INTERACTIONS OF POLYACRYLAMIDES USED FOR ENHANCED OIL RECOVERY AND RESERVOIR ISOLATES OF THE SULFATE-REDUCING BACTERIUM DESULFOVIBRIO

By

GUY W. SEWELL

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Oklahoma State University

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Thesis approved:

Mary M. Grula
Thesis Advisor

K D Berlin

Helen S. Vishniac

Gary T. Stewart

John W. Wien

Norman N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

The purpose of this research was to examine the possible interactions of sulfate-reducing bacteria of the genus Desulfovibrio, which are easily isolated from many petroleum reservoirs, especially those undergoing water flooding, with solutions of partially hydrolyzed polyacrylamides. Polyacrylamide polymers are useful in the oil industry in enhanced oil recovery processes. These polymers are water-soluble, and form very viscous solutions at relatively low concentrations. Viscous solutions are effective in moving oil out of the pores of the rock, where it is found, toward the wellbore, where it can then be pumped out of the ground. Polymers also can be used in conjunction with surfactants (micellar-polymer floods) to improve oil recovery.

Since the effectiveness of polymer solutions depends on maintenance of the viscosity of the solution, any agent, chemical, physical, or biological, that could destroy or significantly reduce the viscosity would have an adverse effect on oil recovery. There is circumstantial evidence that certain microorganisms can, in the environment of the oil reservoir, directly or indirectly reduce the viscosity of polymer solutions. An example of this is in the produced

water samples from the Wilmington Field, Long Beach, CA, where a polymer flood was in progress (Grula and Sewell, 1982.) Produced water is the water fraction of the water-oil mixture which is recovered from oil wells.

During the course of this flood it was observed that a loss of viscosity (and possibly the polymer itself) and a decrease in oil production was accompanied by a tremendous increase in hydrogen sulfide production. This increase in hydrogen sulfide was not seen in areas of the field which were not associated with the polymer flood. At that point Dr. Mary Grula was contacted by the supervisor of the polymer project, because of the suspected bacterial nature of the problem and her previous work in the area of microbial degradation of compounds used in enhanced oil recovery (chemical flooding) technologies. As this coincided with my entry into the research phase of my program and my interest in petroleum microbiology, I was assigned to work on the project.

Produced water samples from the production wells involved in the polymer flood area yielded high numbers of Desulfovibrio, whereas samples from production wells in the Wilmington field which were not involved in the micellar-polymer project yielded few or no sulfate-reducers. It appeared to us that the presence of polyacrylamide solution in situ might actually encourage or enhance the growth of sulfate-reducing bacteria (SRB's) in some entirely unknown way. Also, it seemed to be possible that the presence of a

high count of SRB's in a reservoir (in addition to other undesirable effects) might somehow be destroying the viscoelastic and viscous properties of the polymer solution, properties which make it useful in increasing the recovery of oil. To confirm the association between the existence (and number) of sulfate-reducing bacteria (SRB's) and the presence of polyacrylamide in oil reservoirs would require extensive testing on samples from various sources. In our limited testing of produced waters from wells in Illinois, Oklanoma, and Texas, we have always found SRB's in reservoirs undergoing polyacrylamide flood. This of course does not mean that they could not be there without polyacrylamide, but in our experience there does appear to be a correlation between the presence of polyacrylamide and numbers of sulfate-reducing bacteria in oil reservoirs.

We undertook this project because we felt that it was important to have a better understanding of the relationship between growth of sulfate-reducing bacteria and polyacrylamide degradation.

Properties of Sulfate-Reducing Bacteria

Sulfate-reducing bacteria have long been a bane of the petroleum industry. Major works in the field of petroleum microbiology (Beerstecher, 1954., Davis, 1967., Atlas, 1984.) deal extensively with problems related to sulfate-reducing bacteria in the petroleum industry. SRB's cause biodeterioration problems in the form of corrosion of

production equipment, and plugging of injection systems and reservoir formations. Allred et al. (1959) reported that SRB's were responsible for as much as 80 percent of the corrosion in oil production equipment seen in some fields. Other investigators have linked these bacteria to corrosion in specific areas, such as steel well casings (Doig and Wachter, 1951), storage tanks (Pankhurst, 1968), injection systems, and transport systems (Atlas, 1984).

The classification of sulfate-reducing bacteria has undergone a major change in the last few years bacause of the discoveries of new metabolically and morphologically distinct bacteria, which reduce sulfur compounds. Bergey's Manuel of Systematic Bacteriology, (Widdel and Pfennig, 1984) lists 9 genera in tnis "pnysiological assemblage of morphologically diverse" bacteria.

Until recently, the sulfate-reducing bacteria isolated from petroleum reservoirs were almost exclusively of the genus Desulfovibrio (Atlas, 1984). However, Antloga and Griffin (1985) have used high pressure anaerobic chambers to isolate Desulfotomaculum from produced waters. All of our isolates to date have been of the genus Desulfovibrio.

The genus Desulfovibrio is described as curved or straight, non-endospore forming, gram negative, motile, obligately anaerobic rods, which possess a respiratory type of metabolism witn sulfate, thiosulfate, sulfite, tetrathionate or other inorganic sulfur compounds as the terminal electron acceptor.

Energy Metabolism

It is the energy metabolism of sulfate-reducing bacteria that is most interesting and unique. Basically it is an oxidative type of metabolism, in an obligately anaerobic habitat. Many parallels can be drawn between anaerobic respiration in Desulfovibrio and a generalized aerobic respiration system (Postgate, 1984). In both systems the catabolism of the carbon source generates electrons, ATP (from substrate level phosphylation) and oxidized end products. Electron transport leads to the generation of additional ATP with oxygen acting as the terminal electron acceptor in aerobic respiration and sulfate, or other more reduced sulfur compounds, serving the same function for Desulfovibrio under anaerobic conditions (Postgate, 1965). In this light, anaerobic respiration seems a more appropriate description of the process than the term sulfide fermentation which is sometimes used (Gottschalk, 1979).

Desulfovibrio oxidizes a limited number of low molecular weight organic acids and some alcohols (Postgate, 1984). These compounds are usually oxidized incompletely to the level of acetate. Many strains can also utilize molecular hydrogen as an electron donor with acetate-CO_2 or yeast extract serving as a carbon source for cell biosynthesis (Odom and Peck, 1984). Desulfovibrio has not been reported to utilize complex organic compounds or to produce extracellular enzymes for their breakdown.

In Postgate's medium C (Postgate, 1984), Desulfovibrio oxidize 2 lactate via pyruvate and acetyl-CoA to 2 acetate and 2 CO_2 and 8 Hydrogen atoms. This reaction is coupled to the reduction of sulfate; 1 sulfate and 8 hydrogens form one sulfide and 4 water molecules. Desulfovibrio generate 1 ATP per lactate oxidized by substrate level phosphorylation via the conversion of acetylphosphate to acetate by acetate kinase. Peck (1962) noted another source of ATP must exist since the reduction of sulfate to sulfide required the equivalent of the 2 ATP's generated from the oxidation of 2 lactates (See Figure 1). He also suggested a respiratory chain as the most likely alternative, and in 1966 demonstrated ATP formation from Desulfovibrio particles during the reduction of sulfite under H_2. In aerobic bacteria respiratory phosphorylation involves vectorial proton and electron transfer. Badziong and Thauer (1980) suggested that the periplasmic enzymes in Desulfovibrio, cytochrome C_3 and hydrogenase, generated electrons for vectorial transfer across the cell membrane for cytoplasmic reduction of sulfate. Odom and Peck (1984) proposed the hydrogen cycling pathway which builds on the chemiosmotic scheme of Badziong and Thauer (Figure 2).

They proposed that H_2 released by the oxidation of substrates diffuses throughout the cell membrane to the periplasm. There the H_2 is oxidized by hydrogenase and cytochrome C_3. The electrons generated are transferred back across the membrane for sulfate reduction and the

FIGURE 1. ATP Balance of Substrate Level Phosphorylation and Sulfate Activation in Desulfovibrio. Two molecules of lactate produce two "high energy" phosphate bonds via substrate level phosphorylation which is equivalent to the requirement for sulfate activation (Peck, 1962).

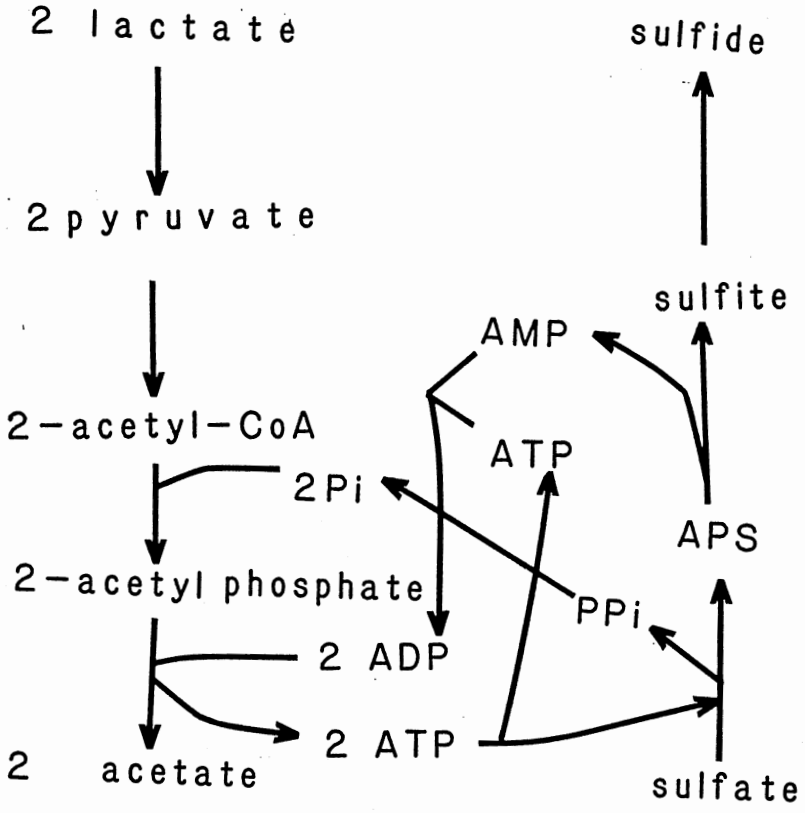
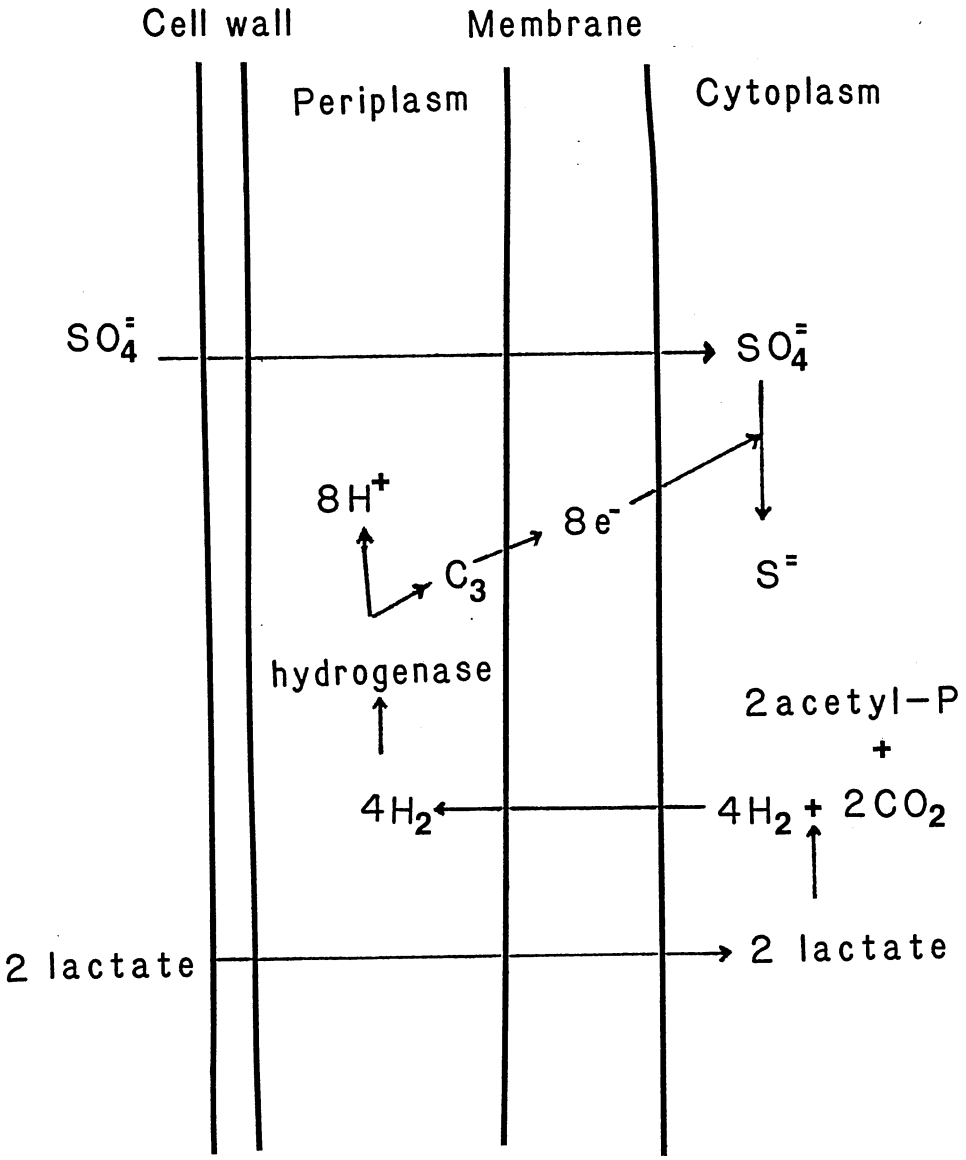

2 lactate
2 pyruvate
2-acetyl-CoA
2Pi
2-acetyl phosphate
2 ADP
2 ATP
2 acetate
AMP
ATP
PPi
sulfide
sulfite
APS
sulfate

FIGURE 2. Hydrogen Cycling in Desulfovibrio. Odom and Peck, 1984.


Cell wall
Membrane
Periplasm
Cytoplasm
$SO_4^=$
$SO_4^=$
$8H^+$
$8e^-$
C_3
$S^=$
hydrogenase
2acetyl-P
+
$4H_2$
$4H_2 + 2CO_2$
2 lactate
2 lactate

protons form a gradent for ATP synthesis. Their observation that spheroplasts of D. gigas are unable to oxidize lactate supports the hypothesis. The hydrogen cycling hypothesis explains the ability of the organism to utilize H_2 produced by other organisms as well as growth under an H_2 atmosphere. Desulfovibrio have been shown to participate in a variety of interspecies hydrogen transfer consortia (Postgate, 1984). It is interesting to note that they seem to walk both sides of the street depending on the environmental conditions, that is, they may be the recipient of the H_2 or the donor. McInerney and Bryant (1981) have reported a co-culture of Desulfovibrio and Methanosarcina barkeri which converted lactate to methane and CO_2 at low sulfate concentrations. In the case reported by Balba and Evans (1980) Desulfovibrio vulgaris was thought to be the recipient of H_2 produced by the utilization of benzoate by Pseudomonas aerugenosa under anaerobic conditions.

Mixotrophic Growth

Early reports in the late 1940's and early 1950's which indicated showed that Desulfovibrio could grow autotrophically, were not confirmed by labelled CO_2 studies (Mechalas and Rittenberg, 1960). This incorrect belief (autotrophic growth) was based on the apparent growth of Desulfovibrio in a mineral media when H_2 and CO_2 were provided. These results are attributed to the ability of the organism to grow mixotrophically and to organic impurities in the medium. Under these conditions, the

organism is able to couple the anaerobic oxidation of H_2, a lithotrophic process, to the assimilation of CO_2. However, preformed organic matter is also required. Sorokin (1966) showed that CO_2 could be assimilated at the expense of H_2 oxidation with sulfate but only with equimolar amounts of acetate. Badziong et al. (1979) showed that the CO_2 - acetate assimilation route was a reductive carboxylation of acetate in the form of acetylphosphate to pyruvate.

Nitrogen Metabolism

Ammonia is the standard source of nitrogen in media for the cultivation of sulfate-reducing bacteria. Nitrate and nydroxylamine are also utilized by Desulfovibrio (Postgate, 1984). Nitrogen fixation, first reported by Sisler and Zobell (1951), is a trait common to many strains of Desulfovibrio. The organic nitrogen metabolism of this organism is not well studied. Postgate (1984) reported no detectable deamination of aspartate, glutamate, L-alanine, arginine, glycine, histidine, isoleucine, leucine, lysine, ornithine, phenylalanine, proline, serine, tyrosine, valine, asparagine or glutamine. Cystine and cysteine were oxidatively degraded to CO_2, NH_3, and H_2S.

Aspects of Oil Recovery

Production of crude oil from underground oil formations requires more than just the drilling of a well. The installation of production facilities and transport of

oil and gas to the market can require enormous sums of money depending on location and conditions. Even so, this may be only the beginning of production costs.

In a large new field the initial production (primary production) normally increases as new wells come on line and may peak in a few months or years after completion of the production system. Thereafter the production rate begins to decline because of decreasing formation pressure, production rates and geological aspects of the formation. Production will continue to decline to a point where it may no longer be economically feasible to continue. This is normally the end of the primary phase of production.

Typically the next step, secondary production, involves the injection of water or gas into the reservoir to "push" oil to the production wells. Eventually, a point is reached where water breaks through the oil bank. From then on water makes up an ever increasing amount of the produced fluids until the amounts of water, relative to oil, is economically unacceptable. This represents the end of secondary recovery. At this point, typically about two thirds (Atlas, 1984) of the original oil in place has not been produced. Even under the best conditions 40-50 percent of the oil is left underground. If this remaining oil were available, or even a fraction of it, it would go a long way toward supplying domestic oil need for many years to come.

It is this oil that is targeted by the third stage of

production, frequently referred to as tertiary recovery. The secondary and tertiary stages of oil production are sometimes linked and referred to as enhanced oil recovery (EOR).

There are a variety of technologies used in tertiary recovery. The two methods which involve the use of polymers as mobility control agents are polymer flooding and micellar flooding.

In polymer flooding the role of the polymer solution is the same as water in water flooding, but with important differences. The polymer is used to avoid or decrease the occurrence of one of the major problems associated with water flooding, that of trapping oil due to the tendency of injection water to channel through areas of high permeability. The increased viscosity of a polymer solution results in a more efficient "pusher" or mobility control agent because of the formation of a more stable displacement front. Expressed another way, the goal of the polymer flood is to lower the mobility ratio of the displacing fluid which in turn improves the sweep efficiency and avoids fingering. The mobility ratio is the mobility of the displacement fluid (polymer water) divided by the mobility of the formation fluids (oil-water). The mobility ratio for a water flood is typically 10:1 to 50:1 or even higher. Ideally, a mobility ratio of 1:1 or even lower would favor oil displacement and improve the sweep efficiency and thus the total amount of oil produced

(Patton, 1971).

A closely related EOR method is the micellar-polymer flood. In this method a water/brine/surfactant/co-surfactant mixture is injected to free residual oil in the formation. This is followed by a polymer-water solution which serves as a mobility control agent to push or sweep forward the oil emulsion which has been formed. Here the polymer solution serves not only as a drive but also as a buffer between the drive water and the emulsion. The viscosity of the polymer-water solution is graded to produce high viscosity immediately behind the emulsion, decreasing to a lower viscosity preceding the water drive by varying the concentration of the polymer (Gabriel, 1979). A graduated polymer slug achieves better mobility ratios and is more cost efficient.

Gabriel (1979) lists the technical requirements of a thickening agent for enhanced oil recovery applications, they are:

> a) the thickener must produce a substantial increase in viscosity at very low concentrations, but it should not produce a viscous gel which would simply clog up the pores of the oil formation;
>
> b) ideally, the thickening agent should be pseudoplastic. In other words a small amount of shear force will produce a marked increase in flow and corresponding decreases in viscosity, but once the shearing forces are removed the thickening effect reasserts itself. It is fairly obvious that this characteristic is most desirable in the oil world, otherwise the products would be very difficult to handle through pumps and filters - as well as in the narrow, oil-filled capillaries of the producing structure itself, without requiring impractical

pressures;

c) the product must be chemically and thermally stable and be effective over a period of years. The physical conditions in an oil well are not congenial, with temperatures approching 100°C or more, together with very high salianity levels from surrounding water bearing formations;

d) the product must also be consistent in quality. It is often said that oilmen will put anything down their wells, if they think they can get more oil out. However, this old adage should not be taken too literally as modern oilmen are quite fussy about any product which is introduced into their well systems;

e) it must be cost effective - measured by the value of the recovered oil over the life of the project.

To this list another consideration must be added: the resistance of the polymer to breakdown by microorganisms. Severe problems have been reported with polymer floods which appear to be bacterial in nature (Grula and Sewell, 1982b). The areas effected have included above ground injection and holding systems and the reservoir itself. Although research has led to methods for controlling bacterial growth at the polymer's expense, conditions and practices at the injection sites are a far cry from the laboratory environment. It was the relationship between the presence of polyacrylamide in EOR and increased SRB associated problems that led to our involvement in the study of these problems.

Two major groups of polymers have been used as thickening or mobility control agents in EOR. The first group consists of the biopolymers. These are, for the most part, high molecular weight natural polysaccharides with

various side groups. The most important of these is xanthan gum, or simply xanthan, which is an extracellular slime produced by the aerobic bacterium Xanthomomas campestris (Gabriel, 1979, Martin, et al., 1982). The positive aspects of xanthan are its high thickening power per unit of concentration, stability to high ionic strength environments and high temperatures, and resistance to shear degradation. The problems associated with this substance are its sensitivity to bacterial degradation both aerobically and anaerobically, production problems in producing a commercially acceptable product with little cellular debris (which could be a potential formation plugging agent) and its relatively high cost.

The other group, the chemical polymers, is best represented by the polyacrylamides (Gabriel, 1979, Martin et al., 1982). These synthetic polymers are chemically produced from acrylamide and then partially hydrolyzed, or produced as a co-polymer with acrylates. The limitations of polyacrylamide polymers in EOR are their shear sensitivity and ionic nature, which result in undesirable effects in high ionic environments and absorption to reservoir material. On the positive side is their ease of synthesis and chemical modification, low cost, high viscosity, and their supposed resistance to anaerobic breakdown by bacteria.

Since both types of polymers have their advantages and disadvantages it has been felt that both types will play a

role in EOR. The most important factor in choosing between them when reservoir conditions are not exclusive is cost. The major advantage of polyacrylamides over xanthan is price, but if unforseen factors affect the cost-effectiveness of polyacrylamides then they may see a decreasing share of EOR projects.

Cost/benefit ratios also determine whether EOR methods may be used at all (Gabriel, 1979). Enhanced oil recovery (EOR) methods are very expensive. Large scale EOR projects may take many years before production profit begins to compensate for the high initial costs. EOR research boomed in the late 1970's in response to embargos of foreign oil (real and threatened) and high crude oil prices. These factors along with the nature and maturity of most domestic fields have led to a greater involvement by U.S. oil companies and the federal government in the use of EOR. The precipitous drop in crude oil prices, beginning in early 1986, to a present level of about $15.00 to $18.00 per barrel and uncertain interest rates make the large capital outlays for EOR methods less attractive. However, there are certain parallels between the increasing dependence of the U.S. on foreign oil in the 1960's, due to the low cost, and the increasing usage of foreign oil now with cutbacks in domestic production and exploration in this era of the "oil glut". Considering the instability of oil prices during the last 20 years, a market for high-priced domestic oil may reappear as quickly as it

disappeared. Research and development in EOR should not be abandoned.

Physical/Chemical Properties of Polyacrylamide

There are several routes to achieving the deserved goal of a lower mobility ratios (Gabriel, 1979): 1) decrease the effective permeability of the reservoir rack to water, 2) increase the effective permeability of reservoir rock to oil, 3) decrease oil viscosity, 4) increase viscosity of injected water.

The first and last methods are involved in the use of polyacrylamides. Increasing injection water viscosity would seem to be a straightforward function for polyacrylamides. However, partially hydrolyzed polyacrylamides produce apparent viscosities during flow in a porous medium (simulated by the screen viscometer) of 5 to 20 times that measured by a conventional viscometer (Dominguez et al., 1977). This difference between shear viscosity and flow behavior has been termed the resistance factor. Two mechanisms have been proposed to explain the observations; one, polymer molecules adsorbed on the pore matrix are hydrophilic and restricted the flow of water, or two, that the polymer reduced permeability by retention do to mechanical entrapment. Although partially hydrolyzed polyacrylamides are anionic and repel the negative charges of the reservoir matrix (Patton and Coats, 1971) there is evidence that the polymer is retained due to mechanical

entrapment and adsorption (Szabo, 1975; Smith, 1970).

Herr and Routson (1974) have studied the anomalous viscosity behavior of high molecular weight polyacrylamides. They suggested that the macromolecular structure is the basis of the observed effects. Supermolecular macro-fibrillar structures were observed by electron microscopic examination of dilute polymer solutions. These structures far exceed the theoretical size of the polymer molecules and are thought to be highly solvated and associated polymer molecules. The nature of the supermolecular structure is not entirely clear. In water the macro-fibrils display considerable polymer-polymer entanglements and/or associations. Alterations in viscosity cause changes in the observed morphology of the polymer. The addition of NaCl causes a decrease in observed entanglements. Mechanically shearing the polymer via filtration resulted in a decrease in size and number of macro-fibrils and an increase in micro-fibrils. Thermal degradation of the polymer produced similar results.

The chemical stability of partially hydrolyzed polyacrylamide has been extensively investigated (Shupe, 1981. Foshee et al., 1976). Metal ions cause severe degradation of polyacrylamides in the presence of oxygen. However, polymer solutions containing metal ions in an anaerobic chamber showed a loss in viscosity of less than 4% over 2 months.

Microbial Degradation of Polyacrylamide

Since polyacrylamides are xenobiotic in nature, it was presumed that they would be resistant to microbial degradation. While the work of Grula and Huang (1981) and Martin et al. (1982) showed that the polymer was not immune to aerobic modifications it was still considered resistant to anaerobic breakdown owing to its covalent carbon-carbon backbone (in contrast to the anhydro-bonds of most biopolymers) and its molecular size. The longest $-CH_2-$ chain subject to attack by hydrocarbon-degrading organisms is approximately 40 carbons in length (Atlas, 1984). Hydrocarbon-degrading microorganisms oxidize a terminal carbon to a primary alcohol, then to an aldehyde and finally to a carboxylic acid, the latter then being subject to B-oxidation which cleaves off two carbon units (Atlas, 1984). Such a process has never been observed with polyacrylamides, whose chain length may be in the range of thousands of carbon atoms. This size precludes the passage of the molecule through the cell membrane.

Field observation by workers who handle these polymers indicate, however, that they somehow enhance microbial growth. In this research we have attempted to examine the interactions of these polymers with Desulfovibrio.

CHAPTER II

MATERIALS AND METHODS

Media

Most of the media use in these studies are those described by Postgate (1979)(see Appendix A). Medium B was used for enrichment, maintenance and also for the most probable number determination for SRB's. We have recovered higher numbers of SRB's using medium B as compared to the API medium or to Allred's medium, both of which are widely used in the petroleum industry. Medium B always contain a precipitate. The blackening of this precipitate is an indication of H_2S production.

Medium C was used for polymer degradation and growth studies. Citrate prevents precipitation of iron and allow photometric measurements of growth. Since medium C contains no reducing agents, it is only used under strict anaerobic conditions.

Medium F is a solid medium used in the form of plates or deeps for the isolation and purification of SRB cultures, which produce characteristic black colonies on it.

Medium D is used for sulfate-free growth studies,

mixotrophy experiments and alternate carbon source testing.

Organisms

The organisms used in these studies are sulfate-reducing bacteria of the genus Desulfovibrio. They most nearly resemble the species D. desulfuricans which is the most common SRB isolated from produced water. Strain SB2 is the most closely studied of our isolates, Table I gives biochemical and other properties useful for identification.

All isolates have been designated Desulfovibrio spp. This identification was based on their cellular morphology, absence of spores, dissimilatory sulfate-reduction, and positive desulfoviridin test (Postgate, 1984). Table II gives a list of SRB cultures, their source and state of purification.

Anaerobic Methods

Anerobic conditions were achieved through the use of a National Appliance Model 365Ø or Forma Scientific Model 1Ø241Ø17 AOM anaerobic chamber. Anaerobiosis is maintained in both chambers by flushing material which is to enter the chamber with N_2 gas to displace oxygen, and by circulating the chamber interior atmosphere, which consists of 1Ø percent H_2 , 5 percent CO_2, and 85 percent N_2, over a palladium catalyst. Any O_2 present reacts with H_2 on the catalyst surface to form water, which is removed by a desiccant compound ($CaSO_4$, $CaCl_2$).

TABLE I

CULTURAL AND BIOCHEMICAL PROPERTIES OF STRAIN SB-2

Strict anaerobe	
Gram (-) short rods, vibrio to spiral in shape	
Motile (fast, progressive movement)	
single polar flagellum	
Salt tolerance Ø-1Ø% (w/v) NaCl	
Reduce sulfate to sulfide	
Non-spore forming	
Desulfoviridin (sulfite-reductase) positive	
Growth in:	
medium B (Postgate)	+
minus lactate	-
minus lactate with Ø.1% J332	-
with Ø.1% J332	+
Growth with	
lactate minus sulfate	-
pyruvate minus sulfate	+
choline minus sulfate	-
Hibitane resistance 5-3Ø mg/l	

TABLE II

SRB CULTURES, SOURCE AND PURIFICATION STATE

Culture	Source	E = enrichment P = pure culture
T - series	Produced water Wilmington Field, CA	P
SB-2, BB	" "	P
U9	Sandpack used for Polymer filtration. Robinson, Ill.	P
API	Desulfovibrio desulfuricans type culture. American Petroleum Institute	P
ATCC	Desulfovibrio desulfuricans type cultures. American Type Culture Collection	P
Slim, Am	Polymer holding tank Texas	E
P - series	Produced water Burbank field, Oklahoma	E/P
L - series	Lake bottom sediment (100ft.), Tenkiller, Oklahoma	E
T-4	Produced water, Texas	P

Anaerobic conditions are monitored through the use of a Forma Scientific Model 190000 Oxygen indicator or by the use of BBL Gaspak disposable anaerobic indicator strips. Both utilize a sugar/methylene blue redox reaction to indicate of the presence of O_2. Methylene blue is completely reduced to the leuco form at a potential of 50mV. Resazurin (Resourufin, Eo -51mV) and Indigo Carmin (Eo - 125mV) based redox systems were also used. Solution redox potentials were measured with an Orion model 98-07 redox electrode.

Inoculation, incubation and most manipulations were done inside the anaerobic chamber whenever possible. When limitations rendered this impossible, certain modifications of the Hungate technique were used (Holdeman et al., 1977). Cultures and media transported outside the anaerobic chamber were contained within screwcap flasks or tubes with tightly sealed teflon closures (Fisher Scientific) or in butyl rubber septum Hungate tubes (Bellco).

Growth Measurements

The measurement of growth of sulfate-reducing bacteria involves certain innate problems. Initiation of growth requires an oxidation-reduction potential of approximately -100mv. Thus, in nonreduced media, initiation of growth is somewhat unpredictable. Also SRB's rarely (if ever) exhibit exponential growth (Postgate, 1984). This may be because one of the major metabolic products (sulfide) is

toxic to the cell, and also the relatively low potential energy of sulfate reduction. Sulfide also interacts with one of the key media constituents, ferrous iron, to produce an insoluble precipitate which interferes with optical measurement of growth. The presence of polyacrylamide complicates the situation, because, for reasons not understood, the polymer suspends ferrous sulfide in the medium. Sulfate-reducing bacteria do not grow well on solid media (we get much higher most probable number (MPN) counts than plate counts on the same samples). This limits the use of plate counts as a method for enumeration. We have used a modified MPN procedure with some success. In the modified MPN procedure heavey blackening of Postgate's medium B is used as a positive indication of SRB growth. The presence of sulfide ions and polyacrylamide render protein assays difficult. Where protein concentrations were determined the method of Lowry et al. (1951) was used.

The concentrations and nature of medium components and metabolic products also cause difficulties with dry weight measurements. Particulate iron sulfide could not be removed from the cells by washing.

The use of Postogate's medium C allows optical density measurements, since citrate, which form a very stable chelate with Fe^{++}, is incorporated in the medium. This prevents the precipitation of FeS on the walls of vessels to some extent. However the concentration of iron sulfide, which in most cases is proportional to growth (Spurry et

al., 1957), always has some effect on the observed O.D. reading. Because of such problems, we have placed more emphasis on the time of initiation and initial rates of growth in our studies. Medium C is a nonreduced medium and as such does not generate the necessary low redox potential for initiation of SRB growth. Several factor influence the initiation of growth under these conditions; the size of the inoculum, the age of the culture used, and the redox conditions in the anaerobic chamber. Desulfovibrio cultures tend to become heterogenous with age due to clumping of the organism and particulate iron sulfide. Do to these factors and also because the amounts of observed stimulation may be small, we used the following set of standard procedures; 3 to 10 replicates for each set of experimental conditions were used, the results from each experiment were considered individually and were not compared to those run on different days, all replicates of all experimental groups in a given experiment recieved the same inoculum with regard to amount and source and were inoculated in a random sequence, all experiments were repeated two or more times.

Gas Chromatography

Gas Chromatography was used to analyse the metabolic products of Desulfovibrio. The procedures used were basically those of Holdeman et al. (1977) in the 4th edition of the Virginia Polytechnic Institute Anaerobe

Laboratory Manual. Briefly, the analysis of volatile fatty acids utilized an ether extraction of the culture medium after acidification. Analysis of non-volatile acids required the formation of methyl-ester derivatives. This was accomplished by heating the culture medium with methanol and sulfuric acid. The medium was then extracted with chloroform.

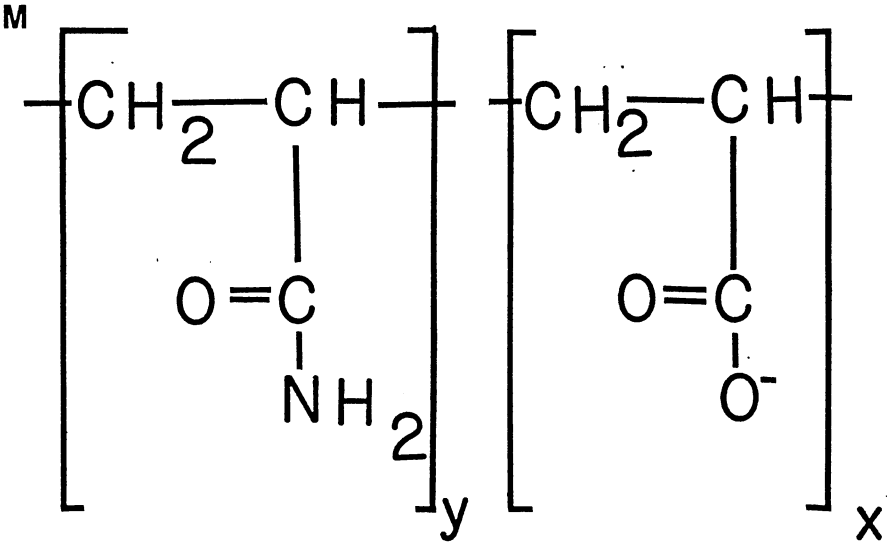
A Tracor model 560 or a Perkin-Elmer Sigma II series gas chromatograph wa used with a Hewlett Packard model 3390A or Perkin-Elmer M2 integrator. Column packing was GP 10% SP-1000/1% H_2PO_4 on 100/120 chromosorb W AW. (Supelco Inc.) with a 6ft x 2mm glass column was used. An isothermic program at 150°C was used with a flame ionization detector at 200°C and an injection temperature of 155°C. Nitrogen was used as the carrier gas at a flow rate of 50cc/min. 1 meq standards of volatile and non-volatile acids were used for identification.

Polymers

The polymers of interest in these studies were partially hydrolyzed polyacrylamides which consist of long chains of methylene groups with amide or carboxyl side groups on alternating carbons. See Figure 3. The molecular weights of the polymers used ranged from 3×10^6 to 1×10^7 (Dowell Division, Dow Chemical Company). The degree of hydrolysis ranged from 0 to 100 percent. The degree of hydrolysis is the ratio of carboxyl side groups

FIGURE 3. Chemical Structure of Partially Hydrolyzed Polyacrylamide. The degree of hydrolysis is the ratio of acid to amide side groups (x/y).

HPAM



to amide side groups. In this context "Hydrolysis" refers to the reaction of the amide group with water to form a carboxylic acid and ammonia, which may then react to form a carboxylate group and ammonium ion. The ammonia released may serve as a source of nitrogen for some bacteria (Grula and Huang, 1981). A completely unhydrolyzed polymer is referred to as "polyamide", whereas the homopolymer with all carboxyl side groups is polyacrylic acid. Table III gives a detailed description of the polymers used in these studies.

Preparation of Polyacrylamide Solutions

The method of preparation of polyacrylamide solutions has a great effect on their final viscosity. Several techniques are available for solution preparation. We have used a modification of the procedure outlined by Foshee et al. (1976), as follows: A laboratory magnetically driven stirrer is used to create a vortex in the appropriately sized beaker of cold glass distilled water, using as large a magnetic stirring bar as can be accomodated by one half the diameter of the beaker. The vortex is generated slightly off center by the positioning of the beaker on the stirrer such that the bottom of the vortex is destabilized by the beaker wall and does not reach the bottom of the container. Beakers are preferable to flasks because they generate more surface area per volume. The dry polymer (powder) is then sprinkled uniformly onto the shoulder of

TABLE III

POLYACRYLAMIDES USED IN STUDIES

Designation	Molecular Weight	Degree of Hydrolysis
J332	$9\text{-}10 \times 10^6$	25-35%
J333	$6\text{-}7 \times 10^6$	25-35%
J334	$3\text{-}4 \times 10^6$	25-35%
J279	3×10^6	1-4%
Non-Hydrolyzed (PAM)	?	-
Polyacrylic Acid (PAC)	?	(100%)
Cationic (CPA)	4×10^6	-

the vortex. The addition of the polymer should be completed in 60 seconds or less to prevent the developing viscosity from interfering with the proper wetting and disperson of the dry polymer crystals. As soon as all the polymer has been added the stirrer is slowed to approximately 60 rpms to avoid mechanical shearing. The solution is stirred at this rate for 5 to 10 minutes until the partially dissolved particles, referred to as "fish eyes", have essentially disappeared. The polymer solutions are then placed, covered, in the cold room over night or until needed. We have experienced no detectable microbial growth on cold stored polymer-water solutions stored as long as 2 months. Nevertheless no solutions were used after 2 weeks of storage. Before the polymer stock was used to make polymer medium solutions or diluted for physiochemical studies, it was warmed to room temperature and passed through Spectra/Mesh Nylon screen of 70 micron mesh openings (Fisher) to produce a homogenous solution. Copper and bronze screens of 100 to 200 mesh were also tried and produced similar results, but were discarded in favor of an inert screen because we feared the possible interactions of the polymer with metal ions. These polymer stocks could then be used as the base for polymer/media solutions by the addition of 10x medium components which were dispersed by gentle stirring. The pH adjustment of polymer medium is time-consuming because the solution viscosity affects the response time of the electrode.

Polymer/medium and polymer/water solutions of concentrations up to 0.2 percent (w/v) of polyacrylamide were prepared in this manner. For the higher concentrations used in the nutritional studies (up to 1.0 percent) another procedure was used. 800 ml of distilled water was added to a Waring blender and, with the machine operating at the highest setting, 6 to 10 grams (0.6 to 1.0 percent) of polymer was added. The blender was allowed to run another 60 seconds. To this solution was added an additional 200 ml of distilled water. This mixture was then stored in the cold room. To insure final mixing and a usable viscosity range, the solution was treated by sonication with a Branson Sonifier Model 350, at 50% power, for varying lengths of time. The solution was then filtered as previously described.

This treatment invariably induced shearing of the polymer and therefore was not used for viscosity reduction experiments.

Viscosity Measurement

Because of sensitivity of solution viscosity to the degradation of polymer molecules and also since viscosity is the key parameter in whether or not a polymer solution will be useful as a mobility control agent in enhanced oil recovery, we have concentrated heavily on viscosity measurement in these studies. Two types of viscosity measurements have been used to monitor the polymers:

Brookfield viscosity and screen factor. Both have advantages and disadvantages.

Brookfield Viscosity

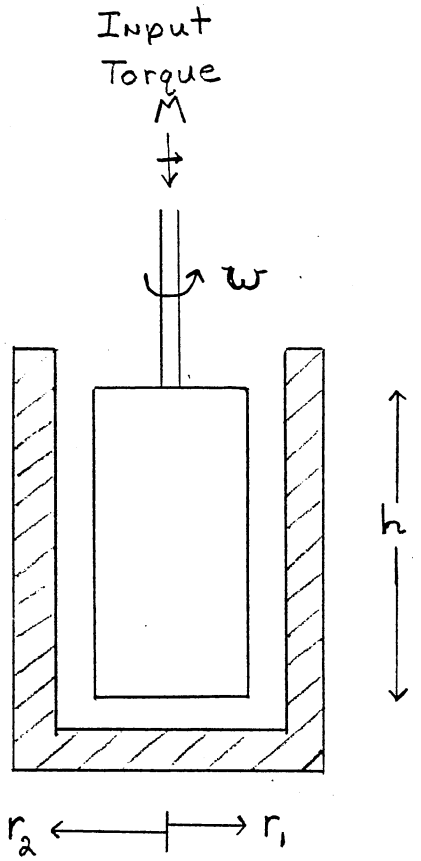
A Brookfield viscometer measures "true" solution or absolute viscosity by rotating a cylinder or disc in a fluid and measuring the torque necessary to overcome the viscous resistance to the induced movement. This is accomplished by driving the immersed element, called the spindle, through a beryllium copper spring. The degree to which the spring is wound is indicated on a numerical scale and is proportional to the viscosity of the fluid for any given speed of rotation and spindle type. Standard calculations then yield solution viscosity (see Figure 4). The Brookfield viscometer used was a model LVT with or without an ultra low viscosity adaptor. The advantages to the Brookfield are that it is relatively fast, and once the operational techniques are learned, it is easy to operate. It also requires no sample preparation. The disadvantages are its decreased sensitivity as compared to screen factor, limitations on sample size and increased difficulties in operation in an anaerobic chamber.

Screen Factor

Screen factor is not a measurement of true solution viscosity; rather, it is a measurement of the viscoelastic flow properties of the polymer solution. It has been shown

FIGURE 4. Brookfield Viscometer.
The Brookfield viscometer measures solution (shear) viscosity.

Brookfield Viscometer



$$\eta = \frac{M}{4\pi h \omega}\left(\frac{1}{r_1^2} - \frac{1}{r_2^2}\right)$$

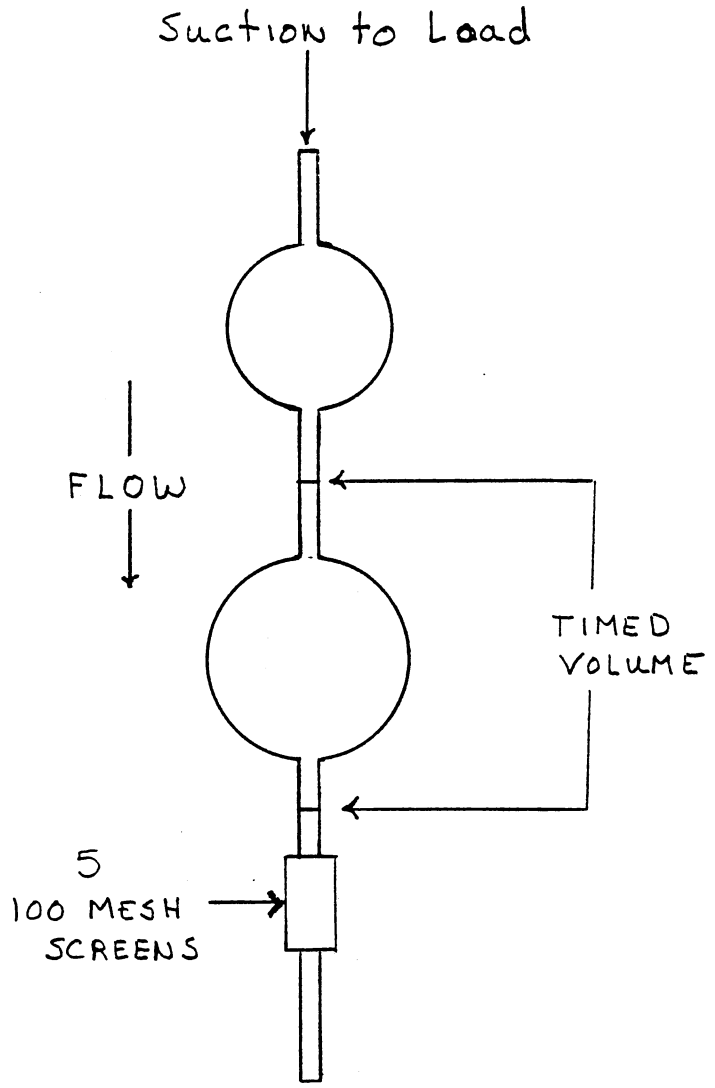
that the "screen factor" of a solution, determined with a screen viscometer, is empirically correlated with the resistance effect produced in reservoir formations, and that the screen factor is more sensitive to changes in polymer quality than is solution viscosity. A screen viscometer measures the time it takes a given volume of a polymer solution to flow through a set of five small, closely positioned, 100 mesh screens (see Figure 5). The screen factor of a solution is defined as the flow time of the polymer solution divided by flow time of the same volume of water at the same temperature. A major disadvantage is that all solutions must first be passed through a 100 to 200 mesh screen to remove particles which might clog the screens. Another problem is the excessive time needed to clean the viscometer between samples and in the run time itself. It is not unusual to accomplish only 1 to 2 measurements per hour. The screen viscometers used in these studies were a generous gift of the Dowell division, Dow Chemical Company, U.S.A.

Analysis of Polyacrylamide Solutions

The concentration of partially hydrolyzed polyacrylamides in solution was determined by the "bleach" method as outlined by Foshee et al. (1976). Polyacrylamide reacts with sodium hypochlorite (bleach) at low pH, forming insoluble chloramide reaction product which produces a stable colloidal suspension. The resulting measurable

FIGURE 5. Screen Viscometer.

SCREEN VISCOMETER



$$\text{SCREEN FACTOR} = \frac{\text{FLOW TIME POLYMER SOLUTION}}{\text{FLOW TIME WATER}}$$

turbidity is a function of polymer concentration and is not strongly influenced by molecular weight or by degree of hydrolysis. Some corrosion inhibitors and demulsifiers will interfere with these measurements. The procedure is accurate over a range of Ø to 5ØØ ppm and is as follows. One ml of polymer solution is mixed gently with 1 ml 5N acetic acid and 1 ml of 1.31% sodium hypochlorite (25% v/v Clorox). Let stand for 5 minutes. Read at 47Ønm in a Bausch and Lomb Spectronic 2Ø spectrophotometer. This method was designed for use with brine/polymer solutions and works well with produced waters.

Ammonium ion concentration in polyacrylamide solutions was determined by the indophenol blue reaction as outlined by Hansen and Phillips (1981).

CHAPTER III

RESULTS

Examination of Produced Water Samples

500 ml samples of produced fluids were shipped to us from the Wilmington Field by the project engineer. These samples were mostly water with a 10 to 20 percent (v/v) overlay of oil. The samples were not refrigerated in transit and for this reason the numerical analysis of SRB's must be held somewhat in doubt, as this could have created conditions for their growth. The results of Most Probable Number Counts of SRB's are given in Table IV. The T-series wells were production wells in the micellar-polymer project area, the others although water flooded were not exposed to the polymer. Figures 6-9 are electron micrographs of SRB isolates from the Wilmington field taken by Dr. Sue Woods.

At this time we began to receive produced water samples (solicited and otherwise) from wells involved in polymer floods from around the country. All polymer flooding operators we contacted reported increased SRB related problems. Produced water samples from polymer floods invariably yielded large numbers of SRB's from

TABLE IV

MOST PROBABLE NUMBER COUNTS OF SULFATE-REDUCING BACTERIA IN PRODUCED WATER SAMPLES FROM THE WILMINGTON FIELD

Well	MPN-SRBs/ml Produced Water
T-1Ø0	$4.9 \times 1Ø^{2}$
T-1Ø1	$2.2 \times 1Ø^{6}$
T-1Ø2	$7.9 \times 1Ø^{1}$
T-1Ø3	$4.9 \times 1Ø^{5}$
T-1Ø4	$3.5 \times 1Ø^{4}$
T-1Ø5	$4.9 \times 1Ø^{3}$
M 486	Ø
M 526	Ø
M 711 E	24

Heavy darkening of Postgate's medium B under anaerobic conditions was used as positive for SRB growth. Tubes were incubated anaerobically at 37°C for 2 weeks or until positive darkening was seen. T - series wells were involved in the polymer flood. M - series wells were of the same reservoir formation but in areas of the field isolated from the polymer flood.

FIGURE 6. Desulfovibrio sp, T-103 SB-2. (30,000x) isolated from produced water, Wilmington Field, California.

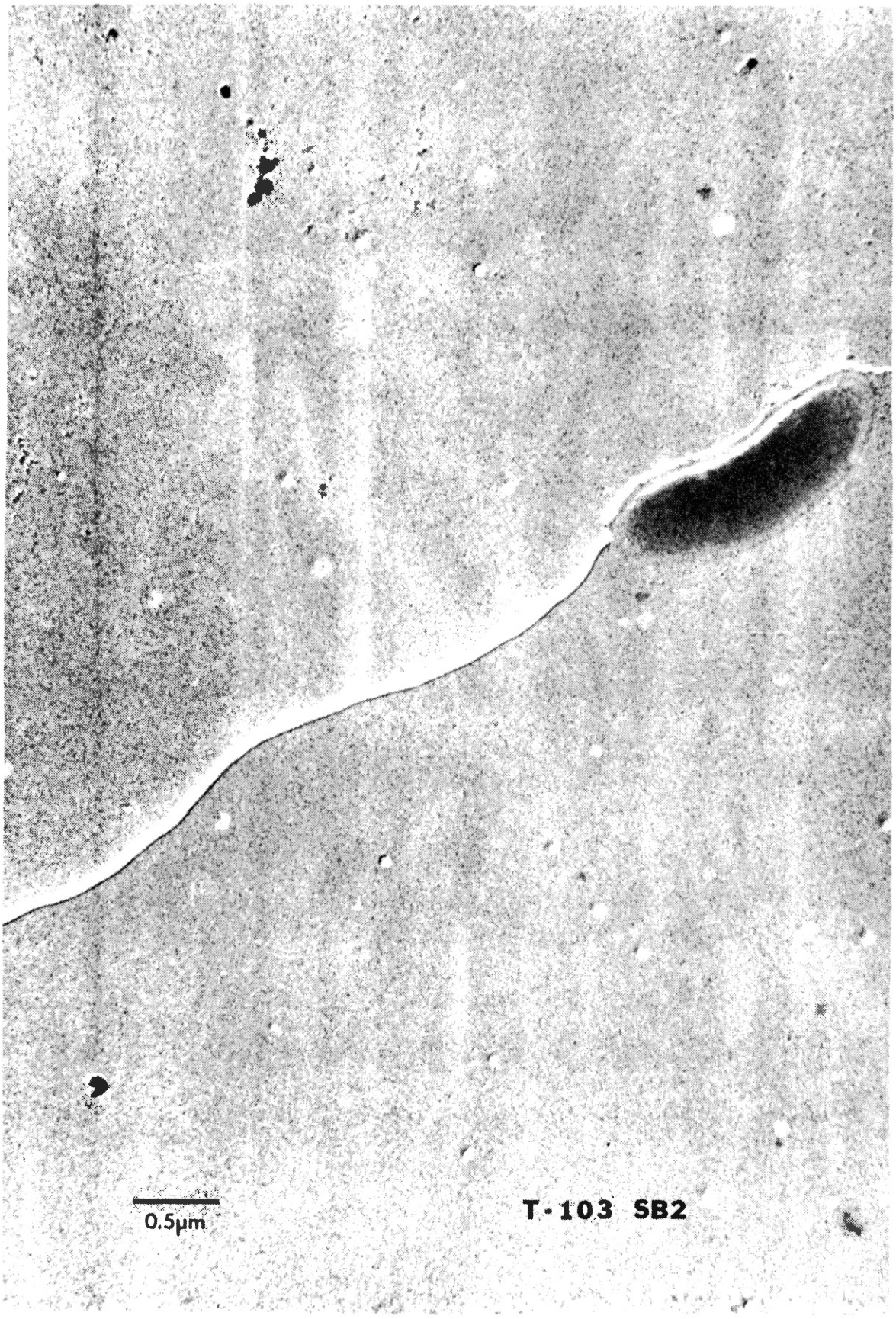

0.5µm
T-103 SB2

FIGURE 7. Desulfovibrio sp, T-104 P. (30,000x) isolated from produced water, Wilmington Field, California.

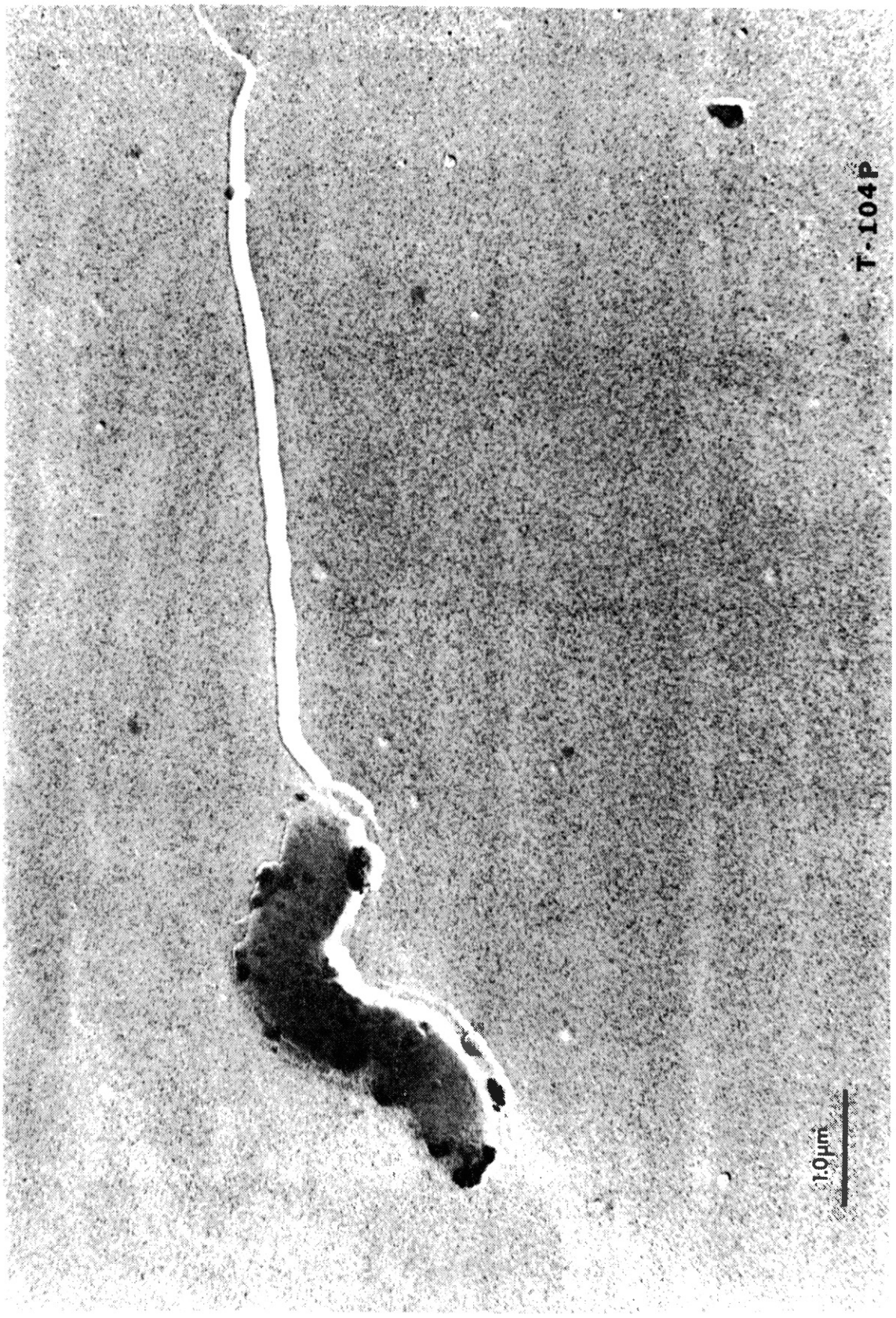

T-104P
1.0μm

FIGURE 8. Desulfovibrio sp, T-103 BB. (50,000x) isolated from produced water, Wilmington Field, California.

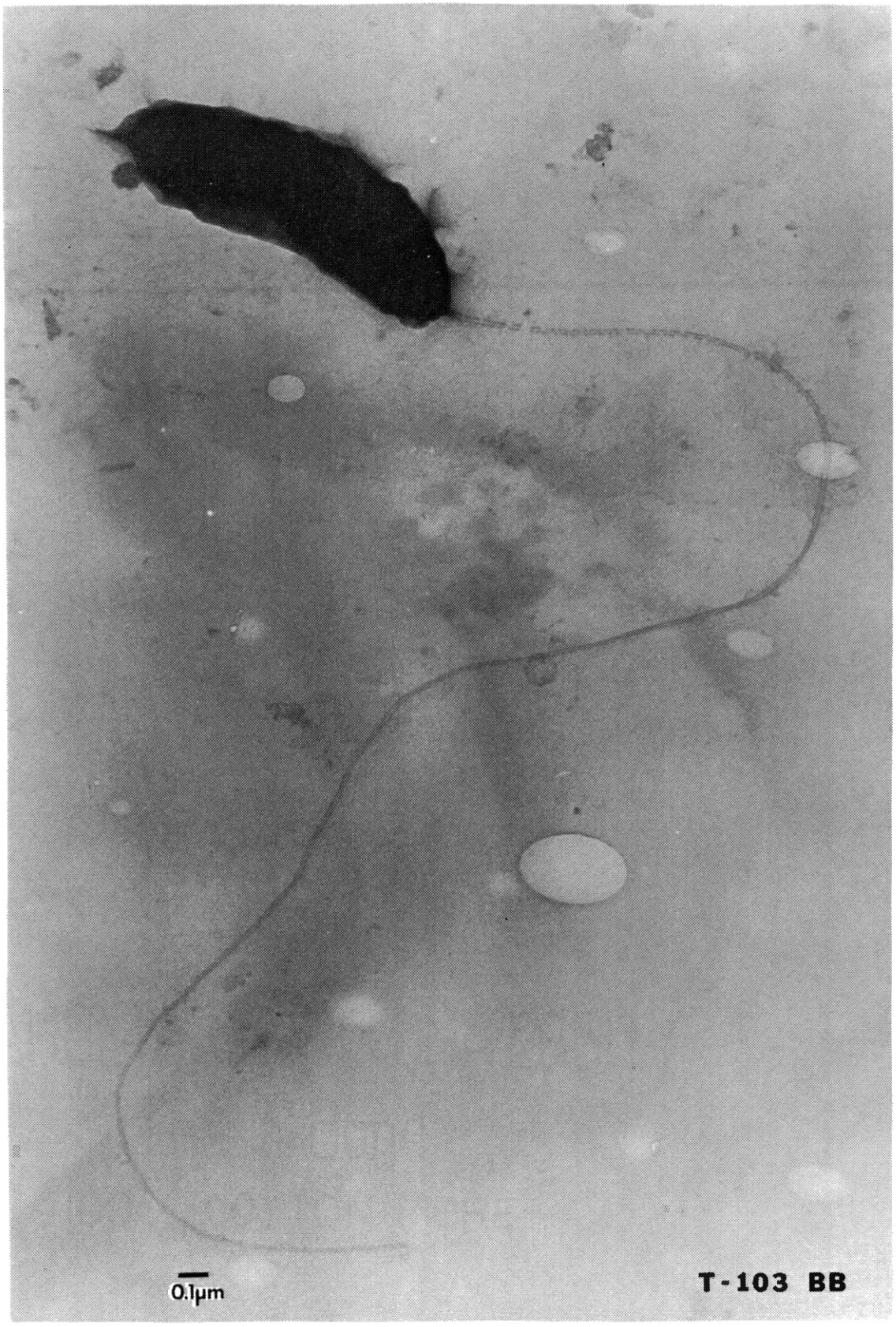
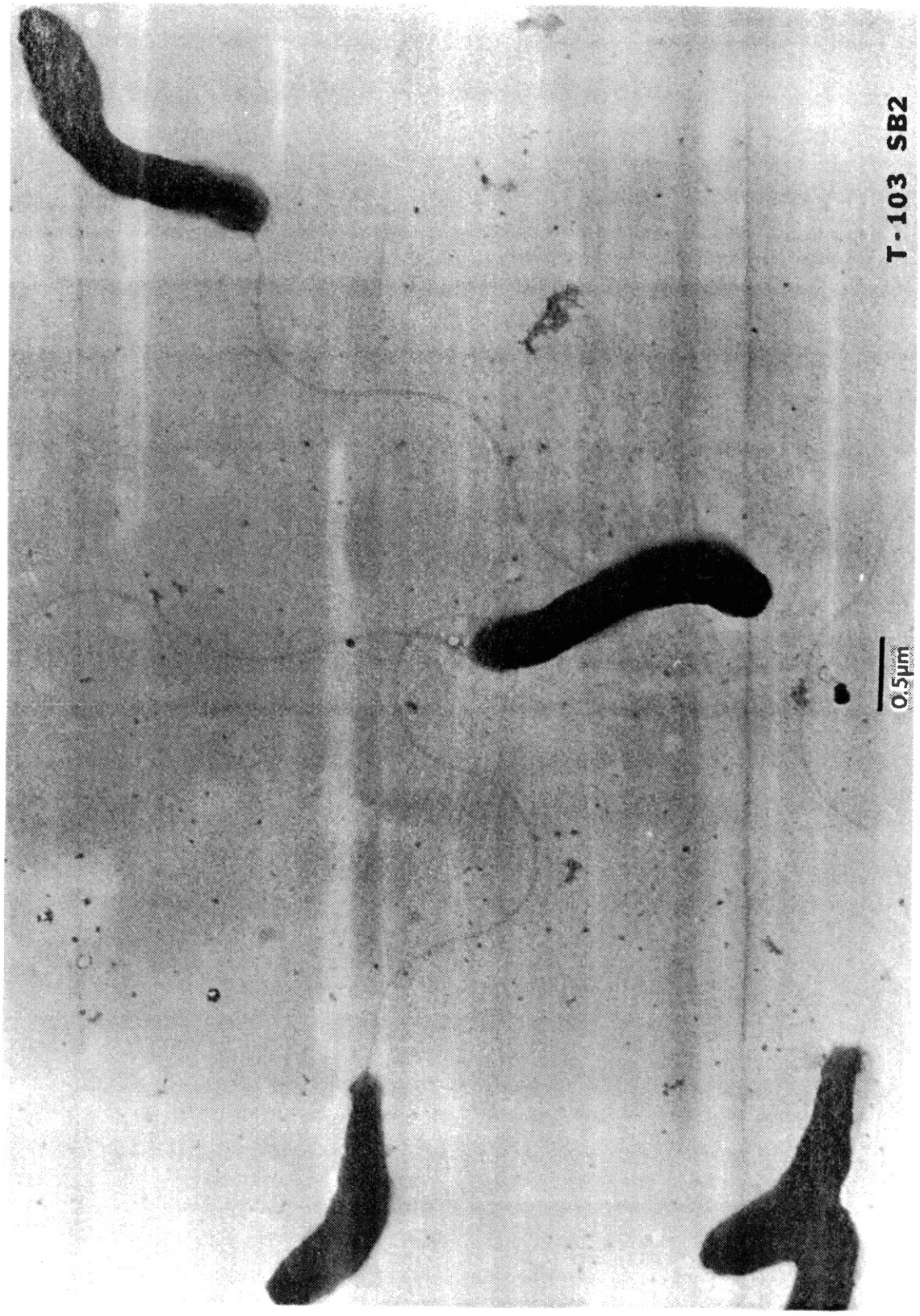

0.1μm
T-103 BB

FIGURE 9. Desulfovibrio sp, T-103 SB-2. (24,000x) isolated from produced water, Wilmington Field, California.


T-103 SB2
0.5µm

routine enrichments while samples from non-polymer associated wells may or may not yield SRB's. Water samples from polymer storage tanks and injection systems also yielded significant numbers of SRB's.

In the fall of 1982 we were invited to collect and examine produced water samples from wells in the Burbank field operated by Phillips Petroleum. Some of the wells tested were involved in a polymer flood. Table V gives a summary of those examinations. Filtered, biocide treated lake water was used for mixing the polymer solution, which was then injected into the reservoir. Off-tract wells are those not involved in the polymer flood. Pre-breakthrough refers to wells in the polymer flood area but which are ahead of the polymer-oil front. Post-breakthrough wells are behind the polymer front and are producing polymer-oil-water mixture. Both Table IV and V indicate a relationship between SRB numbers and polymers.

STIMULATION OF GROWTH

Growth of Desulfovibrio in the Presence of Polyacrylamides

The relationship between the presence of polyacrylamide and the increased numbers of recoverable sulfate-reducing bacteria in produced water samples led to laboratory measurements of the growth of reservoir isolated Desulfovibrio species in the presence of polyacrylamide, in order to determine if the enhancement of growth of the

TABLE V

ENUMERATION OF SULFATE-REDUCING BACTERIA
IN THE BURBANK FIELD

Sample	SRB's/ml
Injection water	Ø.Ø37 + Ø.Ø6
Off-tract or Pre-breakthrough wells	3.8 + 5.3
Post-breakthrough wells	76.6 + 4Ø.4

Maximum recovered SRB's using MPN counts with Postgate's medium B and plate counts with Postgate's medium E and F, Iverson's medium, and API medium. All media were incubated anaerobically at 35°C for 2 weeks or until maximum growth. Black colonies on solid media and heavily darkened tubes of medium B were considered indicative of SRB's. Samples were collected and processed under anaerobic conditions.

bacteria by polyacrylamides could be demonstrated in the laboratory. Polyacrylamides used in enhanced oil recovery vary in molecular weight, and degree of hydrolysis. The amide side groups may also be modified as in cationic polyacrylamide. Table VI represents optical density measurements of the growth of strain SB-2 in the presence of various types of polyacrylamides. These results suggest that it is the partially hydrolyzed polyacrylamides (HPAM) which stimulate growth. These results are from one experiment but the observed stimulation has always been present in the several hundred repetitions we have run. Cationic polyacrylamide, polyacrylic acid (completely hydrolyzed polyacrylamide), and 1 to 4% hydrolyzed polyacrylamide inhibit growth. Cationic polyacrylamide seems to be a particularly effective inhibitor of SRB growth. As little as Ø.Ø1% (w/v) completely inhibited growth of SB-2 in medium C.

Stimulation of Growth of *Desulfovibrio* strain SB-2 by Partially Hydrolyzed Polyacrylamides

Strain SB-2, in *Desulfovibrio* sp. isolated from produced water samples from the polymer flood pilot project area of the Wilmington field, was used as a model organism in these studies as it is typical of the SRB isolates we have recovered.

Partially hydrolyzed polyacrylamides (HPAMs) are the

TABLE VI

GROWTH OF SB-2 IN THE PRESENCE OF VARIOUS POLYACRYLAMIDES

Polymer (degree of Hydrolysis)	O.D.$_{540}$ Ø hr.	24 hr.	4Ø hr.
Control (no polymer)	Ø.Ø2	Ø.35	Ø.52
J334 (25-35%)	Ø.Ø2	Ø.43	Ø.68
J279 (1-4%)	Ø.Ø1	Ø.26	Ø.48
Cationic (substituted)	Ø.Ø2	Ø.Ø1	Ø.ØØ
Polyacrylic acid(1ØØ%)	Ø.Ø1	Ø.Ø7 (±.Ø8)	Ø.18 (±.17)

Postgate medium with Ø.1% Polymers, Incubated anaerobically at 35°C. J334, J279 and Cationic have 3-4 x $1Ø^6$ molecular weights. The molecular weight of Polyacrylic acid is unknown. Standard deviations are ± Ø.Ø2 or less unless otherwise noted. n=1Ø.

type of polyacrylamides used in enhanced oil recovery and most of this research has dealt with interactions of strain SB-2 and the J series HPAMs produced by the Dowell Division of Dow Chemical Company.

Table VII shows the results of growth of strain SB-2 with different partially hydrolyzed polyacrylamides that differ in degree of hydrolysis and in molecular weight. It would seem from these data that the degree of hydrolysis is a key factor in the mechanism of stimulation whereas molecular weight is not. Table VII also demonstrates that mechanically shearing the polymer solution has little effect on the observed stimulation of growth. While the observed stimulation is small, it is always present.

Figure 1Ø is a plot of optical density (O.D.) of strain SB-2 in medium C vs. protein concentration with and without .1% J332. This was done to eliminate the possibility that polyacrylamide was altering the refractive index of the medium and producing an artificial increase in O.D. These data show that the effect on O.D. is minimal.

Initiation and Rate of Growth of SB-2 in the Presence ofJ332

Figure 11 represents the growth of strain SB-2 in medium C with and without .2% J332. These results are typical of the stimulation of growth seen with all reservoir-isolated Desulfovibrio sp. tested. It is interesting to note that while growth is initiated sooner

TABLE VII

GROWTH OF SB-2 WITH VARIOUS PARTIALLY HYDROLYZED POLYACRYLAMIDES

Polymer	MW	Degree of Hydrolysis	O.D.$_{540}$		
		Time (hrs)	Ø	16	25
Control	(no polymer)		Ø.Ø2	Ø.12	Ø.76
J332	9-1Ø x $1Ø^6$	25-35%	Ø.Ø2	Ø.2Ø	Ø.82
J333	6-7 x $1Ø^6$	25-35%	Ø.Ø2	Ø.22	Ø.86
J334	3-4 x $1Ø^6$	25-35%	Ø.Ø1	Ø.25	Ø.8Ø
J279	3 x $1Ø^6$	1-4%	Ø.Ø1	Ø.Ø5	Ø.68
J332	Sheared	25-35%	Ø.Ø2	Ø.21	Ø.83

Postgate's medium C with Ø.1% w/v polymer. Incubated anaerobically at 35°C. Standard deviations ± Ø.Ø5 or less. n=1Ø.

FIGURE 10. Optical Density vs. Protein Concentration SB-2 in Postgate's Medium C. BSA standard. --Δ-- no polymer, —o— with 0.1% J332. r = .985 (or less. ?)

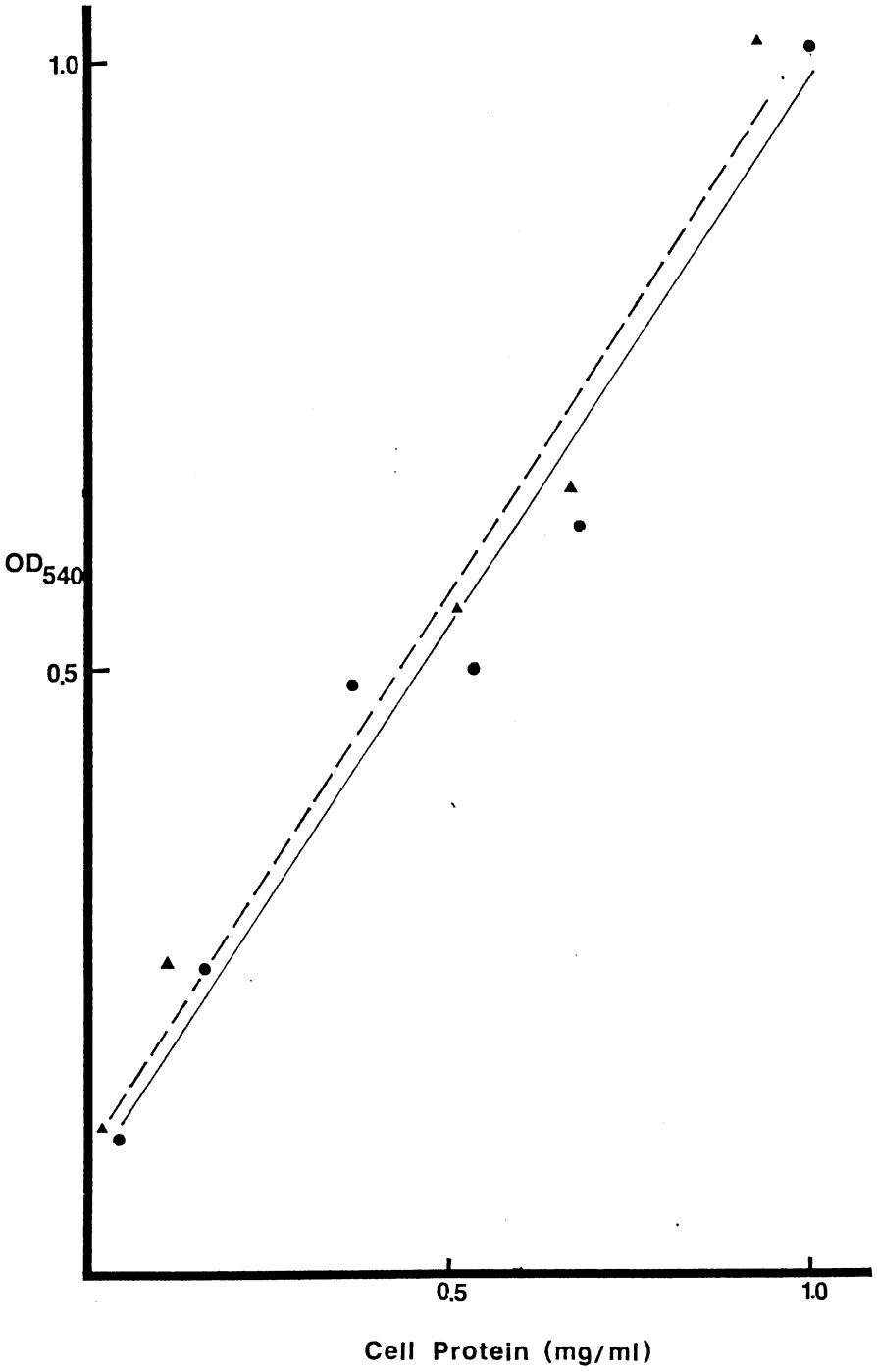
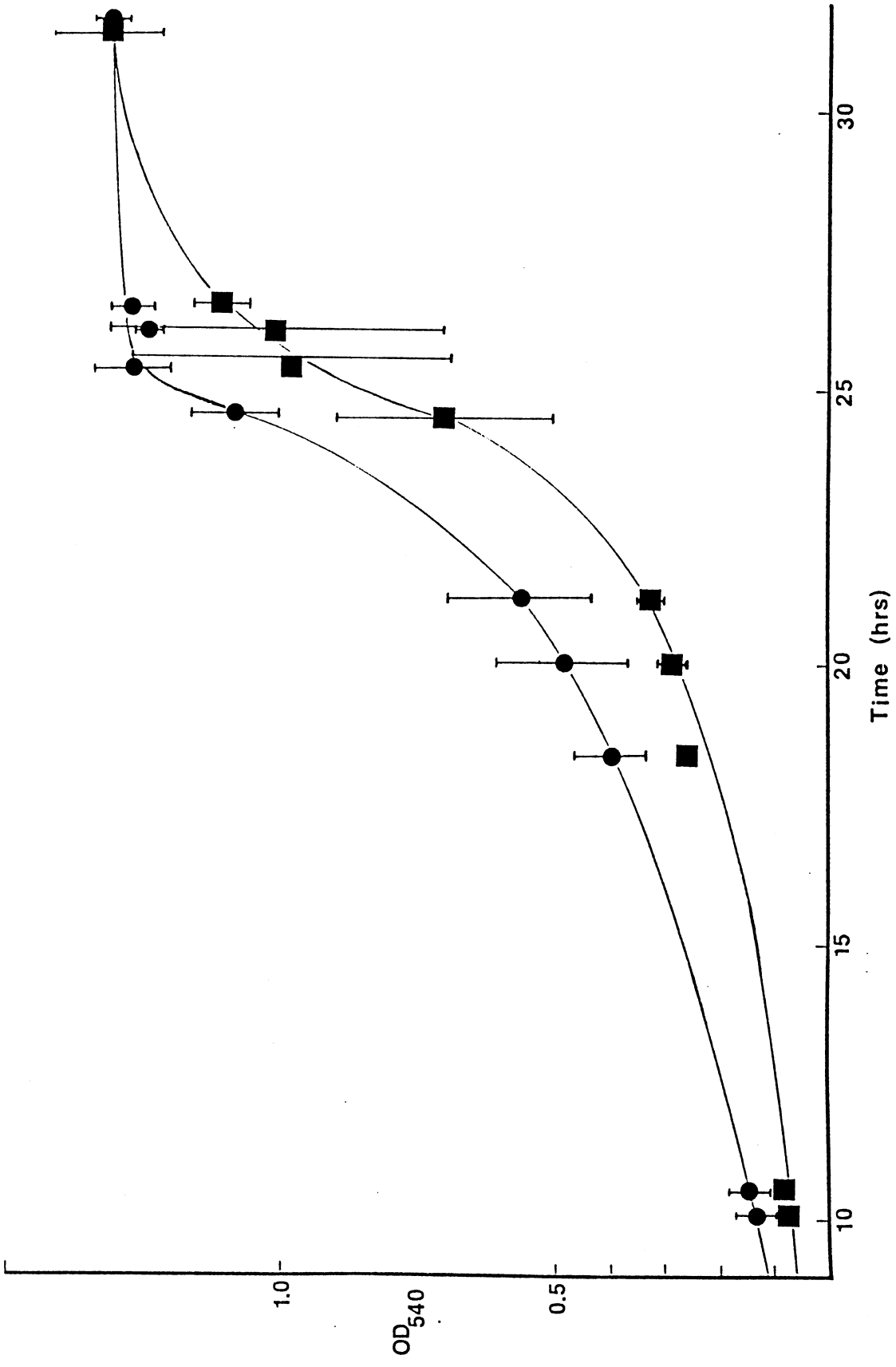

1.0
OD540
0.5
0.5
1.0
Cell Protein (mg/ml)

FIGURE 11. Effect of J332 on Growth of SB-2.
■-Postgate's medium C, ●-Postgate's medium C with 0.2% J332. Incubated anaerobically 35° C. Initial OD = 0.02 for both conditions. Values are average of 10 replicates. n = 10.


1.0
0.5
OD_{540}
10
15
20
25
30
Time (hrs)

in the presence of the polymer and that in the early exponential phase the growth rate is higher, the total growth is roughly equivalent to, or may be slightly higher in the absence of the polymer.

The initiation of growth of Desulvibrio cultures is an event which depends on several factors, the most important of which is the oxidation/reduction potential of the medium. Medium C is a non-reduced medium and as such does not generate the necessary negative potential (-1ØØmV). The nitrogen-hydrogen-carbon dioxide atmosphere present in the anaerobic chamber used in these studies generates a potential in the neighborhood of -5Ømv, thus initiation of growth is not ensured. The most common method of generating a low potential is by the use of large inocula with the resulting carry-over of reducing agents, namely hydrogen sulfide. It is appropriate to note here an unexpected and to this point unexplained observation. In non-reduced media, polyacrylamides seem to affect the oxidation/reduction potential in a concentration-dependent manner. Table VIII shows the measurable O/R potential in uninoculated medium C at various polyacrylamide concentrations after lengthy anaerobic incubation. I have speculated that these differences in O/R potential may be due to the polymer affecting the solubility of gases present in the chamber but have no evidence to support this.

Growth stimulation was also checked as a function of

TABLE VIII

INFLUENCE OF POLYACRYLAMIDE ON OXIDATION REDUCTION POTENTIAL IN MEDIUM C

J332 concentration (w/v)	O/R (mV) potential
Ø.Ø	-5
Ø.Ø1	-95
Ø.Ø5	-12Ø
Ø.1	-125
Ø.5	-135
1.Ø	-155

Uninoculated medium C after incubation in the anaerobic chamber 2 weeks. Polyacrylamide has no effect on initial O/R potential. Initial O/R potential -1Ø mV. O/R determined with redox electrode.

polymer concentration (Table IX). The maximum O.D. at each polymer concentration is eventually the same, however, the initiation of growth appears to be concentration dependent. Although better stimulation was seen at concentrations exceeding Ø.2% (w/v) the production of these media required mechanical shearing of the polymer in solution. Even after shearing, the high viscosity of these concentrations made their use impractical for most purposes.

The inability to control the initiation of growth led to high variance in the absorbance of replicate cultures used in growth measurements during the exponential phase of growth. An example of this can be seen in Figure 11. It is also important to note that less variance is seen in replicate cultures with J332 present.

A Lab-Line Bioengineering model KLF 2ØØØ fermentor was used to quantitatively measure the stimulation of growth of Desulfovibrio by polyacrylamide. Measurements were made in Postgate's medium C+ Ø.2% J333. The reactio vessel was continuously monitored with respect to pH, O/R potential, agitation and temperature. Samples of the medium were collected at various intervals for optical density and dry weight measurements. Head space gas samples were collected for gas chromatographic analysis.

Figure 12 is a semi-logarithmic plot of the optical density of the culture medium at 54Ø nm vs. time. The lag phase of growth is clearly shortest in the presence of the polymer.

TABLE IX

EFFECT OF POLYMER CONCENTRATION ON INITIATION OF GROWTH OF SB-2

Concentration J332		O.D.$_{540}$	
(% w/v)	time (hrs) Ø	48	S.D.
Ø.Ø	Ø.Ø2	Ø.Ø8	± .Ø4
Ø.Ø1	Ø.Ø2	Ø.17	± .Ø5
Ø.Ø5	Ø.Ø2	Ø.29	± .Ø2
Ø.1	Ø.Ø2	Ø.31	± .Ø2
Ø.5	Ø.Ø2	Ø.4Ø	± .Ø4
1.Ø	Ø.Ø2	Ø.71	± .11

Postgate's medium C incubated anaerobically 35°C.
Slow growth due to small inoculum (1 to 1ØØØ).

FIGURE 12. Effect of J332 on Growth of SB-2 Semi-Log Plot.
r = .997 (or more.?) for the exponential phase (data points connected by line) of growth.

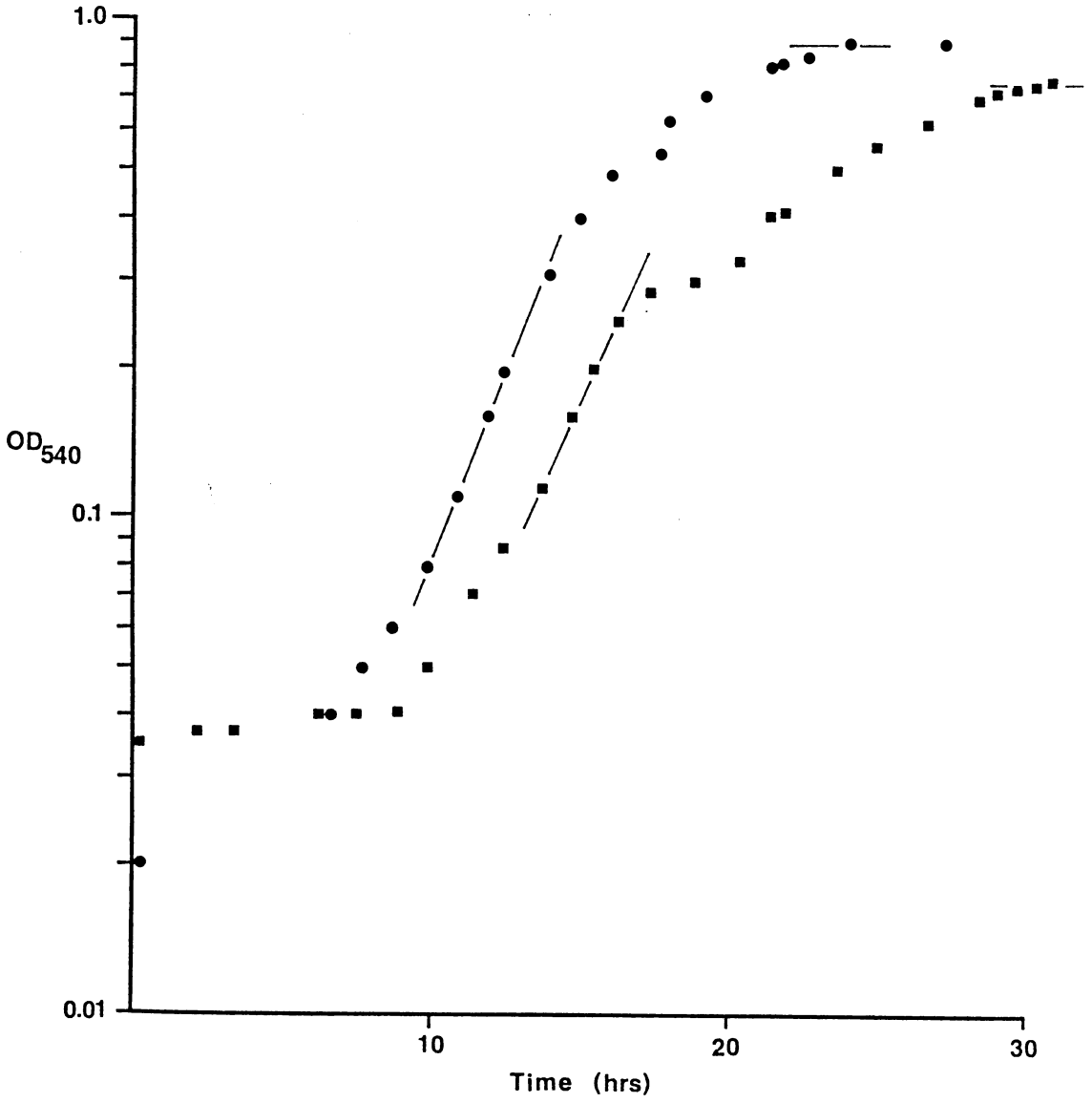

1.0
0.1
0.01
OD540
10
20
30
Time (hrs)

Table X lists the minimum observed doubling times and maximum growth levels with and without polymer, using Desulfovibrio strain SB-2. Figure 13 shows various physical parameters in relation to the growth of the organism with polyacrylamide. Because of the nitrogen flushing, no build-up of CO_2 was detected and our equipment did not allow for H_2S monitoring. Basically the GC analysis yielded N_2 with traces of CO_2 and no O_2. The highly hygroscopic nature of Postgate's medium C resulted in the dry weight data being of no use.

These results show a shorter lag phase and higher maximum growth rate are observed in the presence of partially hydrolyzed polyacrylamide. The results from the batch culture experiments can be accepted with some reservations. This is the only case where we have attempted to compare results from different experiments. The inoculum and conditions in the fermentor were standardized as closely as possible. The experiment was run twice with similar results.

Mechanism of Stimulation

Defining the mechanism of stimulation of growth of Desulfovibrio by polyacrylamides proved to be a more difficult and complex problem than initially assumed. Considering the simplicity of the structure of polyacrylamide and the empirical formula of the repeating units (5H,3C,O,N) there seems a limited number of

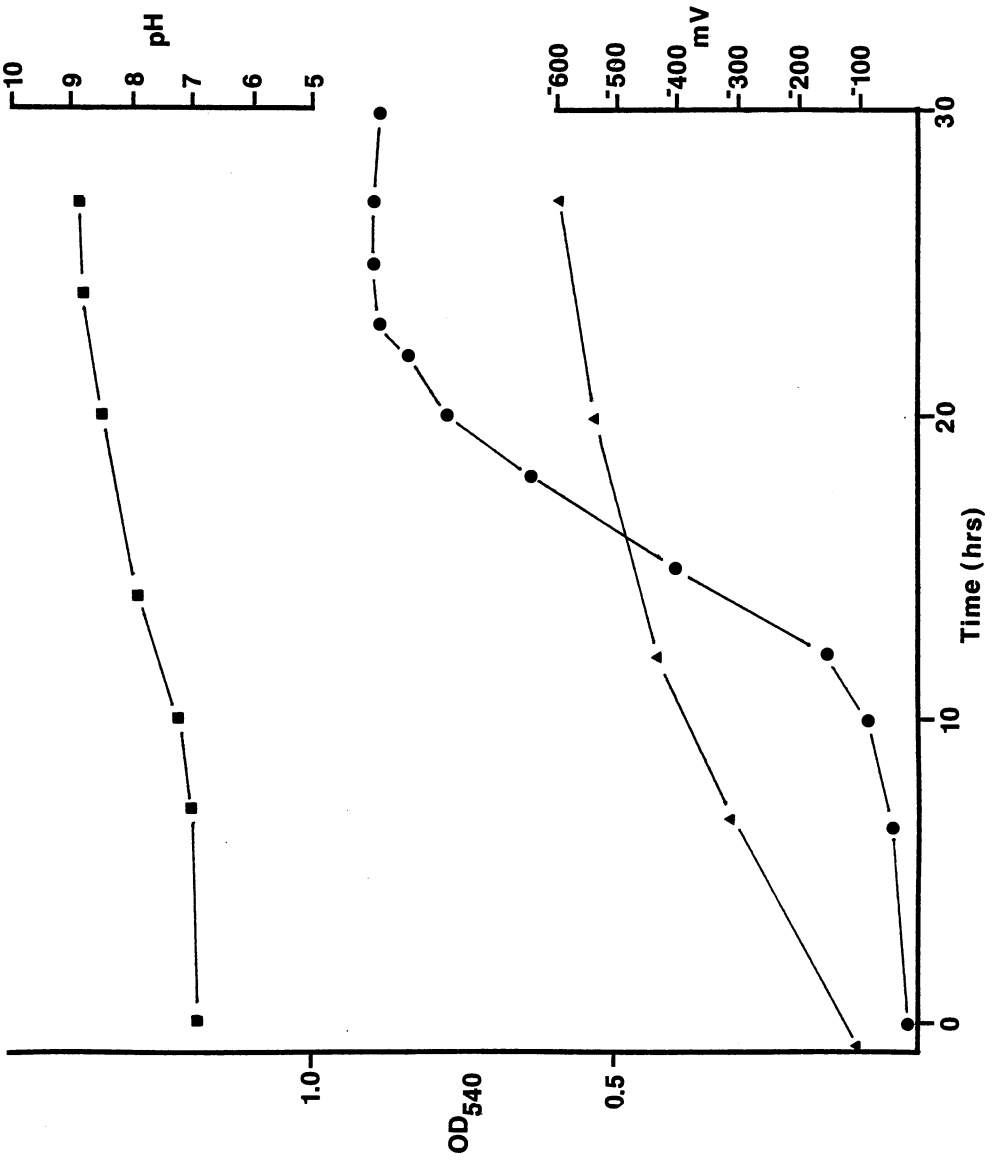
TABLE X

GROWTH RATES AND YIELDS OF SB-2 WITH J333

Measurement	With Ø.2% J332	Without
Minimum observed doubling time (hrs)	1.9	2.6
Max. $O.D._{540}$	Ø.9Ø	Ø.77

Medium C, 35°C, anaerobic

FIGURE 13. Physical Parameters During Growth With J332.
□ - pH
Δ - O/R (mV)
○ - O.D.


pH
10
9
8
7
6
5
mV
-600
-500
-400
-300
-200
-100
1.0
0.5
OD_{540}
0
10
20
30
Time (hrs)

possibilities as to how the polymer could serve as a nutrient. No growth was seen with our test organism when polyacrylamide was substituted for lactate as a carbon-energy source in medium C at concentrations from Ø.Ø5 to 1.Ø percent. We did not consider these results to be surprising because it is assumed that the polymer is immune to cleavage under anaerobic conditions.

Uptake of Labeled Polyacrylamide

^{14}C-Carboxyl labeled polyacrylamide was used to determine if the polymer could be serving as a source of cellular material. Medium C with Ø.1% J334 labeled in the carboxyl carbon was inoculated with strain SB-2. After 4 days of incubation under anaerobic conditions less than Ø.Ø5% of the total recovered radioactivity was cell associated (Table XI). This low level of incorporation indicates that it is very unlikely that the polymer is serving as a source of cellular carbon. Darkening of the medium and the presence of H_2 was taken as a positive indication of the growth of Desulfovibrio.

Analysis of Metabolic Products by Gas Chromatography

Gas chromatography was used to analyze the catabolic products of Desulfovibrio growth in the presence of polyacrylamide. In Desulfovibrio, lactate is oxidized via pyruvate to acetate and CO_2. Pyruvate, glycerol, ethanol

TABLE XI

GROWTH OF SB-2 WITH ^{14}C-LABELED POLYACRYLAMIDE

Fraction	Counts/Minute/ml	Total Counts Recovered	(%)
Uninoculated Medium	3078 ± 1152	304,100	(100)
Supernatant (post incubation)	2462 ± 176	242,500	(79.7)
First wash (100 ml)	391 ± 8	35400	(11.6)
Second wash (100 ml)	54 ± 2	1700	(0.6)
Pellet (10 ml)	49 ± 4	120	(0.04)
Background	37	-	

Postgate's medium C 0.1% J334. Incubated anaerobically 4 days, 35°C. $O.D._{540}$=0.53. 100 ml original volume. Cells were washed with medium C. Total counts recovered is a comparison of the cpm/ml x total volume of the fraction vs the uninoculated medium.

and the acids of the tricarboxylic acid cycle are all converted acetate and CO_2 as major products (Postgate, 1984). A few incomplete substrates yield other products, for example, isbutanol is oxidized to isobutyric acid. Analysis of medium C before and after growth of strain SB2 showed a decrease in lactate and the appearance and increase of acetate with growth. One interesting point is that approximately 10% of the original lactate is present after growth ceases. This is an indication that substrate availability is not the limiting factor on growth under these conditions. Trace amounts of oxalacetic acid and oxalic acid were also observed.

The presence of polyacrylamide (0.1%) did not alter the observed profile before or after growth. No measurable differences were detected in the amounts of product after growth with polymer. The important conclusions of these results are that if the polymer is metabolized it does not alter the product profile of the organism and autoclaving of the polymer in medium C does not produce a detectable substrate for the organism.

Polyacrylamide as a Nitrogen Source

Unhydrolyzed polyacrylamide is approximately 26% Nitrogen by weight and could thus be an available source of this macro-nutrient.

It has been postulated by microbiologists involved in the petroleum industry that polyacrylamide may serve as a

nitrogen source for reservoir bacteria. Work by Grula and Huang (1981) might also suggest that this is possible. In their work soil pseudomonads were isolated which could use polyacrylamide as a nitrogen source.

Using strain SB-2 we have attempted to assay polyacrylamide for the ability to act as a nitrogen source for Desulfovibrio. Because of the idiosyncrasies of SRB growth we have carried out these experiments under a number of conditions. Figure 14 shows growth of SB-2 in medium C with and without ammonium chloride and with and without J332. Yeast extract was also excluded as it may contain from 7 to 10 percent total nitrogen (Bridson and Brecker 1970). The results would seem to suggest that J332 might indeed serve as a nitrogen source, however, because of the uncertainties associated with the poor growth seen under these conditions, additional experiments were carried out.

Since yeast extract stimulates the growth of Desulfovibrio and is a source of vitamins and micro-nutrients, a similar experiment was run with 1 gram per liter of yeast extract present in the medium. The results in Figure 15 show that while the overall levels of growth are higher than in the previous experiment, the polymer is still apparently acting as a nitrogen source. In both of the experiments a small inoculum (1 to 100) is used to prevent carry over of nutrients from the stock cultures.

It was not clear initially as to whether the nitrogen which was available was a contaminant in the polymer

FIGURE 14. Effect of J332 on Growth of SB-2 Under Nitrogen Limiting Conditions. Postgate's medium C (no yeast extract) 0.1% J332, 0.1% NH_4Cl, incubated anaerobically, 35°C.

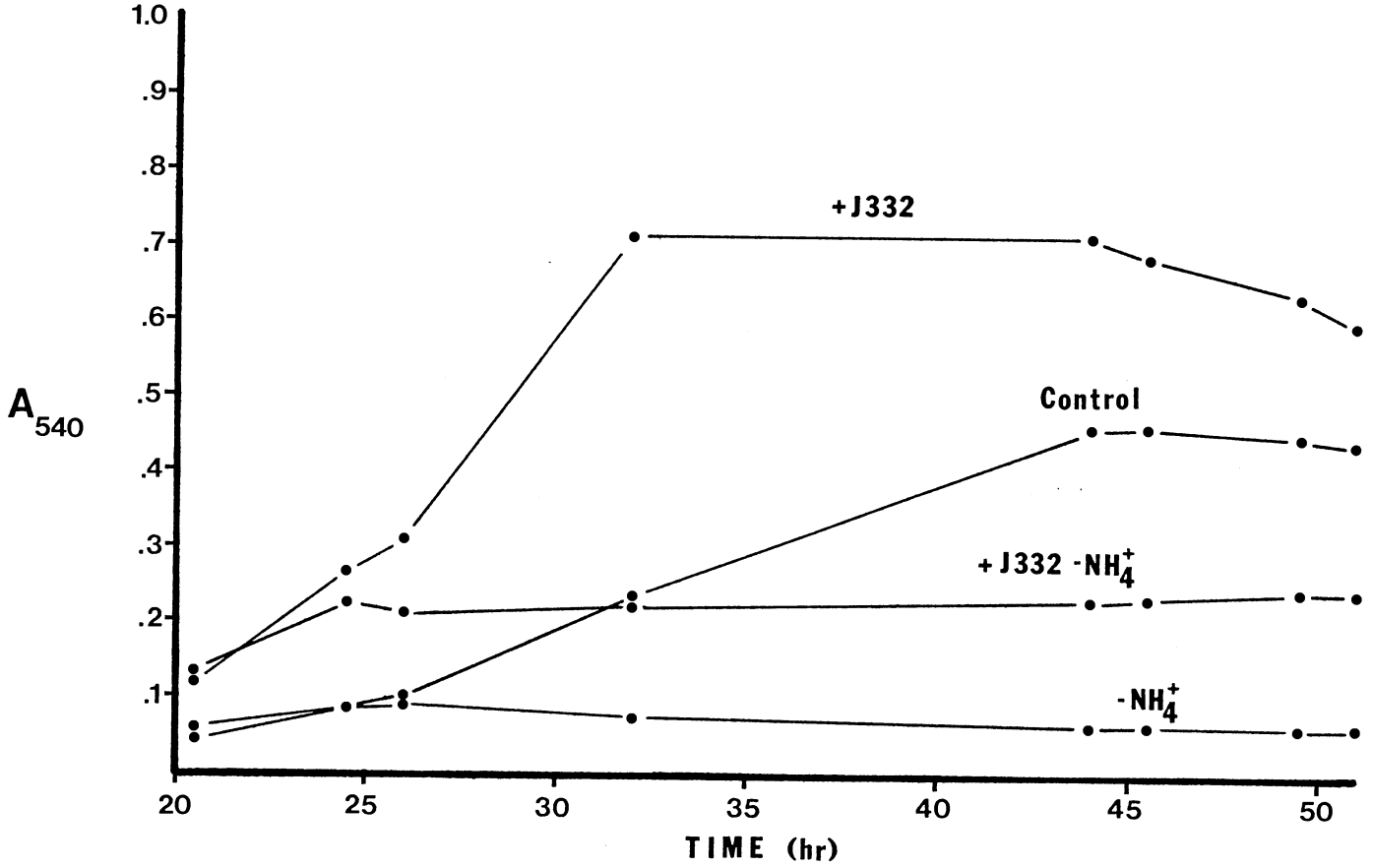
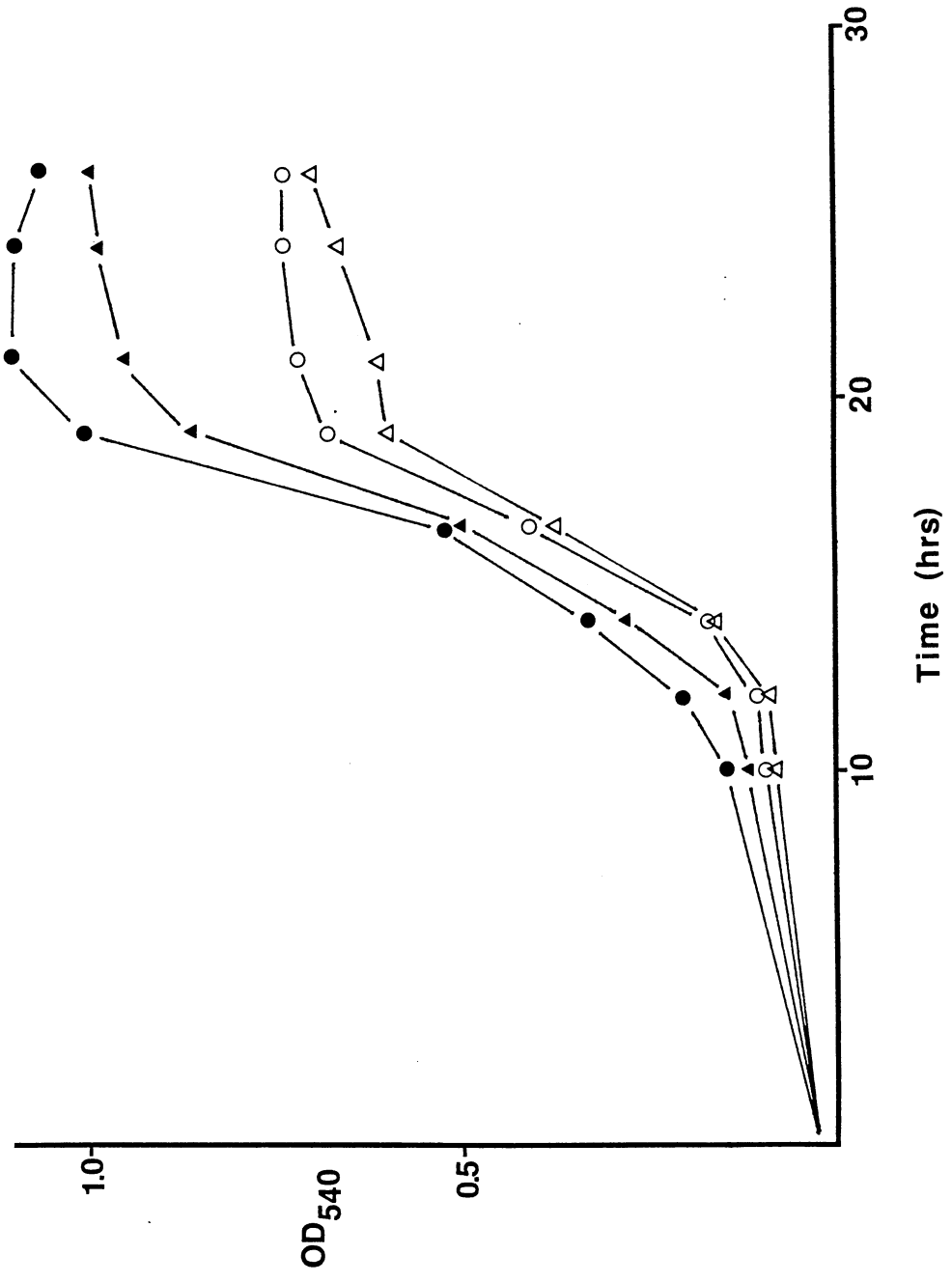

1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
A540
+J332
Control
+J332 -NH4+
-NH4+
20
25
30
35
40
45
50
TIME (hr)

FIGURE 15. Effect of J332 on Growth of SB-2 Under Nitrogen Limiting Conditions With Yeast Extract. Postgate's medium C 0.1% J332, 0.1% NH_4Cl incubated anaerobically, 35°C.

● +NH Cl, +J332
▲ +NH Cl, -J332
○ -NH Cl, +J332
△ -NH Cl, -J332


1.0
0.5
OD_{540}
10
20
30
Time (hrs)

preparation, a carry over from the post-polymerization modification of the polymer used to achieve the desired degree of hydrolysis, a product of spontaneous hydrolysis of the amide group in solution or if it was only available after enzymatic attack on the amide group. Analysis of dilute polyacrylamide solutions for ammonium by the Indophenol blue reaction yielded values of approximately 3ug/ml of nitrogen /gram polyacrylamide/liter. After autoclaving, the values jumped to 11.5 ug/ml of nitrogen per gram of polyacrylamide. In Figure 14 we see a difference in maximum optical density (540nm) when medium C with no ammonium chloride is compared to the medium C (no ammonium chloride) with 0.1% J332 added results of approximately 0.15 absorbance units. This is equivalent to a difference of 0.04 mg protein/ml. Postgate (1984) reported the elemental analysis for nitrogen in Desulfovibrio vulgaris was 12.47%. If we use this figure to apply to cell protein, (when in fact it is probably higher) then the nitrogen available from autoclaved polymer is sufficient for the observed difference in cellular material.

Another possibility is that low molecular weight molecules of the polymer can be utilized by the cell as an organic nitrogen source. Little is known of the organic nitrogen metabolism in Deslfovibrio. However, the lack of detectable uptake of ^{14}C with carboxyl labeled polymer would seem to limit this possibility.

Since low levels of growth were seen in nitrogen free controls, we attempted to assay strain SB-2 for the ability to fix nitrogen. Nitrogen fixation in sulfate-reducing bacteria was first reported by Sisler and ZoBell (1951). Nitrogenase activity in Desulfovibrio is very sensitive to the presence of exogenous nitrogen (Postgate and Kent, 1984). We were hopeful that we would be able to show repression of nitrogenase activity in the presence of polyacrylamide, as it would have been strong evidence that the polymer could serve as a source of nitrogen. However, none of our tested strains (SB-2, U-9) were able to reduce acetylene in measurable amounts. This was true even under the stringent conditions outlined by Postgate and Kent (1985). While diazotrophic growth is widespread in Desulfovibrio, the measurement of nitrogenase activity is often difficult (Postgate, 1984).

Although the polymer seems to be an available source of nitrogen for strain SB-2 we do not believe this is the reason for the observed stimulation of growth under normal conditions (complete medium C, Ø.1% Polyacrylamide). At elevated nitrogen levels (Ø.2% NH_4Cl) J332 was still effective in stimulating the growth of SB-2, although total growth was no higher than the results at Ø.1% NH_4Cl.

Polymer/Iron Interaction

Iron is a critical nutrient in the growth of Desulfovibrio. The respiratory nature of the metabolism of

this organism and the economic/environmental problems associated with its growth have iron as a central focus.

The discovery of a cytochrome (C_3) in an obligately anaerobic organism by J.R. Postgate (1954), was an extremely important event in our understanding of anaerobic respiration. Since that time the list of iron containing hydrogenases and electron transfer proteins has grown considerably (Odom and Peck, 1984). Among those reported for Desulfovibrio spp. are hydrogenases (Fe & NiFe), cytochromes (C_{553} and C_3), ferredoxins (Fe_4S_4, $2[Fe_4S_4]$, and Fe_3S_4) and rubredoxins.

Postgate (1956) determined the optimal concentration of iron for the growth of Desulfovibrio to be 1Ø to 15 mg/l, but in our hands higher levels seemed to stimulate initiation of growth. The iron content of Desulfovibrio cells is difficult to determine since their ability to precipitate iron leads to an excessive iron content in elemental analysis.

Desulfovibrio cells associate with exposed iron surfaces and may cause severe corrosion problems under anaerobic conditions (Bradley et al., 1983). Several theories have been proposed to explain the nature of the chemical and electrochemical processes involved in anaerobic iron corrosion (Postgate, 1984. Miller, 1971. Iverson, 1974).

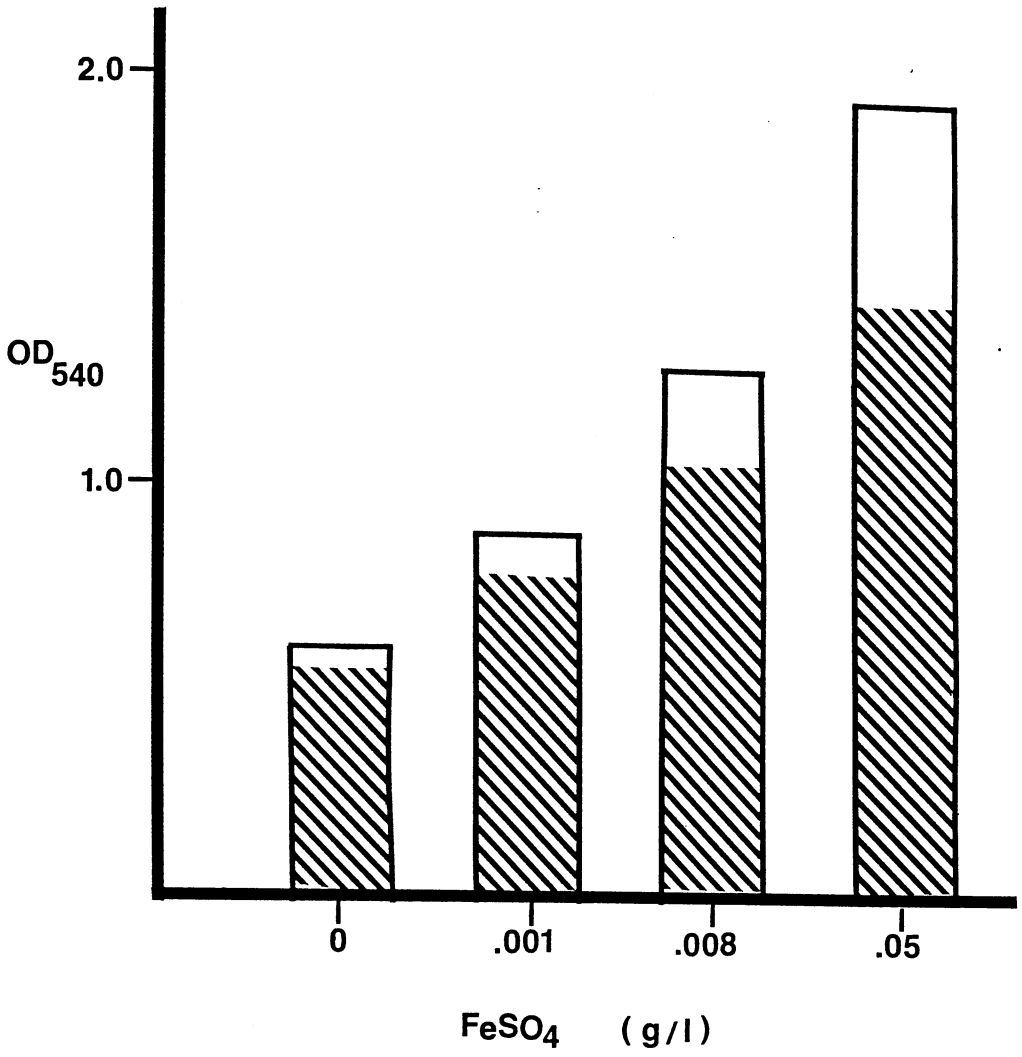
Iron availability is a limiting factor in the growth of Desulfovibrio in Postgate's medium C. Iron in the form

of ferrous sulfide is also the single biggest problem in the study of these organisms. Figure 16 represents growth as a function of $FeSO_4$ concentration. Since anything affecting iron availability would greatly influence the growth of the organism, a large portion of our investigations revolved around the iron/polyacrylamide relationship.

Iron has been reported as a contaminant of commercially available polyacrylamide preparations (Shupe, 1981). Using a colormetric assay based on the iron reagent bathophenanthroline-disulfonic acid, which has a sensitivity of Ø.24 ug/ml we detected no measurable iron present in our polymer preparations (Diehl, 1965).

An early observation was that in a medium with high iron concentration (medium B) the blackening of the medium (FeS formation) did not occur, or clear as the culture aged, if polyacrylamide was included. Culture tubes of this composition are still dark several years later. In an attempt to investigate the iron/polymer interactions, iron (Ferrous) concentration was assayed with bathophenanthroline-disulfonic acid (BPTS) at varying polymer concentrations. Our reasoning was that if the polymer influenced the availability of iron to BPTS we would have strong evidence for an iron-polymer interaction. We also wondered if the polymer prevented precipitation of FeS and thus made iron available for the growth of the organism at high sulfide concentrations. Iron available to

FIGURE 16. Effect of Iron Concentration on Growth.
Postgate's medium C, 18 hr., 35°C, anaerobic
White bar - 0.1% J332
Striped bar - no polymer


2.0
1.0
OD540
0
.001
.008
.05
FeSO4 (g/l)

the BPTS assay was the same with or without polyacrylamide present. Also iron concentrations were equivalent with and without polymers after Na_2S treatment (Table XII). However, one interesting observation was made, the iron became oxidized from the ferrous to the ferric form in the presence of polyacrylamide in non-reduced medium but at a very slow rate (Table XIII). Because of the observed effects of the oxidation state of iron by the polymer, growth was assayed with equimolar amounts of iron added in the three possible forms found under growth conditions: Fe^{++} ($FeSO_4 \cdot 7H_2O$), Fe^{+++}(Ferric citrate), and as the insoluble ferrous sulfide. Each iron form was also checked with polyacrylamide present. The results are shown in Figure 17-19. We were surprised at our results that growth levels were equivalent with an Fe^{++} or Fe^{+++} iron source. There seems to be a lesser amount of stimulation by J332 with the FeS cultures. We had thought that perhaps the polymer would make iron sulfide available to the organism, but apparently this is not the case. Greater stimulation was seen with Fe^{+++} vs Fe^{++} only in the presence of J332. In the absence of polyacrylamide ferrous and ferric iron apear to be equivalently available to SB-2. It is unknown however, what effect the reducing conditions generated during SRB growth have on the oxidation state of the different iron sources.

Citrate is included in Postgate's medium C as an iron chelator to prevent the precipitation of Fe^{++} as FeS

TABLE XII

EFFECT OF J332 ON Fe^{++} CONCENTRATION

J332 % (w/v)	A_{535} 1 μg/ml Fe^{++} as $FeSO_4$	A_{535} 1 μg/ml Fe^{++} as FeS
Ø	.351	.Ø2
Ø.Ø1	.351	.Ø8
Ø.Ø5	.335	.Ø1
Ø.1Ø	.36Ø	.1Ø
Ø.25	.333	.Ø3
Ø.5Ø	.363	.Ø6
Ø.75	.369	.Ø8
1.ØØ	.343	.Ø9

Iron assayed with bathophenanthroline-disulfonic acid.
SD ± .Ø35.

TABLE XIII

EFFECT OF J332 ON Fe^{++} OXIDATION

Concentration J332 % (w/v)	A_{535} Time (hrs) Ø	12
Ø.ØØØ	.351	.359
Ø.Ø25	.333	.166
Ø.Ø5Ø	.363	.Ø49
Ø.Ø75	.369	.Ø49
Ø.1ØØ	.343	.Ø53

Iron assayed with bathophenanthroline-disulfonic acid. 1 μg Fe^{++}/ml. SD ± .Ø35.

FIGURE 17. Effect of J332 on Growth of SB-2 With Ferrous Sulfate. Postgate's medium C, 15 μM $FeSO_4 \cdot 7H_2O$. Incubated anaerobically, 35°C. S.D. ± 03.

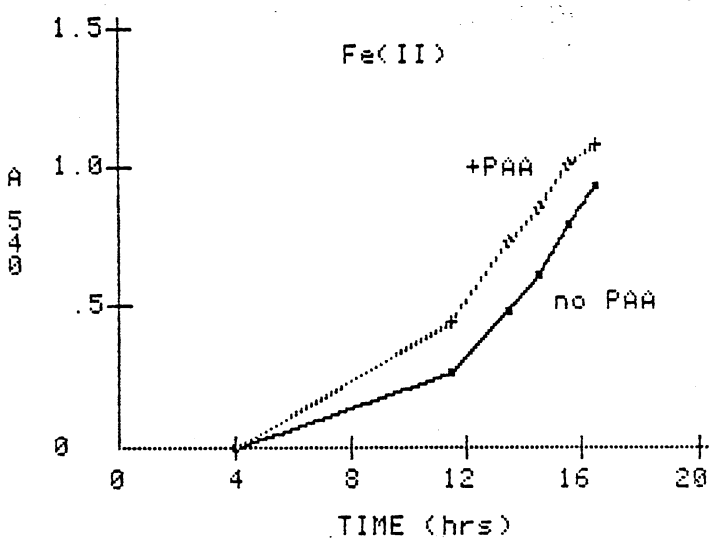

Fe(II)
1.5
1.0
.5
0
A
0450
+PAA
no PAA
0
4
8
12
16
20
TIME (hrs)

FIGURE 18. Effect of J332 on Growth of SB-2 With Ferric Citrate. Postgate's medium C, 15 μM ferrous citrate. Incubated anaerobically, 35°C. S.D. $\pm$ .03.

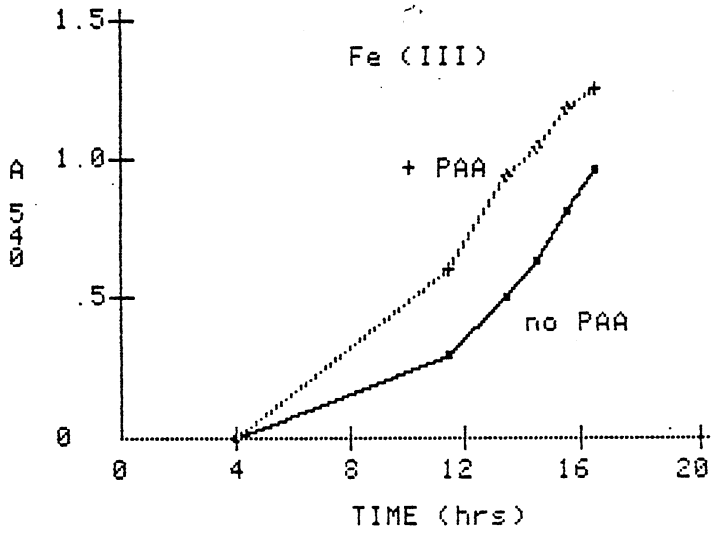
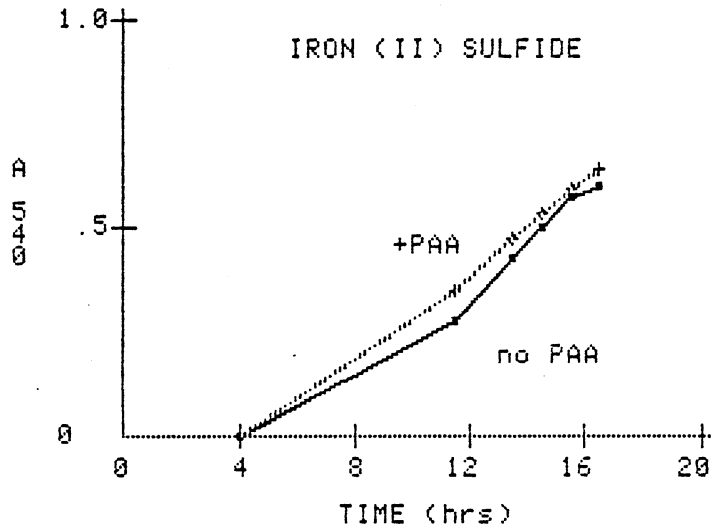

1.5
1.0
.5
0
A
450
Fe (III)
+ PAA
no PAA
0
4
8
12
16
20
TIME (hrs)

FIGURE 19. Effect of J332 on Growth of SB-2 With Ferrous Sulfide. Postgate's medium C, 15 μM ferrous sulfide. Incubated anaerobically, 35°C S.D. ± .03.


IRON (II) SULFIDE
1.0
.5
0
A
4
5
0
+PAA
no PAA
0
4
8
12
16
20
TIME (hrs)

(Postgate, 1984). This is important for mass culture of the organism and optical density measurements of growth. At high concentrations (5 g/l) citrate delays growth of some Desulfovibrio species. With strain SB-2, citrate slowed the growth of the organism but did not interfere with polyacrylamide stimulation of growth (Table XII).

Fermentative Growth With J332

Pyruvate can be metabolized by Desulfovibrio in the absence of sulfate to acetate, CO_2 and H_2 in a classical fermentative manner. Pyruvate can also be oxidatively metabolized if sulfate is present. We have grown SB-2 on pyruvate with and without sulfate with Ø.1% J332, to ascertain if the type of metabolism (oxidative vs. fermentative) is important in the stimulation of growth by the polymer (Figure 2Ø). Under both situations, J332 stimulated growth of strain SB-2. We have no explanation for the low levels of observed growth. Many workers have reported higher yields of Desulfovibrio on pyruvate/sulfate than on lactate/sulfate, although lactate should be energetically more favorable (Postgate, 1984).

Mixotrophic Growth On Polyacrylamide

Sulfate-reducing bacteria exhibit the interesting phenomenon of mixotrophy or the utilization of "incomplete" substrates. An incomplete substrate is one which can serve as an electron source for sulfate-reduction but is not

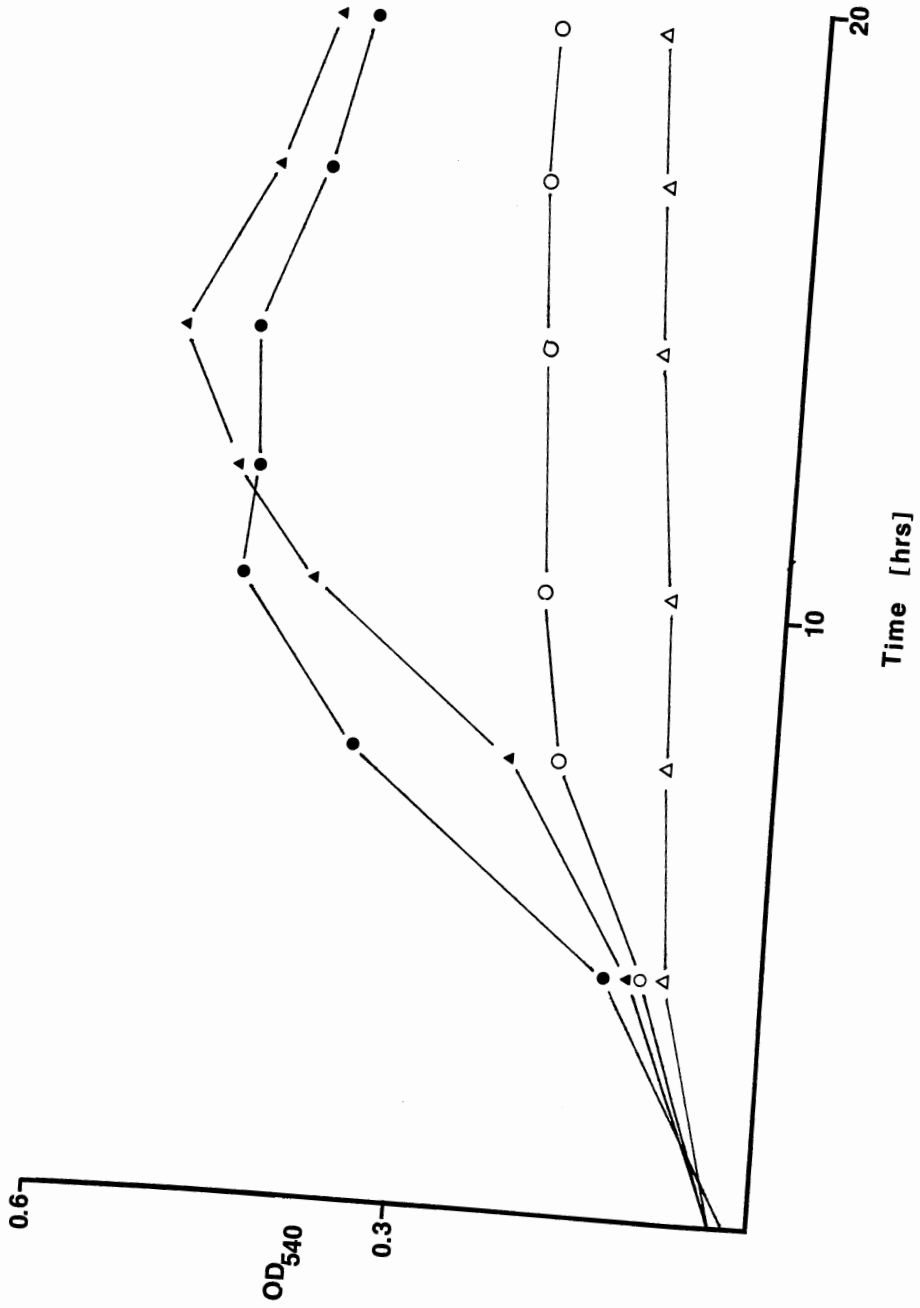
TABLE XIV

EFFECTS OF CITRATE ON GROWTH OF SB-2

concentration (w/v)		$O.D._{540}$	
Citrate	J332	Time (hrs) 0	24
Ø.Ø3	-	.Ø2	.21 ± .Ø8
Ø.Ø3	Ø.1	.Ø2	.33 ± .Ø2
-	-	.Ø1	.29 ± .Ø4
-	Ø.1	.Ø2	.43 ± .Ø1

Postgate's medium C, (no yeast extract) Ø.3g/l sodium citrate, 1g/l J332 incubated anaerobically, 35°C.

FIGURE 20. Effect of J332 on Growth of SB-2 During Fermentative Metabolism.
● - pyruvate/$SO_4^=$ + 0.1% J332
▲ - pyruvate/$SO_4^=$
○ - pyruvate + 0.1% J332
△ - pyruvate

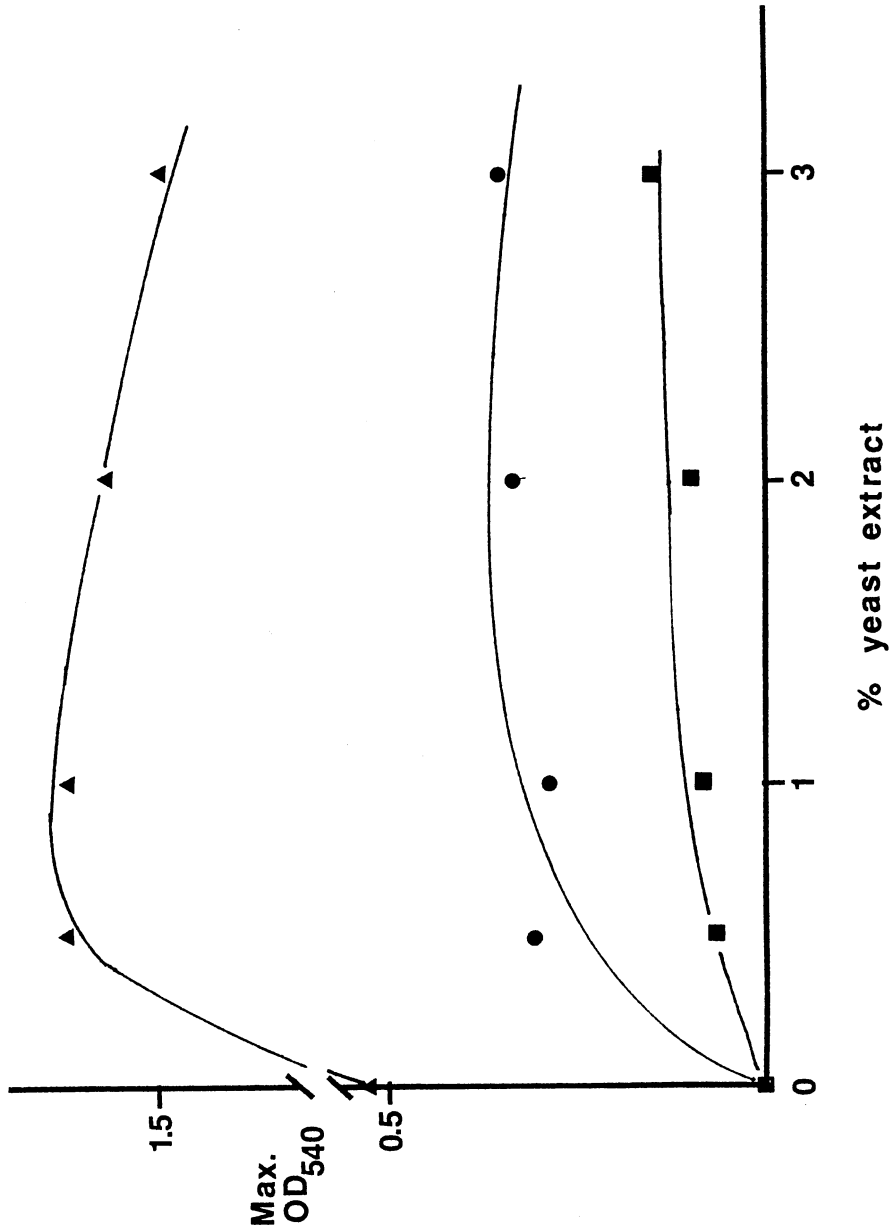

0.6
0.3
OD540
10
20
Time [hrs]

assimilated, i.e., cannot provide a carbon source. Hydrogen (H_2) is such a substance, and certain organic compounds can also act as an incomplete substrate (e.g., 2-butanol). Postgate (1963) also described another interesting incomplete substrate, oxamate, which has functional groups similar to partially hydrolyzed polyacrylamide. Figure 21 indicates the result of growth of SB-2 with 1% J332 (highly sheared) with varying concentrations of yeast extract. These results are typical for an incomplete substrate. There is no growth at Ø% yeast extract in the control or with polyacrylamide. That yeast extract is stimulatory and not essential for growth with complete substrates can be seen in the lactate results in that growth occurs without yeast extract. Clearly the increased growth in the polyacrylamide group over the control results from some fraction of the polymer molecules being available as an energy source. J334, J279, oxamate and formate also act as incomplete substrates for SB-2 (data not shown).

Effects on the Rheological Properties of Polyacrylamide

The second area of investigation involved in these studies has been concerned with the apparent ability of Desulfovibrio cultures to alter the rheological (flow) properties of polyacrylamide solutions. The screen factor of the polymer solution used as a mobility control agent is a key physical characteristic as to its effectiveness in

FIGURE 21. Mixotrophic Growth With J332.
Postgate's medium C with varying concentrations of Yeast Extract.
△ - lactate
○ - J332
□ - no electron donor


1.5
0.5
Max. OD540
0
1
2
3
% yeast extract

that role. Because of the practical importance of potential screen factor loss and observation by field personnel that sulfate-reducing bacteria might somehow be associated with the degradation of polyacrylamide, we undertook a research program to determine if there was a relationship, and if there was, to investigate the nature of the relationship. The first aspect we chose to study was to determine if, under laboratory conditions, any reduction in viscous flow properties of a polyacrylamide solution actually occurred that was specifically the result of growth of sulfate-reducing bacteria.

Screen Factor vs. Brookfield Viscosity

We have employed two methods for monitoring the the rheological properties of polymer solutions; Brookfield viscosity, which measures solution viscosity and screen factor, which measures flow properties. Screen factor is a measure of the viscoelastic charastic of a polymer solution. Viscoelastic fluids are materials that behave like viscous fluids at low rates of flow and partially as elastic solids at high rates. Flow through a porous media (or a screen viscometer) involves both shear forces (the movement of one plane in solution relative to an adjacent plane) and elongational forces (or stretching in the direction of flow). It is the viscoelastic character of the polymer that is measured when the fluid undergoes an abrupt elongation as it flows and accelerates through the

screen opening (Maeker, 1975). Viscoelasltic phenomena depend primarily on the larger molecules of the molecular weight distribution. This is also the molecular population that is preferentially degraded by mechanical degradation. Brookfield viscosity is a measure of shear viscosity. Shear viscosity is a function of the average molecular weight. It is therefore possible to produce large changes in screen factor with negligible changes in absolute viscosity. The distribution of molecular weight of polymer molecules in a 4 x 10^6 average molecular weight, 30% hydrolyzed polyacrylamide solution does not follow a Gaussian distribution (Herr and Routson, 1974). The distribution is weighted toward the high end. Extremely large molecules make up a small but significant fraction of the population (1%, 1.5 x 10^8).

The following experiment illustrates the different responses of screen factor and Brookfield viscosity, measured in centipoises, of a polyacrylamide solution to shearing in a Waring blender.

Polyacrylamide J332 was prepared at a concentration of 0.025% (W/V) in distilled water, and allowed to stand overnight in the cold room. It was then filtered, and allowed to reach ambient temperature. Shearing was accomplished by using the blender at the highest setting, and after various times determining the screen factor using a new screen viscometer, and the absolute viscosity using a Brookfield viscometer with a ULV adapter. Data for this

experiment is given in Figure 22 in which screen factor and Brookfield viscosity are plotted on the same numerical scale, this illustrates the much larger initial rate of decrease of screen factor as compared with absolute viscosity, and also the nature of the curve of decrease in screen factor with time produced by shear forces resembles an exponential curve, indicating a first order type of reaction. Brookfield viscosity, on the other hand changes very little; apparently the average molecular weight was not reduced significantly.

Reduction in Screen Factor

During our investigations our culture collection of reservoir bacterial isolates and enrighments has numbered near 1ØØ. Of those tested, none of the non-SRB cultures have shown the ability to reduce measurably the screen-factor of polymer-media solutions. We have also tested a number of bacteria targeted for potential microbial enhanced oil recovery and again none have shown the ability to reduce screen factor.

Table XV gives an example of the type of results seen in these experiments. Strain SB-2 is a Desulfovibrio sp. isolated from produced waters and is included here as a comparison. All others are non-sulfate-reducing isolates or enrichments.

FIGURE 22. Screen Factor vs. Brookfield Viscosity.
Shear time (seconds) in blender at high speed.

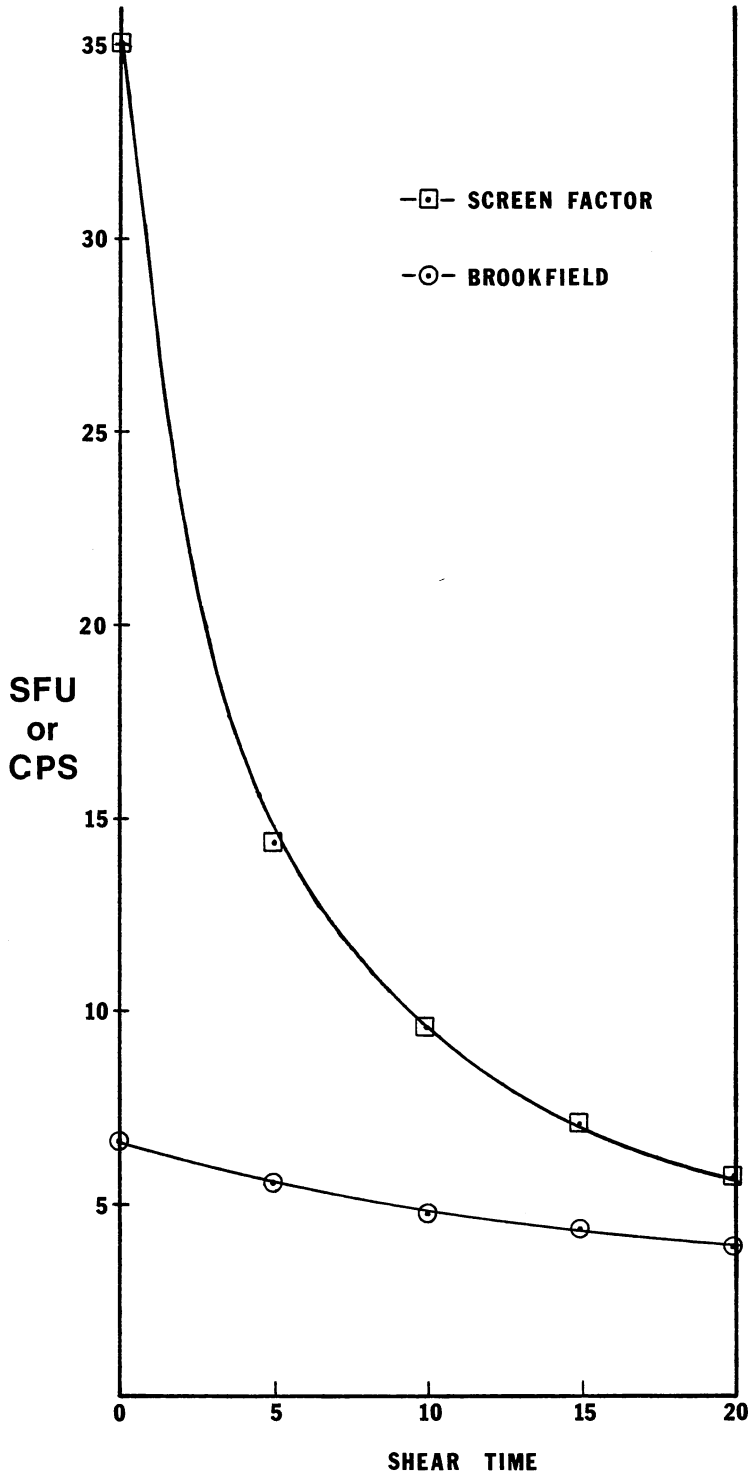

35
30
25
20
15
10
5
SFU
or
CPS
SCREEN FACTOR
BROOKFIELD
0
5
10
15
20
SHEAR TIME

TABLE X V

EFFECTS OF GROWTH OF VARIOUS BACTERIA ON SCREEN FACTOR

Incoculum (1:100)	OD_{540}	Screen factor	% change from control SF
Control (Thymol)	0.02	19.46	-
HR-73	0.32	22.01	+13.1
Cl acetobutylicum	0.25	23.16	+19.0
E. coli	0.62	26.01	+33.6
SEG - 3	0.41	20.13	+ 3.4
Fuzz	0.28	23.21	+19.3
Met	0.47	19.86	+ 2.1
Soil	0.68	25.67	+31.9
C_1	0.57	21.48	+10.4
C_3	0.83	23.71	+21.8
Sewage	0.79	20.52	+ 5.4
SB-2	0.31	15.01	-22.9

Postgate's medium C, with 0.1% J332, not autoclaved. HR-73 and SEG-3 are Clostridium spp, targeted for M.E.O.R. Fuzz and Met are non-SRB reservoir isolates. C_1 and C_3 are contaminates of SRB media isolated in the anaerobic chamber. All samples incubated anaerobically at 35°C for 2 weeks.

Reduction of Screen Factor Under Completely Anaerobic Conditions

It is well known among polymer chemists that polyacrylamides are very quickly degraded in the presence of reducing agent (such as ferrous iron) and molecular oxygen. Therefore it was natural for people in the oil industry to reach the conclusion that any reduction of screen factor that occurs accompanying growth of SRB is the result of a secondary oxidation reaction following the exposure of the system to oxygen. This opinion was expressed to us a number of times. Hence, it became very important to devise techniques, not only for growing the bacteria in the presence of the polymer anaerobically, but also for sampling and measuring viscosity/screen factor under completely anaerobic conditions. This means that samples must be removed from the culture, filtered through 200 mesh screen to remove coarse particles, and screen factor determined, using the screen viscometer, with all steps carried out in the anaerobic chamber.

An experiment of this type was done in which five strains of sulfate-reducing bacteria were grown in medium C with 0.1% polyacrylamide J332. Media were unautoclaved in order to avoid complications with change in molecular structure or loss of screen factor resulting from autoclaving the polymer in the medium.

The strains tested were SB-2, API, U9, P-13 and P-11 (latter two are not pure cultures, but are predominantly

SRB's in an enrichment medium).

All manipulations were done in the anaerobic glove box. Flasks were sealed and incubated at 37 C for one week. They were then opened, anaerobically filtered through 200 mesh screen, and the filtrates were run through the screen viscometer. Optical densities (540nm) were determined for each culture. Maintenance of anaerobic conditions during incubation was monitored using BBL indicator strips and methylene blue indicator tubes. These indicators remained in the reduced form, showing that the O/R potential remained below -50mv. Uninoculated control flasks, containing thymol to prevent bacterial growth, were incubated along with the cultures. Results are given in Table XVI.

Data in Table XVI show that, in every case, growth of sulfate-reducing bacteria was associated with a loss in screen factor under completely anaerobic conditions. The losses are not large, but in every case except possibly P-13, the decrease was significant. How important such a loss in screen factor would be from the standpoint of oil recovery we do not know.

The water samples were run to determine if: 1) bacteria indigenous to oil reservoirs, other than SRB's, randomly picked up, have any effect on screen factor; 2) any bacteria possibly present in medium ingredients (since flasks were not autoclaved) would grow and have an effect on screen factor. Apparently neither happened; since all

TABLE XVI

SCREEN FACTOR REDUCTION UNDER TOTALLY ANAEROBIC CONDITIONS

Inoculum	A540	Screen factor	% increase or decrease from Control SF
Thymol control	0.045	20.12	-
Back flush of injected water	0.53	23.34	+16.0
T-104 water	0.42	23.35	+16.1
Injection water	0.40	20.90	+ 3.9
T-101 water	0.86	21.15	+ 5.1
Sterile water	0.56	22.07	+ 9.7
Medium B (sterile)	0.50	20.95	+ 4.1
API	0.48	14.05	-30.2
SB-2	0.26	15.25	-24.2
U9	0.62	15.08	-25.0
P-13	0.32	18.83	- 6.4
P-11	0.48	17.48	-13.1

Postgate's medium C with 0.1% J332. Not autoclaved. All procedures after media preparation carried out under anaerobic conditions.

flasks with water inocula showed an increase in screen factor. This was true even though bacterial growth (sometimes fairly heavy) occurred in all these cultures. Only the cultures of SRB's (pure or mixed) showed any ferrous sulfide in the medium. A significantly greater reduction occurred in the pure cultures then in the mixed cultures. It is not known what fraction of bacteria in the mixed cultures were actually SRB's.

The Effect of Fermentative Growth on Screen Factor

Ability of Desulfovibrio to lower the screen factor of polyacrylamide solutions when all other types of anaerobic bacteria tested showed no such effect, suggested that their novel metabolism could be a factor. The anaerobic respiration of these organisms produces highly reduced sulfur compounds which we suspect play a key role in this phenomenon. Desulfovibrio also exhibits, under certain conditions, a metabolic pattern analogous to the more common fermentative bacteria. Fermentation in the physiological sense is defined as the anaerobic conversion of a substrate to products more oxidized and more reduced than the original substrate, with the organisms deriving useful energy from the process. When pyruvate is present and no sulfate is available to act as an electron acceptor Desulfobivrio will ferment pyruvate to CO_2, H_2, and acetate. The results of screen factor measurements under

fermentative conditions, with lactate-sulfate medium as a control, are shown in Table XVII. It would thus appear that the respiratory metabolism is necessary for the observed loss in screen factor. This is also supported by the observed screen factor reduction with growth on a pyruvate/sulfate medium. It is possible that sulfate-reduction itself (the process) is involved in the loss of screen factor in a manner analogous to the loss that occurs when oxygen is reduced. This possibility should be investigated in future experiments.

Studies on the Properties of Screen Factor Reducing Agent

Experiments were designed to learn more about the physical properties of the agents resulting in loss of screen factor. To obtain some information as to the molecular weight of the agent Desulfovibrio cultures were separated from a polymer/medium solution via a 10,000 MW limit membrane. After incubation for one week under anaerobic conditions, the screen factor of the polymer/medium was determined. The results shown in Figure 23 indicate that the membrane was ineffective in containing the active agent. The membrane integrity was checked via its ability to act as a barrier to blue dextran (mw 100,000) and the absence of Desulfovibrio in the polymer solution.

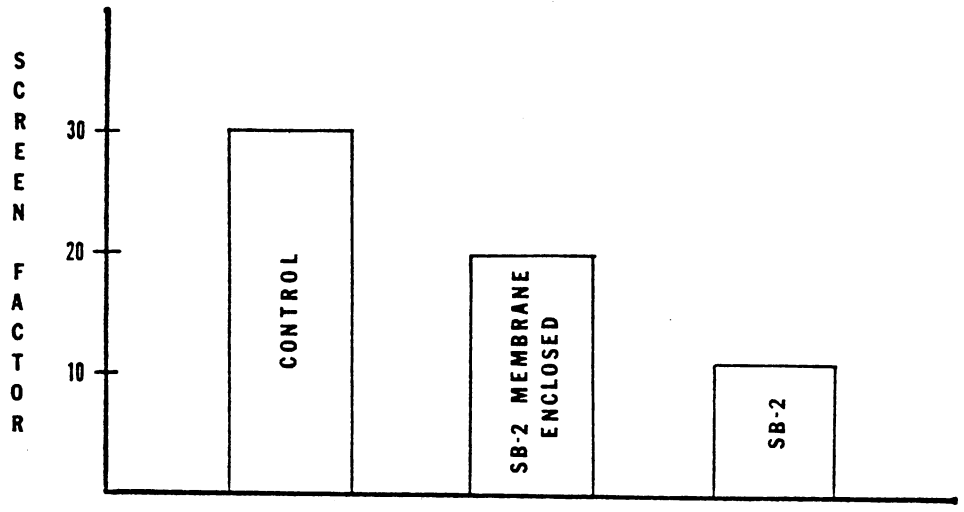
TABLE XVII

EFFECT OF FERMENTATIVE GROWTH ON SCREEN FACTOR

Medium base (Postgate)	% change over control Screen factor (triplicate cultures)
Lactate/Sulfate (C)	-10.1, -12.2, -9.0
Pyruvate (D)	+0.8, +0.2, -0.6
Pyruvate/Sulfate (C with pyruvate)	-12.0, -11.9, -14.2

Postgate's medium D or C with 0.2% J332. Inoculated 1:100 with SB-2, incubated 35°C 1 week anaerobically.

FIGURE 23. Effect of Membrane Segregation on Observed Screen Factor Loss. Postgate's medium C with 0.1% J332. In "SB-2 membrane enclosed" the SB-2 culture was isolated from the polymer/medium solution by a 10,000 mw limit membrane. "Control" is uninoculated, "SB-2" is the bacterium with free access to the polymer.


SCREEN FACTOR
30
20
10
CONTROL
SB-2 MEMBRANE ENCLOSED
SB-2

Measurement of Polymer Concentration

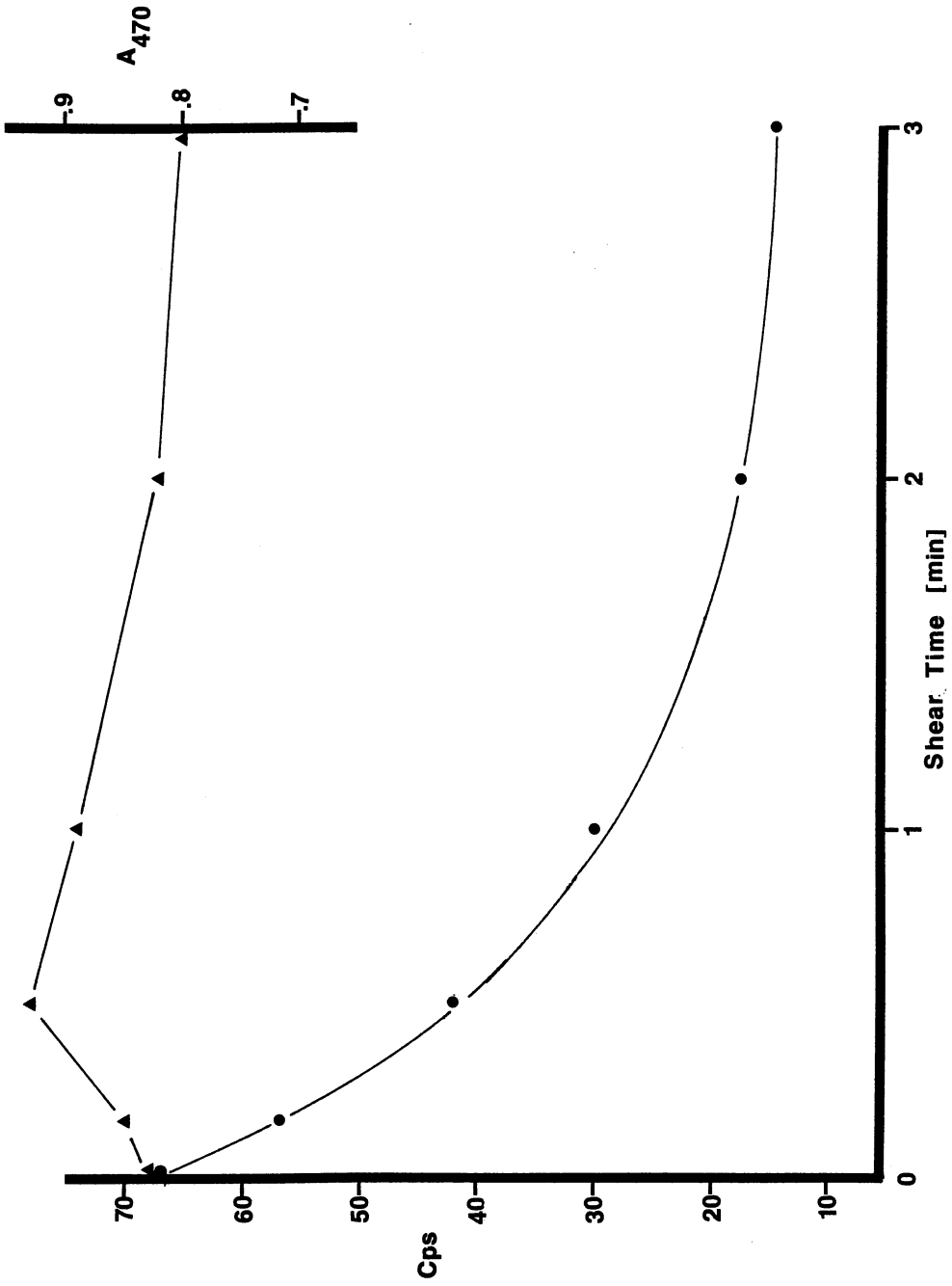
Determination of the concentration of hydrolyzed polyacrylamide in dilute solutions is difficult. Solution viscosity and screen factor have been used to determine concentration by some investigators. However, as we have seen alteration of ionic strength or mechanical shearing can give erroneous results. We have utilized the "bleach" method for polymer concentration determinations (Foshee et al., 1976). This procedure is based on the fact that polyacrylamides react in acid solutions of bleach to form colloidal suspensions which can be measured turbimetrically.

The measurement of polymer concentration after growth of Desulfovibrio showed no loss in concentration as compared to abiotic controls, even though screen factor losses were observed. Although the "bleach" method was responsive to concentration (standard curve) it showed little change with mechanical degradation (Figure 24). These results suggest that the polymer is not metabolized to any major extent but does not rule out a breakdown of large molecules to smaller molecular weight molecules.

Effects of Sulfate and Sulfide Ions on Solution Viscosity

The growth of Desulfovibrio results in a net reduction

FIGURE 24. Effect of Mechanical Shear Degradation on Polymer Concentration.
o - viscosity
Δ - trubidemetric concentration measurement


A470
.9
.8
.7
70
60
50
40
30
20
10
Cps
0
1
2
3
Shear Time [min]

of sulfate ions to sulfide ions under the conditions we have used. Obviously if the viscosity or screen factor of the polymer were comparatively more sensitive to sulfide ions than sulfate ions, this could readily explain our observations.

Under anaerobic conditions sulfate and sulfide produce equivalent viscosity losses per molar concentration. Under aerobic conditions sulfide addition leads to a further loss in viscosity due to a presumed oxidation reaction with O_2. Under anaerobic conditions the observed viscosity losses are apparently ionic strength effects.

Reduction in Screen Factor or Shear Viscosity Unaccompanied by Growth of Bacteria

It was observed early in this work that addition of a small volume of a culture (autoclaved or unautoclaved) of a sulfate-reducing bacterium to a large volume of a polymer solution would significantly reduce the viscosity of the solution. This reduction was not the result of growth of the microorganisms since it occurred under conditions completely unsuitable for growth (aerobic conditions, no culture medium), and appeared shortly after addition of the culture to the polymer solution. A volume as small as 1 ml added to 500 ml polymer solution would produce an appreciable reduction in viscosity in a few minutes, and would nearly destroy it in 24 hours. However, the extreme

decrease in viscosity over time does not occur under anaerobic conditions. This would seem to indicate that the initial decrease is an ionic strength effect while the later effect seen under aerobic conditions is due to interactions of reduced medium components and oxygen.

The lack of significant viscosity loss under anaerobic conditions is not surprising considering our inability to show Brookfield viscosity losses due to the growth of sulfate-reducing bacteria. This is further proof, we feel, that the observed screen factor losses are not due to an enzymatic degradation of the polymer backbone. Rather, we feel that the bacteria are causing an indirect effect possibly through their metabolic end products on the viscoelastic properties of the polymer. Possibly by affecting the molecular interactions between polymer molecules or the structure of those molecules in solution.

CHAPTER IV

DISCUSSION

The role of sulfate-reducing bacteria in biodeterioration problems in the petroleum industry is well documented (Atlas, 1984, Davis 1967), and here is apparently another problem to add to their infamy. Any process which increases the likelihood of bacterial plugging of reservoir formation is definitely to be avoided since such situations may well be irreversible. The ingrained fear of "bacteria" by "oil people" has been a major problem in the development of microbially-enhanced oil recovery and the study of bacteria present in petroleum formations in general. To a large extent, the reservoir engineers interest has been limited to "how do you kill them?", and while petroleum geology and reservoir engineering are highly technical and complex areas, the interaction we have had with personnel who handle actual production of petroleum at the field, has led me to the conclusion that petroleum microbiology is still in its infancy.

The observation of on-site field personnel involved with polymer floods and our sampling of produced water from oil reservoirs subjected (and not subjected) to polymer

flooding established a strong link between polyacrylamides and sulfate-reducing bacteria. This statement does not overlook the fact that sulfate-reducing bacteria are a potential problem in any type of water flooding; however, the problems associated with polymer floods, in particular polyacrylamide floods, are more severe in nature. Nor is the problem limited to the reservoir formation. Any environment which produces anaerobic conditions in the presence of polyacrylamides is susceptible to problems caused by the ubiquitous sulfate-reducing bacteria. I have received physical evidence and reports of SRB problems in above ground polymer storage tanks (H. Russell, personal communication), polymer filtration and transport equipment (Tretolite Corp., personal communications and samples), petroleum storage facilities in areas involved in polymer floods (Phillips Petroleum, samples), and even in the photographic industry where polyacrylamide is used as an emulsifying agent (Polaroid Inc., personal communications).

We have studied several possible mechanisms for the empirically observed stimulation of growth of sulfate-reducing bacteria, using strains of Desulfovibrio isolated from oil reservoirs, by polyacrylamide. These were the ability to act as a nitrogen source, incomplete substrate (electron donor), the lowering of O/R potential, and to a lesser extent the possible role of the depolymerization process on growth.

Our results showed that under nitrogen limiting

conditions, partially hydrolyzed polyacrylamides allow a limited amount of growth - more than nitrogen free control. This indicates that the polymer may serve as a nitrogen source, but we do not believe that this is the only reason for growth stimulation by the polymer. We are not surprised at these findings, as work in this laboratory had already found other organisms which were capable of utilizing the polymer in this manner. We do not believe that this is the mechanism of stimulation seen under normal conditions (medium C, Ø.1% polymer) since increased ammonium chloride levels do not decrease the observed stimulation.

Since ammonium ion is the usual source of nitrogen for sulfate-reducing bacteria (there is very little information on the ability of organic compounds, such as amino acids, or more oxidized forms of inorganic nitrogen such as nitrate) it is a real possibility that polyacrylamides provide nitrogen from the small amount released from the polymer molecule by hydrolysis. If this were the case, unhydrolyzed polyacrylamide or J279, which is only four percent hydrolyzed, would show much less or no stimulation of growth. This proved to be the case, making it likely that more highly hydrolyzed polymers (such as the J332-334 group) produce their limited stimulation by released NH_4 ions.

Many Desulfovibrio exhibit diazotrophy. Our strains apparently do not contain nitrogenase, since we could never

detect acetylene reduction in any of our Desulfovibrio cultures. The low levels of growth observed in nitrogen free media remain unexplained , but is is possible that inpurities in the medium could have allowed the low levels of growth we observed.

Our findings that polyacrylamide could serve as an incomplete substrate for Desulfobibrio was, in my opinion, the most exciting discovery of this research effort. Desulfovibrio sp. exhibit mixotrophy on a variety of compounds (Postgate, 1984), such as isobutyl alcohol, molecular hydrogen, formate and oxamate. The apparent contradiction of a high molecular weight molecule serving as an incomplete substrate can be avoided if we assume that a small fraction of the total population of molecules is being used. This is supported by the low observed yield.

Although until recently Desulfovibrio were considered to be inactive against large polymers, recent studies have shown growth of the bacteria on another molecule with structurally simple repeating units. Dwyer and Tiedje (1986) report that Desulfovibrio desulfuricans could utilize low molecular weight units of polyethylene glycol as a substrate but not the larger units.

The growth of the bacteria under mixotrophic conditions agrees with the results of several other experiments. The fact that labeled polymer is not incorporated into cellular material is consistent with mixotrophic growth as is the failure to detect a decrease in polymer concentrations

after growth of the bacteria. Gas chromatographic analysis of metabolic end products reveals profiles which are equivalent with and without polymer. However GC analysis of produced gases seems to suggest that the presence of the polymer leads to an increase in CO_2 production. This is consistent with what could be seen on incomplete substrates such as oxamate.

These results are consistent with the results of growth measurements and the changes seen in the presence of the polymer. We propose the following mechanism of stimulation. The lag phase of Desulfovibrio growth is related oxidation/reduction potential of the medium. If the O/R potential is too high, resting metabolism, respiration with little growth occurs. If the polymer provides a readily available source of electrons for $SO_4^=$ reduction, this would lead to a lowering of the O/R potential and an earlier initiation of growth, which happens in the presence of polymer. This hypothesis would also explain another observation. The maximum growth in the presence of HPAM is sometimes less than in control media. The limiting factor of SRB growth under some conditions is not substrate availability but sulfide concentration. A higher sulfide concentration per unit growth would be consistent with the model and would agree with semi-quantitative sulfide measurements we have used.

Some of our early experiments indicate that the polymer produced relatively more stimulation of growth in

sulfate deficient media. This is difficult to reconcile with a role of polymer as sulfate-reducing agent unless the actual transfer of electrons to sulfate were more efficient, if source of electrons was polyacrylamide. Under the same conditions sulfide may be toxic for Desulfovibrio. High sulfide may become a limiting factor at low levels of growth, and thus result in relatively poor growth seen with polymer as sole electron donor.

Several unexplained effects or observations occur with polyacrylamide. These include the effect on the measurable O/R potential in non-reduced media held under anaerobic conditions for long periods of time. This observation may result from a viscosity effect on the O/R electrode or may be a physical effect on the solubility of reducing gases (H_2 , H_2S) present in the anaerobic chamber. Another unexplained phenomenon is the iron polymer interactions which are discussed in the viscosity reduction section.

The mechanism of viscosity reduction is not understood. Such a straightforward question seems to call for an equally direct answer. However, all of the data we obtained give only an indistinct view into the nature of the interaction of the polymer with Desulfovibrio. Desulfovibrio growth results in a measurable loss in screen factor under the conditions we have used. It also appears that these bacteria have little or no effect on Brookfield viscosity. These facts are reconcilable since we have shown that the two measurements, although both involving

viscosity do not reflect exactly the same properties of the polymer molecule. Screen factor is more sensitive to polymer degradation and less sensitive to concentration changes than Brookfield viscosity (Foshee, 1976). We have used Brookfield viscosity measurements because they are much simpler to do and because certain industry sources consider them more important than screen factor.
Our findings seem to agree with others who suggest that Brookfield viscosity is not sensitive enough in a high ionic strength medium and that screen factor is a more valid measurement of the properties which influence the polymer's ability to effect mobility ratios (Patton, 1971).

It seems clear that the polymer is not being subjected to enzymatic cleavage of the backbone of the molecule. There is no measurable loss in concentration, and ^{14}C-carboxyl labeled HPAM studies show no measurable uptake of the molecule by Desulfovibrio. These observations do not rule out a co-metabolism of the polymer molecule; however this possibility is limited by the results of the membrane segregation experiment and the fact that autoclaved cultures still show activity. These results indicate that a metabolic product, probably extracellular, is the immediate cause of loss of screen factor. The seemingly insurmountable problem of transport into the cell of an intact molecule with a molecular weight in the 1,000,000 range still exists. Desulfovibrio must produce some extracellular entity that can bring about removal of

electrons from the polymer molecule.

One key in understanding the mechanism of the observed screen factor loss is in its link with Desulfovibrio metabolism. Only Desulfovibrio cultures show the ability to reduce screen factor and more specifically only when carrying out the anaerobic respiratory sulfate-reduction metabolism. The net chemical changes associated with this are the oxidation of lactate to acetate and CO_2 and the reduction of sulfate to sulfide. Under anaerobic conditions sulfide and sulfate appear equivalent in their ability to affect screen factor. The initial viscosity drop seen in polymer/water solutions is most likely a Na^+ ion effect. However, the greater effect of Na_2S over time under aerobic conditions illustrates an important point; polyacrylamide solutions are very susceptible to oxygen, even in trace amounts, in the presence of reducing agents. (Shupe, 1981). This degradation is even more pronounced in the presence of ferrous iron. Polymer solutions under relatively mild reducing conditions with as little as 10 ppm ferrous iron show substantial screen factor loss in only a few hours (Martin et al., 1982). We have run our experiments under anaerobic conditions, with all manipulations and measurements carried out in the anaerobic chamber and still observed substantial screen factor reduction, but we cannot dismiss the possibility that trace amounts of O_2 were present.

Another possible mechanism has been suggested by

polymer chemists concerning the three dimensional structure of the polymer in solution. In an as yet undefined manner, we envision an ionic/physical interaction between the Fe^{++} and S^{2-} ions and the carboxyl groups of polymer. Neutralizing the negative charge of the carboxyl side groups will tend to cause the polymer to fold up upon itself and could lead to the observed viscosity effects. This hypothesis fits several observations made during this work. These are the polymer/iron sulfide slimes which we have found associated with severe sulfate-reducer problems, similar "slimes" generated in the laboratory after SRB growth with polyacrylamide present and the observed darkening of growth medium with polymer present. However, the testing of this hypothesis begins to exit the realm of microbiology and enter the field of polymer chemistry.

We have defined two conditions under which the polymer may be a nutrient for SRB's but whether this entails an actual breakdown of large polymer molecules is not clear. While the polymer may serve as a nitrogen source it is not clear if the nitrogen utilized is a contaminant of the polymer preparation, the result of chemical reactions in solution or is indeed an enzymatic cleavage of the amide side chain. Enzymatic cleavage of the amide side group does not necessarily produce viscosity changes (Grula and Huang, 1981, Grula and Huang unpublished results).

The other condition is the use of the polymer as an incomplete substrate. One percent w/v polyacrylamide

solutions were extensively sheared in their preparation. Considering the levels of growth observed in the mixotrophic studies we suggest that a small fraction of the polymer molecules, most likely the extremely low molecular weight molecules, are being used by Desulfovibrio as an incomplete substrate. However, we feel that this has little bearing on the observed screen factor losses at lower concentrations.

It is interesting to try to apply our observations to the reservoir environment. It is not entirely clear as to whether or not the activities of sulfate-reducing bacteria are localized around the well bores of injection and recovery wells, or are spread uniformly throughout the aqueous phase in the formation. In either case the problems associated with the bacteria would persist. SRB problems are usually associated with FeS-slimes in which the bacteria are found in high numbers (Iverson and Olson, 1984). These slimes are particularly pronounced in the presence of polyacrylamide solution. The concentration of polymer in these biofilms may reach levels to where it could conceivably either serve as a nitrogen source, or act as an incomplete substrate.

The formation of polymer/FeS slimes would adversely affect the performance of the polymer flood, resulting in plugging of the formation and the removal of the polymer from solution. The formation of biofilms is a real problem during water flooding, and more so when SRB's are involved.

The types of bacteria that are isolated from produced waters vary greatly. Two of the most predominant types are SRB's and pseudomonads (Carlson et al., 1961). Besides bacteria of the genus Pseudomonas, many other aerobic bacteria are found. The ecological role of these aerobes in the highly anaerobic reservoir environment is not understood but many studies have implicated interactions with reservoir SRB's (Westlake 1983, Grula et al. 1985, Jobson, 1977). The susceptibility of partially hydrolyzed polyacrylamide to aerobic bacteria is well documented (Martin 1982). Our studies with mixed populations sugges that Desulfovibrio strains were the key organisms in the observed screen factor losses but a very real possibility remains that results could be different with mixed populations under the complex environmental conditions of the reservoir.

The ability to act as a nitrogen source may well be one of the reasons bacterial populations are stimulated by the presence of polyacrylamides even when no viscosity loss is seen (Martin, 1982).

In conclusion, I would like to suggest some possible experiments which might be useful in clarifying some gray areas and resolving some contradictions. There are now available on the market HPLC columns (molecular sieve type) which can be used to determine average molecular weights of polymers in solutions. This could give a quantitative answer as to what extent the polymer is being degraded, and

what the distribution of molecular weights in the degraded polymer population is, or is the observed viscosity loss a physical effect of some produce of Desulfovibrio growth.

Another possible avenue of investigation would be a physical/chemical based study on the interactions of iron, sulfide and polyacrylamide with emphasis on the physical structure of the polymer in solution.

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APPENDIX A

LIST OF MEDIA

American Petroleum Institute (API) medium

Sodium lactate	4.0 ml
Yeast extract	1.0 g
Acorbic acid	0.1 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Dipotassium phosphate (K_2HPO_4 anhydrous)	0.01 g
Ferrous ammonium sulfate ($Fe(SO_4)_2(NH_4)_2 \cdot 6H_2O$)	0.1 g
Sodium chloride (NaCl)	10.0 g
Distilled water	1,000 ml
Agar	15.0 g

Iverson Medium

Trypticase soy agar (BBL)	40.0 g
Agar	5.0 g
Sodium lactate (o.4%)	600 ml
$MgSO_4 \cdot 7H_2O$	2.0 g
Ferrous ammonium sulfate	0.5 g
Distilled water	400 ml

Allred's Medium

Calcium lactate	3.5 g
Yeast extract	1.0 g
Ascorbic acid	0.1 g
Sodium thioglycollate	0.1 g
$MgSO_4$	0.2 g
K_2HPO_4	0.2 g
$Fe(SO_4)_2(NH_4)_2$	0.05 g
NaCl	10.0 g
Agar	15.0 g
Distilled water	1000 ml

Postgate's Media

	Concentration g/l		
Component	Medium B	Medium C	Medium D
KH_2PO_4	0.5	0.5	0.5
NH_4Cl	1.0	1.0	1.0
$CaSO_4$	1.0	-	-
Na_2SO_4	-	4.5	-
$MgSO_4 \cdot 7H_2O$	2.0	2.0	-
Sodium lactate	3.5	3.5	-
Sodium pyruvate	-	-	3.5
$CaCl_2 \cdot 2H_2O$	-	0.06	0.1
$MgCl_2$	-	-	1.6
$FeSO_4 \cdot 7H_2O$	0.5	0.004	0.004
Yeast Extract	1.0	1.0	1.0
Sodium Citrate	-	0.3	-

Postgate's Medium F

Iron sulfite agar (oxoid)	23.0 g
Sodium lactate	3.5 g
$MgSO_4 \cdot 7H_2O$	0.2 h
Distilled Water	1000 ml

APPENDIX B

Reservoir Characteristics
HXa Sand
Willmington Field, CA

Porosity	32%
Interstitial Water	30%
Permeability (air)	439md
Oil Gravity	17 API
Oil Viscosity at Reservoir Conditions	25 cp
Reservoir Temperature (original)	145 F
Original Mobility Ratio	3.2
Project Area	11 acres
Net Sand Thickness	52 ft.
Depth (VSS)	2900 ft.
Current Oil Saturation	38% pv
Pattern Pore Volume	1.42 x 10 Bbls.
Current Oil-in-Place	540,000 Bbls.

VITA

Guy W. Sewell

Candidate for the Degree of

Doctor of Philosophy

Thesis: INTERACTIONS OF POLYACRYLAMIDES USED FOR ENHANCED OIL RECOVERY AND RESERVOIR ISOLATES OF THE SULFATE-REDUCING BACTERIUM DESULFOVIBRIO

Major: Microbiology

Biographical:

Personal Date: Born in Stillwater, Oklahoma on March 27, 1957, son of Vernon L. and Waynell Sewell.

Education: Graduated from Atoka High School, Atoka, Oklahoma, in May 1975; received Bachelor of Science Degree in Microbiology, minor in Chemistry, from Oklahoma State University in May, 1980; completed requirements for the Doctor of Philosophy Degree in Microbiology at Oklahoma State University in July, 1987.

Professional Experience: Graduate Teaching Assistant, Microbiology Department, Oklahoma State University, August, 1980 to May, 1983; Graduate Research Assistant, Microbiology Department, Oklahoma State University, June, 1983, to August, 1986; presentations to Missouri Valley Branch of the American Society for Microbiology, the Society for Industrial Microbiology, the American Society for Microbiology, and the International Conference on Microbial Enhancement of Oil Recovery.

Professional Organizations: Member of the American Society for Microbiology and the Society for Industrial Microbiology.