	21.87
210.9	53.3	
237.7	62.9	24.77
247.3	62.0	
272.7	111.5	23.06
287.3	113.3	

Table 72. Determination of sulfate flux at 35°C, pH = 7.0, and $D = 2.31 \text{ h}^{-1}$. Biofilm thickness was 30-63 μm .

$\text{SO}_4^{2-}{}_{\text{in}}$	$\text{SO}_4^{2-}{}_{\text{out}}$	AVG. $D \times (\text{SO}_4^{2-}{}_{\text{in}} - \text{SO}_4^{2-}{}_{\text{out}})$
22.5	1.9	
22.5	2.3	1.99
83.6	3.8	
82.7	3.6	7.77
134.1	10.3	
136.8	12.2	12.15
193.6	20.0	
173.2	19.7	15.99
330.0	84.5	
318.2	84.1	23.45
489.5	200.0	
493.6	207.3	28.15
667.3	199.1	
670.9	199.1	24.87 ^a

^a Dilution rate was 1.25 h^{-1}

Table 73. Determination of sulfate flux at 35°C, pH=7.0, and $D=3.25 \text{ h}^{-1}$. Biofilm thickness was 40-65 μm .

$\text{SO}_4^{2-} \text{ in}$	$\text{SO}_4^{2-} \text{ out}$	AVG. $D \times (\text{SO}_4^{2-} \text{ in} - \text{SO}_4^{2-} \text{ out})$
21.1	3.8	2.32
20.4	4.0	
80.9	8.2	10.11
80.5	6.3	
99.1	21.5	10.59
97.7	21.4	
160.9	50.9	15.11
163.2	53.6	
237.3	74.1	21.48
226.4	77.3	
242.7	82.3	21.94
242.7	84.1	
290.0	130.5	22.76
298.2	126.8	
331.8	171.8	22.01
324.5	164.5	

Biofilm Kinetic Experiment I**Table 74.** Biofilm phase analyses of a RotoTorque reactor for determination of μ_b and q_{dx} : $D = 2.2 \text{ h}^{-1}$, Temp. = 35°C . (Mean $\pm$ SD)

Time (hour)	L_f (μm)	x_b (g-C/m^2)	Cell # ($10^7/\text{m}^2$)
0.0	6.7 ± 1.8	0.088 ± 0.013	8.22 ± 1.86
1.3	6.4 ± 1.8	0.118 ± 0.005	9.50 ± 1.06
4.0	11.7 ± 2.3	0.186 ± 0.001	17.3 ± 4.95
7.0	22.8 ± 3.9	0.293 ± 0.009	34.3 ± 4.70
9.3	34.6 ± 4.4	0.341 ± 0.021	49.3 ± 10.0
12.0	45.8 ± 8.2	0.522 ± 0.006	63.5 ± 17.9
15.0	60.2 ± 12.4	0.712 ± 0.011	69.8 ± 12.4
18.0	54.1 ± 9.2	0.215 ± 0.007	15.6 ± 4.84

Table 75. Liquid phase analyses of a RotoTorque reactor for determination of μ_b and q_{dx} : $D = 2.2 \text{ h}^{-1}$, Temp. = 35°C . (Mean $\pm$ SD)

Time (hour)	Lac. (mg/L)	S^{2-} (mg/L)	Bio-C (mg-C/L)	Cell # ($10^7/\text{mL}$)
0.0				2.20 ± 0.00
1.3	489.1 ± 2.7	27.7	3.15 ± 1.80	2.89 ± 0.82
2.3		25.8		
3.2		21.8		
4.0	472.7 ± 9.1	29.1	3.33 ± 2.74	3.67 ± 0.97
5.0		32.1		
6.5		38.6		
7.0	392.3 ± 0.9		4.50 ± 1.73	3.86 ± 1.15
8.0		43.8		
9.0		52.9		
9.3	309.1 ± 4.6		4.03 ± 1.35	6.10 ± 1.60
10.0		60.1		
11.0		63.8		
12.0	180.7 ± 1.2	69.8	4.43 ± 1.70	5.61 ± 1.24
13.0		76.9		
14.0		71.1		
15.0	91.2 ± 0.3	72.6	2.55 ± 1.03	12.6 ± 2.70
18.0	74.1 ± 1.4	80.5	41.6 ± 3.30	57.20 ± 10.40

Table 76. A summary of the determined μ_b and q_{dx} at $D = 2.2 \text{ h}^{-1}$.

Time (h)	Cell # Basis		Bio-C Basis	
	μ_b (h^{-1})	q_{dx} (h^{-1})	μ_b (h^{-1})	q_{dx} (h^{-1})
0.0				
1.3	0.384	0.276	0.451	0.226
4.0	0.442	0.224	0.355	0.189
7.0	0.349	0.129	0.300	0.151
9.3	0.268	0.114	0.180	0.116
12.0	0.184	0.090	0.245	0.087
15.0	0.165	0.134	0.148	0.046
18.0	-	-	0.173	0.530

Biofilm Kinetic Experiment II**Table 77.** Biofilm phase analyses of a RotoTorque reactor for determination of μ_b and q_{dx} : $D = 3.5 \text{ h}^{-1}$, Temp. = 35°C . (Mean $\pm$ SD)

Time (hour)	L_f (μm)	x_b (g-C/ m^2)	Cell # ($10^7/\text{m}^2$)
0.0	3.8 ± 2.1	0.186 ± 0.005	4.35 ± 0.50
2.0	6.3 ± 3.0	0.141 ± 0.010	6.19 ± 1.64
4.5	12.0 ± 3.0	0.117 ± 0.005	7.91 ± 1.59
8.0	13.8 ± 3.0	0.180 ± 0.012	16.1 ± 3.90
11.0	21.8 ± 4.8	0.315 ± 0.017	30.3 ± 9.03
13.5	27.8 ± 6.4	0.421 ± 0.016	40.6 ± 7.17
17.0	31.1 ± 7.9	0.560 ± 0.032	68.0 ± 11.20
21.0	70.5 ± 11.1	0.847 ± 0.047	111.0 ± 11.10
25.5	60.8 ± 10.4	0.925 ± 0.009	121.0 ± 16.50
32.5	45.6 ± 7.2	0.465 ± 0.032	69.7 ± 10.70
59.0	33.2 ± 9.6	0.415 ± 0.011	26.1 ± 5.00

Table 78. Liquid phase analyses of a RotoTorque reactor for determination of μ_b and q_{dx} : $D = 3.5 \text{ h}^{-1}$, Temp. = 35°C . (Mean $\pm$ SD)

Time (hour)	Lac. (mg/L)	SO_4^{2-} (mg/L)	S^{2-} (mg/L)	Bio-C (mg-C/L)	Cell # ($10^7/\text{mL}$)
0.0					
1.0			5.4		
2.0	577.3 ± 13.7	665.5	5.5	0.55 ± 1.13	0.44 ± 0.15
3.0			5.6		
4.0			6.6		
4.5	552.3 ± 2.3	539.2		1.73 ± 1.16	0.32 ± 0.14
5.0			6.8		
6.0			8.1		
7.0			9.5		
8.0	494.3 ± 5.7	465.5	11.1	1.78 ± 1.79	1.06 ± 0.34
9.3			12.9		
11.0	442.1 ± 10.3	470.0	17.2	1.58 ± 0.96	0.86 ± 0.40
12.0			27.4		
13.5	423.9 ± 7.9	445.5	37.0	2.93 ± 1.53	1.53 ± 0.41
15.0			46.0		

Table 78. Liquid phase analyses of a RotoTorque reactor for determination of μ_b and q_{dx} : $D = 3.5 \text{ h}^{-1}$, Temp. = 35°C . (Mean $\pm$ SD) (Continued)

Time (hour)	Lac. (mg/L)	SO_4^{2-} (mg/L)	S^{2-} (mg/L)	Bio-C (mg-C/L)	Cell # ($10^7/\text{mL}$)
17.0	357.8 ± 5.8	470.1	41.2	1.45 ± 0.82	2.06 ± 0.53
19.0			51.4		
21.0	213.6 ± 4.6	446.4	54.3	5.90 ± 0.47	7.54 ± 2.24
23.0			48.7		
23.5	244.3 ± 10.2	383.7	42.7	2.78 ± 1.24	8.40 ± 1.95
32.5	221.7 ± 3.5	380.0	54.7	9.25 ± 1.12	18.00 ± 3.16
59.0	273.6 ± 10.0	409.1	50.3	6.30 ± 1.36	2.03 ± 0.37
60.0	264.1 ± 1.4	410.0	50.4	6.68 ± 2.44	2.34 ± 0.45
61.0	261.4 ± 11.4	411.8	53.6	2.10 ± 0.86	2.36 ± 0.46

Table 79. A summary of the determined μ_b and q_{dx} at $D = 3.5 \text{ h}^{-1}$.

Time (h)	Cell # Basis		Bio-C Basis	
	μ_b (h^{-1})	q_{dx} (h^{-1})	μ_b (h^{-1})	q_{dx} (h^{-1})
4.5	0.170	0.072	0.070	0.138
8.0	0.282	0.087	0.285	0.165
11.0	0.262	0.057	0.275	0.093
13.5	0.166	0.050	0.206	0.091
17.0	0.192	0.047	0.140	0.059
21.0	0.201	0.081	0.181	0.079
23.5	0.116	0.097	0.085	0.065
32.5	0.122	0.199	0.031	0.126
59.0	0.253	0.287	0.242	0.246

Biofilm Kinetic Experiment III

Table 80. Biofilm phase analyses of a RotoTorque reactor for determination of μ_b and q_{dx} : $D = 1.7 \text{ h}^{-1}$, Temp. = 35°C . (Mean $\pm$ SD)

Time (hour)	L_f (μm)	x_b (g-C/ m^2)	Cell # ($10^7/\text{m}^2$)
0.0	3.7 ± 2.0	0.068 ± 0.002	7.91 ± 1.08
5.5	5.5 ± 1.3	0.156 ± 0.012	19.8 ± 3.30
11.0	17.3 ± 4.4	0.255 ± 0.001	48.2 ± 5.60
17.0	33.4 ± 5.7	0.662 ± 0.066	74.3 ± 8.70
23.0	54.3 ± 12.1	0.729 ± 0.032	77.6 ± 9.70
30.0	30.0 ± 7.4	0.659 ± 0.015	53.0 ± 6.04
37.0	43.3 ± 6.5	0.745 ± 0.099	61.3 ± 9.00
44.0	32.0 ± 6.6	0.604 ± 0.049	53.9 ± 7.00
50.0	44.5 ± 10.4	0.773 ± 0.015	69.5 ± 7.00
56.0	41.7 ± 8.7	0.886 ± 0.003	71.5 ± 8.10
62.0	40.3 ± 7.3	0.775 ± 0.063	98.6 ± 9.20
70.0	34.1 ± 7.4	1.036 ± 0.042	98.7 ± 10.9

Table 81. Liquid phase analyses of a RotoTorque reactor for determination of μ_b and q_{dx} : $D = 1.7 \text{ h}^{-1}$, Temp. = 35°C . (Mean $\pm$ SD)

Time (hour)	Lac. (mg/L)	SO_4^{2-} (mg/L)	S^{2-} (mg/L)	Bio-C (mg-C/L)	Cell # ($10^7/\text{mL}$)
0.0	411.4 ± 2.3	501.4	24.2 ± 2.9	2.65 ± 1.36	3.49 ± 0.72
2.0			22.8 ± 0.4		
4.0			26.2 ± 0.1		
5.5	378.2 ± 3.7	472.1		4.23 ± 3.18	1.46 ± 0.46
7.5			32.3 ± 0.2		
10.5			37.0 ± 0.2		
11.0	212.8 ± 3.7	430.5		2.85 ± 0.65	2.78 ± 0.84
17.0	57.3 ± 0.5	366.9	64.0 ± 0.1	9.50 ± 0.25	14.7 ± 2.02
23.0	54.1 ± 0.5	358.6	69.1 ± 4.3	6.30 ± 0.78	16.2 ± 2.60
30.0	56.6 ± 2.5	356.9	84.6 ± 4.2	15.3 ± 1.48	19.2 ± 2.10
37.0	51.2 ± 1.2	373.7	71.3 ± 5.0	14.3 ± 1.64	23.1 ± 3.90
44.0	36.8 ± 1.8	338.2	77.8 ± 0.8	5.50 ± 0.36	9.69 ± 1.35
50.0	24.6 ± 1.9	341.9	78.4 ± 1.0	11.3 ± 0.75	29.2 ± 4.40
56.0	32.5 ± 0.7	353.3	68.4 ± 2.5	17.1 ± 2.05	20.2 ± 2.03
62.0	39.3 ± 0.7	357.2	76.4 ± 1.4	3.80 ± 0.70	10.1 ± 2.01
70.0	23.9 ± 1.2	362.4	80.9 ± 1.5	23.4 ± 1.40	29.3 ± 4.10

Table 82. A summary of the determined μ_b and q_{dx} at $D = 1.7 \text{ h}^{-1}$.

Time (h)	Cell # Basis		Bio-C Basis	
	μ_b (h^{-1})	q_{dx} (h^{-1})	μ_b (h^{-1})	q_{dx} (h^{-1})
0.0				
5.5	0.266	0.110	0.359	0.217
11.0	0.197	0.045	0.199	0.111
17.0	0.180	0.109	0.248	0.100
23.0	0.146	0.139	0.090	0.074
30.0	0.132	0.185	0.098	0.112
37.0	0.274	0.254	0.159	0.141
44.0	0.160	0.179	0.061	0.091
50.0	0.276	0.234	0.129	0.088
56.0	0.232	0.228	0.142	0.120
62.0	0.165	0.112	0.052	0.074
70.0	0.145	0.144	0.148	0.112

APPENDIX G

Determination of Cellular and EPS Carbon
Concentrations by LPS Method

Determination of Cellular Carbon and LPS Concentrations

Cellular Carbon Calibration

Table 83. Cellular carbon and LPS contents in *D. desulfuricans* cells from chemostat cultures at various dilution rates: Temp. = 35 °C, pH = 7.0.

Cell # (10 ⁷ /mL)	Cell-C (mg/L)	LPS (mg/L)	C/cell (pg-C/cell)	LPS/cell (pg-LPS/cell)	C/LPS
<u>D=0.05 h⁻¹</u>					
5.40	4.00	1.284	0.074	0.026	2.85
5.83	4.15	3.853	0.071	0.066	1.08
<u>D=0.08 h⁻¹</u>					
4.50	3.80	1.963	0.084	0.044	1.91
4.94	5.70	2.660	0.115	0.054	2.13
<u>D=0.10 h⁻¹</u>					
4.90	4.95	1.752	0.101	0.036	2.81
6.15	5.60	1.881	0.091	0.031	2.94
<u>D=0.12 h⁻¹</u>					
5.29	5.05	2.832	0.095	0.054	1.76
		1.038	0.095	0.020	4.75
3.56	4.65	1.413	0.131	0.040	3.28
		1.017	0.131	0.029	4.52
4.12	4.35	1.564	0.106	0.038	2.79
		1.831	0.106	0.044	2.41
<u>D=0.24 h⁻¹</u>					
4.67	4.70	2.486	0.101	0.037	2.73
4.23	3.70	1.743	0.087	0.041	2.12

Comparison of LPS and EPI Methods

Biofilm Experiment

Table 84. Typical progression of biofilm cellular carbon and EPS carbon areal densities determined by the LPS assay and the epifluorescence method: $D=2.2 \text{ h}^{-1}$, Temp. = 35°C , and $\text{pH}=7.0$. (Mean $\pm$ SD)

Time (h)	LPS Method		EPI Method	
	Cell-C (g-C/m ²)	EPS-C (g-C/m ²)	Cell-C (g-C/m ²)	EPS-C (g-C/m ²)
0.0	0.004 $\pm$ 0.004	0.083 $\pm$ 0.010	0.027 $\pm$ 0.006	0.061 $\pm$ 0.0 4
1.3	0.023 $\pm$ 0.044	0.096 $\pm$ 0.044	0.031 $\pm$ 0.004	0.087 $\pm$ 0.060
4.0	0.009 $\pm$ 0.004	0.177 $\pm$ 0.004	0.056 $\pm$ 0.016	0.129 $\pm$ 0.017
7.0	0.046 $\pm$ 0.024	0.247 $\pm$ 0.025	0.112 $\pm$ 0.016	0.181 $\pm$ 0.018
9.0	0.166 $\pm$ 0.063	0.175 $\pm$ 0.065	0.161 $\pm$ 0.033	0.180 $\pm$ 0.039
12.0	0.127 $\pm$ 0.061	0.395 $\pm$ 0.061	0.207 $\pm$ 0.058	0.315 $\pm$ 0.059
15.0	0.150 $\pm$ 0.066	0.562 $\pm$ 0.066	0.228 $\pm$ 0.041	0.484 $\pm$ 0.042
18.0	0.046 $\pm$ 0.020	0.170 $\pm$ 0.020	0.051 $\pm$ 0.016	0.165 $\pm$ 0.018

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