

## UTILIZATION OF CARBON DIOXIDE FROM FOSSIL FUEL - BURNING POWER PLANTS WITH BIOLOGICAL SYSTEMS

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### ABSTRACT

Currently available physical-chemical processes for CO<sub>2</sub> capture and disposal from fossil fuel-fired power plant flue-gases are very expensive. Biological systems for CO<sub>2</sub> utilization involve plant photosynthesis and conversion of the biomass produced to fuels that can substitute for fossil fuels. Photosynthesis by many plants increases with higher CO<sub>2</sub> levels suggesting that flue gas fertilized greenhouses or even flue gas dispersal into open plant stands could increase biomass production. However, such systems are neither effective nor practical. Only for submerged aquatic plants - microalgae, seaweeds, and some higher plants - does a high concentration of CO<sub>2</sub> as present in flue-gases, result in large increases in productivity. Microalgae have the potential for high productivities and ready conversion to gaseous and liquid fuels. A cost-analysis of such a process suggests that if high productivities are indeed achievable, overall costs could be much lower than currently available methods for CO<sub>2</sub> flue gas capture and disposal. Limitations are the relatively large land areas required, a maximal reduction in CO<sub>2</sub> outputs of only 25 to 30% of total emissions, and the relatively undeveloped state of this technology.

### INTRODUCTION

Reducing CO<sub>2</sub> loads on the atmosphere is required to forestall potentially catastrophic consequences of the greenhouse effect. Although the possible consequences are highly uncertain, reducing the current and projected rise in CO<sub>2</sub> concentration appears to be a prudent course of action. Several European countries and Japan are proposing to reduce current levels of CO<sub>2</sub> emissions from fossil fuels by 20% to 25%. Natural processes already remove 50 to 60% of anthropogenic CO<sub>2</sub> emissions, thus a 25% reduction would actually slow atmospheric CO<sub>2</sub> increases by between 40% to 50%, assuming current conditions continue. This would significantly reduce the probabilities of the catastrophic consequences of the greenhouse effect (Benemann, 1992). Fossil fuel-burning power plants generate about 25% of all fossil fuel derived atmospheric CO<sub>2</sub> inputs. Thus they are a major target in plans to reduce CO<sub>2</sub> accumulation in the atmosphere.

Reducing the CO<sub>2</sub> outputs of fossil fuel-fired power plants could be accomplished through a number of methods, such as increasing efficiency in fossil fuel utilization, substitution of fossil fuels with energy sources that do not produce net CO<sub>2</sub> emissions, establishing remotely sited reforestation projects that would sequester CO<sub>2</sub> into standing biomass (Marland, 1988), and recovery and subsequent sequestration of CO<sub>2</sub> directly from flue (stack) gases of fossil fuel-burning power plants. The latter options, CO<sub>2</sub> sequestration from flue gases using presently available chemical scrubbing systems, appear to be much more expensive than the former. Recent cost-analysis of the costs of

CO<sub>2</sub> removal and concentration from stack gases suggests that this would essentially double current electricity costs, and the amount of fossil fuel used. This does not include ultimate disposal of the sequestered CO<sub>2</sub> (in the ocean depths, depleted oil and gas wells) which add to costs and uncertainties (Herzog et al., 1991; Fluor Daniels, 1991).

### BIOLOGICAL CO<sub>2</sub> MITIGATION OPTIONS

A variety of microbes utilize CO<sub>2</sub>. But all, except for the microalgae, require some inorganic reducing agent (H<sub>2</sub>, H<sub>2</sub>S, NH<sub>3</sub>, pyrites, etc.). Such substrates are unlikely to be available in the quantities required for CO<sub>2</sub> removal from power plants. If nuclear or solar (photovoltaics) electricity generation were to allow economical H<sub>2</sub> production, it could be plausibly converted to a C-based fuel using CO<sub>2</sub>. The methanogenic bacteria are able to convert H<sub>2</sub> and CO<sub>2</sub> into CH<sub>4</sub>. However, it is likely that methanol would be the preferred product, as it would be useable as a liquid fuel, favoring chemosynthetic processes. Thus, only photosynthetic processes based on water as the electron source for CO<sub>2</sub> reduction are likely applicable for CO<sub>2</sub> mitigation.

Photosynthetic processes are able to convert CO<sub>2</sub> into biomass, which can be used or in turn converted to biomass fuels that can replace fossil fuels, either for electricity production or in other sectors of the economy (e.g. transportation) (Benemann, 1980). Plant photosynthesis is already a major world-wide source of fuels, with biomass fuels representing about 15% of all primary energy consumption (Scurlock and Hall, 1987). Biomass fuels could displace a major fraction of current fossil fuel consumption, particularly if CO<sub>2</sub> mitigation were to become a policy and economic goal.

Biomass production takes place in the presence of atmospheric levels of CO<sub>2</sub>, the concentrated CO<sub>2</sub> present in flue gases is not required. Nevertheless, it is well known that plants exhibit higher productivities under elevated levels of CO<sub>2</sub>. In greenhouses, elevated levels of CO<sub>2</sub> are routinely used to increase plant production. The cultivation of algae, both the seaweeds and the smaller microalgae, requires an enriched source of CO<sub>2</sub>, as the transport of CO<sub>2</sub> from the atmosphere into the growth ponds is not sufficient to support their growth. These possibilities were reviewed in the present evaluation of biological systems for direct utilization and mitigation of stack-gas CO<sub>2</sub> sources.

### PHOTOSYNTHETIC PROCESSES FOR FLUE GAS CO<sub>2</sub> UTILIZATION

The use of higher plants, either in greenhouses or in the open air, for the utilization of flue gas CO<sub>2</sub> has been proposed (Bassham, 1977) but does not appear practical nor feasible. CO<sub>2</sub> fertilization can increase plant productivities by a significant factor (20 to 30% are typical enhancements, although higher values are reported, see Benemann, 1982, for references). However, comparisons with open air cultivation are not as favorable because of the reduction in light intensities in greenhouses due to glazing - which typically are in the same range as the CO<sub>2</sub> fertilization effects. Thus, overall, greenhouse agriculture is, in principle, not significantly more productivity than open air systems. However, greenhouse agriculture does exhibit high productivities (Wilson et al., 1992), but for other reasons: greater control over water supply and fertilizers, higher management inputs, and, of course, temperature control to overcome low temperatures in unfavorable climates. However, greenhouse crops cost typically over ten times more to produce than open air crops. Thus, greenhouses would not be a suitable method for biomass fuels production, the objective of any CO<sub>2</sub> mitigation program.

An alternative possibility would be to fertilize open air stands of plants (trees, row crops) with flue-gas CO<sub>2</sub>, dispersed through distribution pipes. The major factors to consider are the effects of wind, turbulence, etc. on the dispersal plume and the effects of highly variable CO<sub>2</sub> concentrations on plant productivity. Experimental systems are being operated, to study the effects of CO<sub>2</sub> on natural stands (e.g. outside of greenhouses) in which dispersal is through distribution pipes which discharge CO<sub>2</sub> from various points in a stand, computer controlled to adjust for variations in wind direction, intensity, daytime, etc. In a large stand, encompassing many square miles, such systems may indeed achieve a relatively good dispersal. However, the incremental productivities, estimated at 20 to 30% from those observed without CO<sub>2</sub> supplementation, and most likely only half those, would not likely justify the extensive distribution piping and control systems required. Also, the actual utilization factor for the CO<sub>2</sub> is likely to be low. This preliminary analysis suggests that such a process could not be justified.

This leaves the submerged plants - microalgae, seaweeds and some higher plants - as the only biological systems which could benefit from the use of flue gas levels of CO<sub>2</sub> (typically about 10% by volume). The transfer of CO<sub>2</sub> from the atmosphere into a pond, assuming essentially zero CO<sub>2</sub> in the ponds, would only support 1 to 2 g of biomass production/m<sup>2</sup> per day, a small fraction (< 5%) of potential productivity. Energetically it is not feasible to supply CO<sub>2</sub> by bubbling air through the cultures, to provide CO<sub>2</sub> (Steinberg, 1991). Only a highly enriched source of CO<sub>2</sub>, e.g. flue-gas, could supply submerged plants with the CO<sub>2</sub> required. Thus, among biological systems only submerged plants could make use of flue gas CO<sub>2</sub> sources.

#### SUBMERGED HIGHER PLANTS AND SEAWEEDS

Of the three alternatives - microalgae, seaweeds, and higher plants - the latter two have significant limitations. Higher submerged plants exhibit relatively low productivities, even under optimal conditions of nutrient and CO<sub>2</sub> supply (Murry and Benemann, 1980). The reasons for this is primarily due to hydrodynamic factors; it is difficult to get good water exchange in dense stands of such submerged plants. In water CO<sub>2</sub> diffusion is over 1,000 times slower than in air, and thus not only the transfer into the ponds but also from the water phase to the leaves is a major limitation in such systems. Creation of sufficient turbulence to overcome diffusion limitations does not appear practical.

Seaweeds exhibit relatively higher productivities than submerged higher plants, and have been produced commercially in near-shore, shallow ocean systems and have been considered for energy production (See Bird and Benson, 1987, for reviews). In such systems the C required is provided from seawater, and the relatively high water exchange in such open systems. Seawater, at pH 8.2 and 2.3 meq. l<sup>-1</sup> alkalinity, contains almost 40 mg of available CO<sub>2</sub> (assuming an upper pH of about 10 for seaweed growth). A 1 m deep culture system thus would be able to support a production of about 20 g/m<sup>2</sup> of biomass (organic dry weight assuming 50% C), suggesting a once to twice a day water turn-over to supply the required C. Thus, seaweed culture, as currently practiced in near-shore environments, would not be limited by C, and would not require flue gas CO<sub>2</sub> injection.

In on-shore, shallow ponds, water exchange would become a limiting factor, depending on lift (head losses). From the above, it would require approximately 50,000 m<sup>3</sup> of seawater to supply the C required for one ton of biomass, which would make seawater an uneconomical source for this nutrient for on-shore systems. The advantages of on-shore,

vs. near-shore, cultivation is that a higher control over cultivation conditions is feasible, including predations, competing algae, diseases, etc. Also, other nutrients (N, P, Fe, etc.) can be supplied without the large losses experienced in near-shore farming techniques. And, perhaps most important, the losses experienced due to storms would be minimized. However, seaweed culture in open ponds has not been developed except on an experimental basis. The reason for this are the hydrodynamic constraints on such cultures: the rather dense seaweed cultures require considerable mixing and turbulence to allow effective transfer of nutrients (particularly CO<sub>2</sub>) to the plants (Wheeler, 1988). Such mixing and turbulence requires considerable energy inputs, which would make such systems impractical, at least for the production of fuels. Although preliminary, this evaluation suggests that on-shore seaweed cultivation is not a favorable approach to flue gas CO<sub>2</sub> utilization. These mixing limitations do not apply to the smaller microalgae.

#### MICROALGAE FOR CO<sub>2</sub> MITIGATION

Microalgae culture technology has been developed for over 40 years (Burlaw, 1953). The concept of using the CO<sub>2</sub> in power plant flue gases for producing microalgae and converting the biomass to fuel, was first studied over thirty years ago by Oswald and Golueke at the University of California Berkeley. They proposed using municipal sewage to grow algae in large open ponds into which flue gas would be injected, harvesting the biomass by settling, and digesting it to methane gas, which would be used by the power plant. The digester residues (containing the nutrients and residual organic and inorganic C) and water would be recycled, allowing system size expansion well beyond that feasible from the production of algae for waste water treatment alone. A laboratory-scale system, involving algae growth, digestion to methane, and recycle of the nutrients, was successfully demonstrated (Golueke and Oswald, 1959). A preliminary analysis concluded that with favorable assumptions this process could be economically competitive with nuclear power (Oswald and Golueke, 1960). This concept was further refined by Oswald and colleagues and others during the 1970's, with research sponsored in large part by the U.S. Department of Energy (U.S. DOE). Conceptual engineering designs and cost estimates (Benemann et al., 1982) supported the conclusion that, in principle, algal biomass cultivation in open ponds could be relatively inexpensive.

About a decade ago, the "Aquatic Species Program" (ASP), was initiated at the Solar Energy Research Institute (SERI, now NREL, National Renewable Energy Laboratory, a U.S. DOE facility). The ASP emphasized the development of algae systems for the production of liquid transportation fuels (specifically vegetable oils) (Neenan et al., 1986). This program, supported many basic research projects, including isolation of a large number of algal strains and investigation of biochemical and genetic aspects of lipid production in microalgae. Three outdoor algal production facilities were supported by the ASP, in California Hawaii and New Mexico. These outdoor projects, together with an updated engineering and cost feasibility analysis (Weissman and Goebel, 1987), and the considerable experience from commercial operations for microalgae production for food supplements (Benemann et al., 1987), and use of microalgae in waste water treatment (Oswald and Benemann, 1980), support the conclusion that, in principle, it is possible to produce microalgae in large-scale outdoor ponds at both high productivity and at relatively low cost. Microalgae are now being studied for CO<sub>2</sub> mitigation in the U.S. and Japan (Laws and Berning, 1991, Negoro et al., 1991, for examples). However, considerable R&D is still required and many aspects of this technology remain to be demonstrated, from species control and stability, to harvesting and algal processing.

## COST ANALYSIS OF MICROALGAE CO<sub>2</sub> MITIGATION

Table 1 summarizes the overall cost estimates for a large-scale (appx. 1,000 ha) microalgae production system for liquid-fuels using flue gas CO<sub>2</sub> from a power plant. Using a "CO<sub>2</sub> mitigation credit" of \$16/tCO<sub>2</sub>, fuel costs of about \$40/barrel are projected. These cost estimates are based on prior studies (Benemann et al., 1982; Weissman and Goebel, 1987), and reflect numerous favorable assumptions about both the engineering and biological aspects of such a system. For example, the individual growth ponds would be "raceway" designs, with a single central baffle and 10 ha in size, over ten times larger than any operated previously, and mixed with paddle wheels. For economy, the ponds would be earthwork construction without plastic liners, with a clay sealer to minimize percolation. The water source (such as seawater) must contain sufficient alkalinity to allow some CO<sub>2</sub> storage. CO<sub>2</sub> would be supplied via diffusers and sumps. Major design factors are the depth of the sumps (which determines transfer efficiency), the mixing velocities (typically 20 - 30 cm/sec), the number of carbonation stations (which depend on the CO<sub>2</sub> storage, pH range for operations and outgasing rates), the depth of the pond culture (typically 20 to 30 cm). These factors are interactive and must be optimized.

Many aspects of this process require R&D. Harvesting involves "bioflocculation", in which the algae spontaneously flocculate and sediment in settling ponds. Although a well known natural process which has been demonstrated in waste grown algae (Benemann et al., 1980), its applicability to large-scale systems needs to be demonstrated. The extraction and processing of the vegetable oils from the algal biomass was cost mated based on soybean processing as no relevant data for algal biomass is available. The algal oils would be produced by limiting the algal cultures for nitrogen, which has been demonstrated at the laboratory scale (Benemann and Tillett, 1987) but not yet in open ponds. The residue from the oil extraction would be fermented to produce methane gas.

Most important, in Table 1 two different productivities were assumed, about 109 and 219 metric tons/ha/yr, corresponding to about 5 and 10% solar energy conversion efficiencies, for favorable sites in the U.S.. The lower productivity is based on present experience. The higher productivity will require the development of algal strains that have a lower pigment content, allowing better overall light utilization in dense cultures (Benemann, 1990). If the higher productivities are indeed achievable, and the other assumptions on which this cost estimate is based are verified, then such a process could produce biomass fuels using flue gas CO<sub>2</sub>, with a relatively modest CO<sub>2</sub> mitigation credit (\$16/t CO<sub>2</sub>), within the range of those presently discussed (Lashoff and Tirpak, 1989).

A major constraint on such systems, besides the R&D issues, is the availability of sufficient land and water near the power plant. A 1,000 MWe power plant would require as few as 6,000 ha (Table 2). Also, only about 30% of the CO<sub>2</sub> emissions from the power plant could be captured, as the system would be sized to utilize most of the CO<sub>2</sub> produced during peak summer daytime utilization, wasting night and much of the winter CO<sub>2</sub> outputs. However, as pointed out in the introduction, such a rate of CO<sub>2</sub> capture would mitigate most of the potential adverse effects of CO<sub>2</sub> released from such a power plant. And the land area required is a small fraction, less than one tenth, that required for other biomass systems (tree farms). Perhaps most important, such systems would provide over 3 million barrels of fuel per year. Although much R&D is still needed, no insurmountable problems are apparent and no "breakthroughs" are required. Microalgae systems could become an affordable process for CO<sub>2</sub> removal from flue gases.

## REFERENCES

- Bassaham, J.A., in A. Mistui et al., eds., Biological Solar Energy Conversion, Academic Press, New York, p. 151 (1977).
- Benemann, J.R., Plant and Soil, in press (1992).
- Benemann, J.R., D.M. Tillett, and J.C. Weissman, Trends in Biotechnology, 5, 47 (1987).
- Benemann, J.R., R.P. Goebel, J.C. Weissman, D.C. Augenstein. Microalgae as a Source of Liquid Fuels, Final Report U.S. Department of Energy, pp. 202 (1982).
- Benemann, J.R., in Algal Biotechnology, in R.C. Cresswell, T.A.V. Rees, and N. Shah, eds., Longman, London pp. 317 (1990).
- Benemann, J.R., B.L. Koopman, J.C. Weissman, D.E. Eisenberg and R.P. Goebel, in G. Shelef, and C.J. Soeder, eds., Algal Biomass, Elsevier, Amster., p. 457 (1980).
- Benemann, J.R. Intl. Energy Journal, 1, 107 (1980).
- Benemann, J.R., and D.M. Tillett, in D. Klass, ed., Symp. Proc. Energy from Biomass and Wastes XI, Institute of Gas Technology, Chicago, Ill. (1987).
- Bird, K.T., and P.H. Benson (eds.), Seaweed Cultivation for Renewable Resources, Elsevier, Amsterdam (1987).
- Burlew, J., Algae Culture: From Laboratory to Pilot Plant, Carnegie Institute, Washington D.C. (1953).
- Fluor Daniel, Inc., Engineering and Economic Evaluation of CO<sub>2</sub> Removal from Fossil-Fuel Power Plants, Electric Power Research Institute, Palo Alto, California, EPRI IE-7365 (1991).
- Golueke, C.G., and W.J. Oswald, App. Microbiol., 7, 219 (1959).
- Herzog, H., D. Golomb, and S. Zemba, Environmental Progress, 10, 64 (1991).
- Lashof, D.A., and D.A. Tirpak, Policy Options for Stabilizing Global Climate, U.S. Environmental Protection Agency, Washington D.C., February (1989).
- Laws, E.A., and J.L. Berning, Biotech. Bioeng., 37, 936 (1991).
- Marland, G., The Prospect of Solving the CO<sub>2</sub> Problem Through Global Reforestation, Oak Ridge Associated Universities, Department of Energy, Washington D.C. DOE/NBB-0082 (1988).
- Murry, M.A. and J.R. Benemann, in T.A. McClure and W.S. Lipinski, eds., Handbook of Biosolar Resources. C.R.C. Press, Boca Raton, FL., III, p. 407 (1981).
- Neenan, B., et al., Fuels from Microalgae: Technology Status, Potential, and Research Requirements, Solar Energy Research Institute, Golden, Colorado, SERI SP-231-2550 (1986).
- Negoro, M., N. Shioji, M. Miyamoto, and Y. Miura, Biochem. Biotech., 28/29, 877 (1991).
- Oswald, W.J., and C.G. Golueke, in S. Pietro, ed., Biochemical and Photosynthetic Aspects of Energy Production, p. 59 (1980).
- Oswald, W.J., and C.G. Golueke, Adv. Appl. Microbiol., 11, 223 (1960).
- Scurlok, J.M.O. and D.O. Hall, Biomass 21: 75 (1990).
- Steinberg, M., J. Lee, and S. Morris, An Assessment of CO<sub>2</sub> Greenhouse Gas Mitigation Technologies, Brookhaven National Lab., Upton, NY, BNL 46045 (1991).
- Weissman, J.C., R.P. Goebel, and J.R. Benemann, Bioeng. Biotech., 31: 336-344 (1988).
- Weissman, J. C. and R. P. Goebel, Design and Analysis of Pond Systems for the Purpose of Producing Fuels, Solar Energy Research Institute, Golden Colorado SERI/STR-231-2840 (1987).
- Wheeler, W.N., Prog. in Phycological Res., 6, 23 (1988).
- Wilson, J.W., D.W. Hand, and M.A. Hannah, J. Exp. Botany, 43, 363 (1992).

TABLE 1. MICROALGAE SYSTEM CAPITAL AND OPERATING COSTS

PRODUCTIVITY ASSUMED: Average Daily: (ash-free dry weight)	30 g/m <sup>2</sup> /d Annual: 109 mt/ha/yr	60 g/m <sup>2</sup> /d 219 mt/ha/yr
<b>CAPITAL COSTS (\$/ha):</b>		
Ponds (earthworks, CO <sub>2</sub> sumps, mixing)	27,500	33,000
Harvesting (settling ponds, centrifuges)	12,500	17,000
System-wide Costs (water, CO <sub>2</sub> supply, etc.)	30,000	40,000
Processing (oil extraction, digestion)	10,000	20,000
Engineering, Contingencise (25% of above)	20,000	27,500
<b>TOTAL CAPITAL COSTS (\$/ha)</b>	<b>100,000</b>	<b>137,500</b>
Capital Costs \$/t-yr	920	630
Barrels of Oil/y (@ 3.5 bar./t)	380	760
<b>CAPITAL COSTS \$/Barrel/y</b>	<b>260</b>	<b>180</b>
<b>OPERATING COSTS (\$/ha/yr):</b>		
Power, nutrients, labor, overheads, etc.	10,000	15,500
Credit for methane	- 3,000	- 6,000
<b>Net Operating Costs \$/ha/yr</b>	<b>7,000</b>	<b>9,500</b>
Net Operating Costs \$/barrel oil	18	13
CO <sub>2</sub> Mitigation Credits (\$16/tCO <sub>2</sub> )	-10	-10
Annualized Capital Costs (0.2 x Capital)	52	36
<b>TOTAL COSTS \$/BARREL</b>	<b>60</b>	<b>39</b>

TABLE 2. LAND REQUIREMENTS FOR ALGAE CO<sub>2</sub> UTILIZATION

Assumptions: 30% CO<sub>2</sub> average annual CO<sub>2</sub> utilization  
 1,000 MW power plant, 0.88 kgCO<sub>2</sub>/kwh (Herzog et al., 1991).  
 Composition: 50% lipid, 25% carbohydrate, 25% protein.  
 Heat of Combustion: 7.5 Kcal/g (60% C in biomass).  
 Avg. Annual Solar Insolation: 500 Langleys, 45% visible.  
 Production: 1.05 x 10<sup>6</sup> mt/yr biomass; 3.7 x 10<sup>6</sup>/yr barrels oil.

<b>PRODUCTIVITY ASSUMPTIONS:</b>		
Avg. Ash free dry weight g/m <sup>2</sup> /d	30	60
Annual Productivity mt/ha/yr	109	219
Lipid fuels barrels/ha/yr	380	760
Solar Conversion Efficiency (appx.)	5	10
Fixation C mt/ha/yr	66	131
Fixation CO <sub>2</sub> mt/ha/yr	241	482
<b>LAND AREA REQUIREMENTS:</b>		
,000 Ha required growth ponds area	9.6	4.8
,000 Ha total area (ponds x 1.25)	12	6

that *T. denitrificans* may be readily cultured aerobically and anaerobically in batch and continuous reactors on gaseous H<sub>2</sub>S under sulfide-limiting conditions. A microbial process for the removal of H<sub>2</sub>S from gases have been proposed based on contact of the gas with a culture of *T. denitrificans* [3]. Sublette and Sylvester [3,4] have shown that sulfide concentrations as low as 100 to 200 μM inhibit the growth of the wild-type strain of *T. denitrificans* (ATCC 23642) on thiosulfate. Complete inhibition was observed at initial sulfide concentrations of 1 mM. However, a sulfide- and glutaraldehyde-resistant strain (strain F) of *T. denitrificans* has been isolated by enrichment from cultures of the wild-type [7]. This strain grows at inorganic sulfide concentrations in excess of 1000 μM and glutaraldehyde concentrations of 25 to 40 ppm. These concentrations are lethal to the wild-type.

*T. denitrificans* strain F has been successfully grown in co-culture with the sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, both in liquid culture and through Berea sandstone cores without the accumulation of sulfide [8]. The presence of the sulfide-resistant strain F also controlled microbial sulfide production in an enrichment from an oil field brine. The effectiveness of strain F is due to its ability to grow and use sulfide at levels which are inhibitory to the wild-type strain of *T. denitrificans*.

The ability of *Thiobacillus denitrificans* strain F to control H<sub>2</sub>S production in an experimental system using cores and formation water from a gas storage facility was investigated. Strain F and nitrate were added to nutrient amended formation water and injected into the core system. It is important to note that the objective was not to control the concentration of sulfate-reducing bacteria. Strain F does not inhibit the growth of sulfate-reducing bacteria; it simply removes the unwanted product of sulfate reduction, sulfide [8]. The test was therefore considered successful if the sulfide concentration of the effluent of the core treated with strain F was lower than that found before strain F treatment.

## MATERIALS AND METHODS

### Formation Water

Formation water was collected daily from well Davis-6 of the Northern Natural Gas Co. gas storage in Redfield, Iowa. The chemical composition of the water was as follows (in mg/L): iron (0.6), sulfide (9), chloride (420), sulfate (450), phosphate (1.8), hardness (960), alkalinity (660), and total dissolved solids (718).

### Core System

The core system used in these experiments was assembled by Bioindustrial Technologies, Inc. (BTI, Grafton, NY) and was previously used to test the effectiveness of biocide formulations in controlling sulfide production by sulfate-reducing bacteria in the cores. BTI operated the core system with a feed of Davis-6 formation water for approximately ten weeks. Following the completion of the BTI studies, the core system was flushed with formation water at approximately 75 mL/hr in the treated core (see below) and at approximately 14 mL/hr in the control core for seven days before the experiments described here were initiated.

The core system contained cylindrical cores of St. Peter sandstone with dimensions of about 2.5 cm diameter and 7.5 cm length, each of which was mounted in polyvinyl chloride (PVC) tubing. Two sets of three cores each were connected in series using stainless steel tubing and compression fittings. Each set of cores had its own feed pump to inject fluids. The intake line of each core system had a course 5 μm membrane

filter to remove suspended solids (iron sulfides) from the fluid before injection into the cores. A sampling port was located at the inlet side of each core in the set. The porosity of the St. Peter sandstone was 30%. From the porosity of the cores and the volume of the tubing, the liquid volume of the core system was estimated to be 240 mL.

As noted above, one set of cores had previously been treated with biocides while the other set of core set served as the control. The injectivity of the control set of cores was much lower than that of the biocide treated set. Because of this, the flow rates of the two core sets were very different; the flow rate of the control core set was 14 mL/h while that of the treated core set was 75 mL/h. These were the maximum flow rates that could be obtained without leakage due to an excessive pressure. The hydraulic retention times were 3.2 and 16.7 h for the test and control cores systems, respectively.

#### Stock Cultures

Stock cultures of *Thiobacillus denitrificans* strain F were maintained anaerobically in thiosulfate medium described previously [4]. In this medium, thiosulfate is the energy source, nitrate is the terminal electron acceptor, bicarbonate is the source of carbon and ammonium ion is a source of reduced nitrogen. Stock cultures were transferred every 30 days and stored at 4°C until used.

#### Growth of Cells for Core Injection

*T. denitrificans* strain F cells were grown anaerobically in thiosulfate medium in 2-L cultures in a B. Braun Biostat M at 30°C and pH 7.0. The culture received a gas feed consisting of 30 mL/min of a gas mixture containing 5% CO<sub>2</sub>, with the balance being N<sub>2</sub> to ensure that the culture did not become carbon limited. When the OD<sub>460</sub> of the culture medium reached approximately 1.0 (about 10<sup>9</sup> cells/mL), the cells were harvested by centrifugation at 5000 x g and 25°C. The cells were then washed with 15 mM phosphate buffer, pH 7.0, and shipped as a wet pellet by overnight delivery service to the test site. Sufficient medium (without thiosulfate) was used to resuspend the pellet in a five-liter beaker so that the suspension was only slightly turbid. The viable cell concentration of the suspension was estimated by end-point dilution method using the above medium with thiosulfate. Because of the lack of facilities on site, medium that was injected into the core system was not sterilized.

#### Core Experiments

The objective of the first experiment (E1) was to determine whether indigenous microbial populations capable of oxidizing sulfide and using nitrate as the electron acceptor were present in the core system. Formation water supplemented with 40 mM sodium nitrate was injected into the test core set only for 24 h. Formation water with sodium nitrate was then injected into both sets of cores for another 24 h. After each 24 h period, a sample was collected from the sample port located upstream of the first core of each set and from the tubing exiting each core set. The samples were immediately analyzed to determine the concentrations of sulfide, sulfate-reducing bacteria, acid-producing bacteria, and strain F. The remainder of each sample was frozen and analyzed for nitrate, nitrite, sulfate, and sulfite at a later date.

In a second experiment (E2), *T. denitrificans* growth medium without thiosulfate was injected into each core set for 40 h to determine whether the addition of nutrients would stimulate the production of sulfide in the core system. Samples for chemical and microbiological analyses were taken after 24 and 40 h of medium injection.

In a third experiment (E3), the test core was inoculated with strain F to determine the effectiveness of this organism in preventing the production of sulfide in a continuous flow system. Approximately  $10^9$  viable cells/mL of strain F suspended in growth medium (without thiosulfate) was injected into the test set of cores for 6 h (about 0.5 L). This was followed by the injection of growth medium without thiosulfate for 24 h. This cell inoculation procedure was repeated once. During the inoculation procedure, growth medium without thiosulfate was injected into the control core set. Samples for chemical and microbiological analyses were taken every 24 h.

In a fourth experiment (E4), a mixture of growth medium and formation water starting with 90% (vol/vol) growth medium without thiosulfate and 10% (vol/vol) formation water with 40 mM sodium nitrate was injected into both sets of cores. Every 12 h, the fraction of formation water with nitrate injected into the core system was increased by 10% until only formation water with nitrate was injected into the core system. When the fraction of formation water injected into the core was 30, 60 and 80%, the test core was again treated with a cell suspension of strain F in growth medium without thiosulfate for a period of six hours, followed by a 6 h treatment of the appropriate combination of formation water with 40 mM sodium nitrate. When the percentage of formation water with nitrate reached 100%, the amended formation water (with 40 mM nitrate) was injected into both cores sets for 24 h. Samples for chemical and microbiological analyses were taken every 12 h.

During the time that 100% amended formation water was injected into the core systems, samples from both sets of cores contained a compound that interfered with the detection of sulfide. This suggested that a nutrient may be limiting the growth of strain F which would result in the incomplete oxidation of sulfide or the incomplete reduction of nitrate. Because of this problem and after receiving 100% amended formation water for 24 h, the test core was treated with growth medium (without thiosulfate) for 6 h and then with formation water with 10 mM sodium nitrate containing (in g/l)  $\text{KH}_2\text{PO}_4$  (1.8),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.4),  $\text{NH}_4\text{Cl}$  (0.5),  $\text{CaCl}_2$  (0.03),  $\text{NaHCO}_3$  (1.0). In experiment E5, this nutrient amended formation water with the lower nitrate concentration was injected into both sets of cores for 32 h, after which time the fluid flow to both sets of cores was stopped. After 12 h of incubation without fluid flow, the injection of nutrient-amended formation water with 10 mM nitrate was reinitiated. Samples for chemical and microbiological analyses were periodically taken during this treatment.

Twelve hours after fluid flow was reinitiated, the fluid injected into the control set of cores was changed to formation water without any nutrient or nitrate amendments. The core system was operated in this manner, i.e., with nutrient-amended formation water with 10 mM nitrate injected into the test set of cores and formation water only injected into the control set of cores for an additional 48 h.

#### Microbiological and Chemical Analyses

Concentrations of *T. denitrificans* strain F, sulfate-reducing bacteria, and acid-producing bacteria were estimated using the end-point dilution method. One milliliter of the sample was diluted in the respective growth medium. The inoculated bottles were then incubated at 30°C and checked for growth on a daily basis.

Strain F was enumerated using the growth medium given previously described [4]. Sulfate-reducing bacteria and acid-producing bacteria were enumerated using BTI-SRB medium and BTI-APB medium (Bioindustrial Technologies, Inc., Grafton, NY).

Samples were analyzed for sulfide immediately by the methylene blue method using Hach Chemical (Loveland, CO) field kits. Sulfate, nitrate, and nitrite were determined by high pressure liquid chromatography (HPLC) using anion exchange column and a conductivity detector as previously described [8].

## RESULTS

The addition of nitrate alone to the formation water injected into the core systems resulted in lower effluent sulfide levels (Table I). Concomitant with the reduction of sulfide was the decrease in nitrate concentrations in the core effluent, suggesting the presence of indigenous microbial populations capable of oxidizing sulfide using nitrate as the electron acceptor. However, in an earlier study of microbial activities in the subsurface at this site, BTI did not identify any indigenous organisms capable of sulfide oxidation [9]. The addition of nitrate did not affect the numbers of sulfate-reducing bacteria and acid-producing bacteria. Strain F-like organisms were not detected in the core effluents. In the test core system, where the flow rates were about five times faster than the control core system, the sulfide levels were reduced by about 40%, while in the control core system sulfide levels were decreased by 98%. The efficacy of the nitrate treatment clearly depended on the residence time of the liquids in the core system. It is interesting to note that the sulfide levels in the influent and the effluent before treatments began were similar. This suggested that little or no sulfide production occurred within the core system. (No organic nutrients were added to the formation water to support the growth of sulfate-reducing bacteria). In earlier studies at this site, BTI personnel observed that a change in microbial activities occurred when nitrate was detected in the produced water [9]. These investigators found that nitrate can be used as an electron acceptor by the majority of the community members. As a consequence, a reduction in sulfide concentration in the formation water was observed when nitrate was present [9].

The injection of nutrients for *T. denitrificans* did not stimulate sulfide production in the core systems. Although the numbers of sulfate-reducing bacteria were not affected, the influent and the effluent sulfide levels were low when only medium was injected into the core systems. This again suggested that little or no sulfide production actually occurred within the core system. Significant numbers of strain F cells were detected in the first two cores of the test core system after the first treatment with strain F. The number of strain F cells increased with the subsequent treatment with cells followed with medium injection. Thus, cells of strain F were maintained in the test core system when growth medium was used.

Preliminary studies suggested that the formation water contained a compound inhibitory to the growth of strain F (data not shown). Therefore, as noted above, the fraction of formation water injected into the core was increased in steps in order to acclimate strain F (experiment E4). Relatively high concentrations of strain F were detected in samples of the effluent and from each intermediate sampling port even when the influent contained 80% formation water with nitrate. When the influent was 100% formation water with nitrate, the levels of strain F decreased, but complete washout of strain F was not observed. During these experiments the effluent from the control core system contained a compound that interfered with the detection of sulfide. This problem plus the fact that the control core and the test core systems operated at different flow rates made definitive comparisons between the two cores systems difficult. However, throughout this period, the concentration of sulfide in the effluent of the test core system was consistently lower than the influent concentration. There was also a concomitant reduction in nitrate levels in the test core system suggesting that these two processes were linked. Interestingly, the sulfate concentrations in the effluent relative to the

influent concentration of the test core system increased after strain F inoculation. This was not observed in the control core system and suggested that strain F was oxidizing endogenous sulfur compounds (such as iron sulfides) that had accumulated in the core system. This would explain why the concentration of nitrate in the effluent of the test core system was much lower than expected if the sulfide present in the influent was the only source of electrons for nitrate reduction.

During this experiment, strain F was consistently detected in all cores of the test core system. This suggested that strain F was active and growing in the test core system. However, when the influent was shifted completely to formation water, the concentration of strain F in the test core system decreased substantially and interferences in effluent sulfide analyses were observed. This suggested that some essential nutrient may be limiting the growth of strain F which would result in the incomplete oxidation of sulfide or in the incomplete reduction of nitrate. In subsequent treatments, the concentration of nitrate was decreased from 40 mM to 10 mM and nutrients were added to the formation water as noted in the Materials and Methods section.

The treatment of the test core system with strain F and the subsequent injection of formation water with reduced nitrate concentration and nutrient amendments resulted in the reestablishment of strain F in the test core system. Concomitant with the increase in strain F was the disappearance of the interfering substance from the effluent of the test core. A reduction in sulfide concentration in the effluent compared to the influent concentrations in the test core was also observed. The levels of sulfide in the effluent of the test core compared to the influent concentration were reduced by 84 to 99%. There was a substantial reduction in the levels of nitrate and a substantial increase in the levels of sulfate in the effluent compared to the influent of the test core system. This suggests that, in the core system, strain F was oxidizing the sulfide present in the formation water to sulfate using nitrate as the electron acceptor. However, the amount of sulfate detected in the effluent of the test core system was much higher than that expected if strain F completely oxidized only the sulfide present in the formation water. As noted above, this suggests that strain F may have metabolized sulfur compounds that had accumulated within the core system. These sulfur compounds may have been iron sulfides or other sulfide precipitates which accumulated in the core sections during previous experiments. Strain F has been observed to utilize as an energy source iron sulfide precipitates produced by sulfate-reducing bacteria in media containing  $Fe^{3+}$  [8].

## DISCUSSION

Several lines of evidence support the conclusion that treating the test core system with *Thiobacillus denitrificans* strain F, nitrate, and certain inorganic nutrients was effective in controlling sulfide production. After the strain F treatments, the effluent sulfide concentration in the test core system was 84 - 99% lower than the influent concentration. Also, after strain F treatment, the effluent sulfide concentration in the test core system was 90 to 99% lower than the effluent sulfide concentrations before the test began. And lastly, following strain F treatment the sulfide concentration in the test core effluent was 86 to 97% lower than when the test core system was treated with formation water plus 40 mM nitrate. Since the control core system had a much slower flow rate and samples from this core system contained compounds that interfered with the detection of sulfide, direct comparisons between the test and control systems are not possible. However, it is clear that less sulfide was detected in the effluent samples of the test core system after strain F treatment.

High concentrations of strain F were observed in the effluent and at each sampling port in the test core system after inoculation and injection of nutrient supplemented formation

water with 10 mM nitrate. This indicates that strain F was able to colonize the core system and successfully compete with the indigenous microbial populations over a long period of time. The growth of strain F in the core did not result in any significant increase in the pressure drop through the system. The presence of high levels of strain F at the time when effluent concentrations of sulfide and nitrate decreased, and sulfate increased suggests not only that strain F was maintained in the system, but that it was metabolically active. Strain F-like organisms were not detected in samples from the control core system suggesting that the changes observed in the test core system were the result of strain F treatment.

Concomitant with the reduction of sulfide in the effluent was a decrease in the effluent concentration of nitrate and an increase in the effluent concentration of sulfate in the test core system. These changes suggest that as sulfide was used, nitrate was reduced and sulfate was produced. Little or no change was observed in the effluent concentrations of sulfate and nitrate compared to the influent concentrations of these compounds in the control core system. Since the control core system was not inoculated with strain F and strain F-like organisms were not detected in the control core system, this suggests that the changes in the effluent concentrations of nitrate and sulfate observed in the test core system were the result of the activity of strain F.

The addition of nitrate alone to the formation water did result in the reduction of sulfide in the core system. This was most pronounced in the control core system where little or no sulfide was detected in the effluent after nitrate treatment. However, the effectiveness of this treatment is difficult to determine since the samples from the control core system contained a compound that interfered with detection of sulfide. In the test core system, the addition of nitrate alone was not as effective in reducing sulfide concentrations compared to that observed in the control core system. These data suggest that the efficacy of nitrate addition clearly depended on the retention time of liquids in the system. At the shorter retention times that occurred in the test core system, the addition of nitrate was not as effective in reducing sulfide concentrations as was the strain F treatment.

The fact that the effluent sulfate concentration of the test core system after strain F treatment was almost twice the influent core system suggests that sulfur-containing compounds had accumulated within the test core system and were being oxidized to sulfate by strain F. This would explain why the such a large decrease was observed in the effluent concentrations of nitrate after strain F treatment. One possible source of endogenous sulfur compounds may have been iron sulfide precipitates. Iron sulfide precipitates were clearly visible in the formation water and the tubing entering the core systems. Montgomery *et al* [8] showed that strain F can metabolize sulfide in the form of iron sulfide resulting in a clearing of the medium and removal of blackened areas in sandstone cores. Iron sulfide precipitates that form as a consequence of sulfide production can plug pores in porous rock and lead to the loss of injectivity. The fact that strain F is able to use these precipitates should increase the permeability and injectivity of oil and gas wells.

## CONCLUSIONS

Once inoculated into the test core system, *Thiobacillus denitrificans* strain F was maintained for a long period of time. After inoculation with strain F, a 84 to 99% reduction in the sulfide levels in the effluent compared to the influent concentration of the test core system was observed. Effluent sulfide levels of the test core system were much lower than those observed before the test began or after treating the core with nitrate amended formation water. Concomitant increases in effluent sulfate levels and

decreases in effluent nitrate levels suggest that strain F metabolized sulfide to sulfate while using nitrate. These data will support the conclusion that strain F was metabolically active and effective in controlling the level of sulfide in the test core system.

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#### REFERENCES

1. Orr, W. L. 1977. Geologic and geochemical controls on the distribution of hydrogen sulfide in natural gas. *Advances in Organic Geochemistry 1975* (R. Campos and J. Goni, eds.), pp. 571-597, Enadimsa, Madrid, Spain.
2. Westlake, D. W. S. 1991. Microbial ecology of corrosion and reservoir souring. *Microbial Enhancement of Oil Recovery--Recent Advances*. (E. C. Donaldson, ed.), pp. 257-263, Elsevier, Amsterdam.
3. Sublette, K. 1987. Aerobic oxidation of hydrogen sulfide by *Thiobacillus denitrificans*. *Biotech. Bioeng.* 29: 650-659.
4. Sublette, K. and Sylvester, N. D., 1987a. Oxidation of hydrogen sulfide by *Thiobacillus denitrificans*. *Biotech. Bioeng.* 29: 245-257.
5. Sublette, K. and Sylvester, N. D. 1987b. Oxidation of hydrogen sulfide by continuous cultures of *Thiobacillus denitrificans*. *Biotech. Bioeng.* 29: 753-758.
6. Sublette, K. and Sylvester, N. D., 1987c. Oxidation of hydrogen sulfide by mixed cultures of *Thiobacillus denitrificans*. *Biotech. Bioeng.* 29: 759-761.
7. Sublette, K. and Woolsey, M. E. 1989. Sulfide and glutaraldehyde resistant strains of *Thiobacillus denitrificans*. *Biotech. Bioeng.* 34: 565-569.
8. Montgomery, A. D., McInerney, M. J., and Sublette, K. 1990. Microbial control of the production of hydrogen sulfide by sulfate reducing bacteria. *Biotech. Bioeng.* 35: 533-539.
9. Dzielwski, David M., 1990. A Case Study of Biological Souring in a Gas Storage Field Operation, paper presented at the Third International Symposium on Gas, Oil, Coal and Environmental Biotechnology, New Orleans, LA (Dec. 3-5, 1990).

**TABLE I. Summary of the effects of strain F inoculation and nitrate addition on sulfide production in test core system.**

TREATMENT	EFFLUENT SULFIDE ( $\mu\text{M}$ )	CELL CONCENTRATIONS (CELLS/ML)		
		SRB	APB	STRAIN F
None	160	$10^5$	$10^5$	0
Nitrate	110	$10^5$	$10^7$	0
Nitrate, strain F, and nutrients	3-16	$10^7$	$10^7$	$10^7$