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Chemical Technology Division

CRADA Final Report
for
CRADA Number ORNL93-0208

**Advanced Bioreactor Concepts
for Gaseous Substrates:
Conversion of Synthesis Gas to
Liquid Fuels and Removal of
SO_x and NO_x from Coal
Combustion Gases**

E. N. Kaufman
P. T. Selvaraj

Date Published: October 1997

Prepared by the
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831-6226
managed by
Lockheed Martin Energy Research Corp.
for the
U.S. Department of Energy
under contract DE-AC05-96OR22464

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Final CRADA Report* ORNL93-0208

Oak Ridge National Laboratory
and
Bioengineering Resources, Inc.

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* This work was supported by the Advanced Research and Technology Development Program of the Office of Fossil Energy, U.S. Department of Energy under contract DE-AC05-96OR22464 with Lockheed Martin Energy Research Corp.

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EXECUTIVE SUMMARY

The objectives of this Cooperative research and Development Agreement project between Oak Ridge National Laboratory (ORNL) and Bioengineering Resources, Inc. (BRI), were to investigate the production of fuels and chemicals from coal synthesis gas and to research the removal of NO_x and SO_x from coal combustion gases using advanced bioreactor systems. BRI had the lead role in the conversion of coal synthesis gas, and ORNL developed advanced bioreactors for both flue gas removal and synthesis gas conversion to fuels. The work was conducted over 3 years, and this report summarizes the accomplishments during the period.

The major achievements by ORNL in the effort of flue gas desulfurization were the development of process culture (mixed culture of sulfate-reducing bacteria, or SRB), the use of sewage digest as a low-cost feedstock for the process culture, and the use of immobilized cell bioreactors for increased volumetric productivity in flue gas reduction. These factors were found to have significant impact on the economics of the process for commercial applications. Mixed cultures of SRB were isolated and developed from municipal sewage sludge. The feedstock was generated by simple sewage digestion in a continuous reactor which produced fermentable substrates of 5 g/L. A columnar reactor with SRB cells immobilized in porous polymeric beads exhibited the highest productivity of the reactors studied (16.5 mmol sulfite/h•L, 32 kg/d•m³). Coal synthesis gas was then identified as an alternative low-cost feedstock in a situation where sewage digest feedstock would be non-economical due to the transportation of sewage solids from sewage treatment plant to the utility plant. However, in the system with synthesis gas feedstock, mass transfer was the limiting factor due to the low solubility of synthesis gas in aqueous medium. A continuous stirred tank (CSTR) and trickle bed reactors were studied to improve mass transfer property of the synthesis gas. Trickle bed reactor showed better productivity (9.5 mmol sulfite/h•L). In addition, synthesis gas was fed as microbubbles to enhance the mass transport doubled the productivity in CSTR.

Studies in batch stirred reactors using bacteria capable of converting synthesis gas to ethanol resulted in ethanol concentrations of 27 g/L with 90% utilization of CO , and 80% utilization of H_2 with a gas retention time of less than 10 minutes. Both bioprocess technologies demonstrated economic competitiveness and are in the process of licensing and commercialization.

INTRODUCTION

The purpose of the proposed research program was the development and demonstration of a new generation of gaseous substrate-based bioreactors for the production of liquid fuels from coal synthesis gas and the removal of NO_x and SO_x species from coal combustion flue gas. Expeditious commercialization of this advanced technology was implemented by a joint effort encompassing both Oak Ridge National Laboratory (ORNL) staff and that of an industrial firm, Bioengineering Resources, Inc. This work was supported by the Advanced Research and Technology Development Program of the Office of Fossil Energy, U.S. Department of Energy under contract DE-AC05-96OR22464 with Lockheed Martin Energy Research Corp.

Advanced biotechnology for a variety of industrial uses, including processing fossil fuels, has been acknowledged as an important area for research, development, and industrialization in the recently identified Presidential Biotechnology Initiative [1]. The importance of these advanced bioprocessing concepts to the nation's economic well-being and competitiveness was pointed out in the recent report by the Federal Coordinating Council for Science, Engineering, and Technology that was entitled "Biotechnology for the 21st Century." This report specifically indicated that biocatalysts for the conversion of coal to liquid and gaseous fuels and bioprocesses for the removal of hazardous components in coal-derived effluent streams were important future objectives that could have a significant impact on the future use of fossil materials for energy production [1]. The recent Energy Policy Act of 1992 also emphasizes research, development, and demonstration of the conversion of coal to gaseous and liquid fuels and the control of sulfur and nitrogen oxides in an innovative approach to the use of bioprocessing concepts that will have utility in both of these identified areas.

Our large coal reserves may be used to provide precursors for alternative liquid fuels through indirect liquefaction. In such a process, coal is thermochemically converted to synthesis gas consisting mainly of carbon monoxide, hydrogen, and carbon dioxide. Conventional catalytic upgrading of synthesis gas into alcohols or other oxychemicals is subject to several processing problems. Additional constituents in the synthesis gases (e.g., sulfides such as H_2S and COS) are potent poisons to conventional catalysts and must be removed in costly preprocessing procedures. These inorganic catalysts for upgrading also require strict CO/H_2 ratios to maintain a particular product distribution and yield. This may necessitate costly gas recompression and shift reaction conversion procedures. Conventional catalytic conversion is performed at high temperatures and pressures which increase processing cost and raise safety issues. Finally, product specificity is often poor, resulting in a large product spectrum, decreased yields, and increased separation costs.

Recently isolated and identified bacterial strains capable of utilizing CO as a carbon source and converting CO and H₂ into mixed alcohols offer the potential of performing synthesis gas conversion using biocatalysts [2]. While biocatalytic conversion with such microorganisms is generally slower than that achieved with inorganic catalysts, such methods would offer several advantages over conventional synthesis gas upgrading. Feed gas components that would poison conventional catalysts do not interfere with the bioconversion in sulfur tolerant strains of the microorganisms, and enzyme-mediated reactions in biocatalytic conversions are not bound to strict substrate ratios. Thus, synthesis gas preprocessing would be eliminated. Further, many enzymatic reactions are essentially irreversible. This would help eliminate product stability issues that arise in conventional catalytic upgrading. Finally, biocatalytic reactions may take place at or near ambient temperatures and pressures, reducing both capital and operating expenses.

Several biocatalytic schemes have been proposed for the production of alcohols from synthesis gas. *Clostridium ljundahlii* catalyzes the conversion of CO, H₂, and CO₂ into acetate and ethanol. The chemical environment can be modified to either optimize bacterial growth or to favor ethanol production. This microbial strain has been used in a two-CSTR scheme in which growth is favored in the primary reactor, and ethanol production is favored in the secondary reactor [3]. The ethanol production rate in this stepped reactor mode was 250 - 300 mmol/g cell•day with a product concentration of 0.3%.

The efficient biocatalytic production of liquid fuels from synthesis gas is constrained by several factors that are often interacting. The solubility of the substrate in the liquid phase is very low. Mass transport of the substrate from the gas phase into the liquid media and to the solid biocatalyst may also be reaction-limiting. High cell density in the reactor is desired for complete and timely substrate conversions, and plug flow hydrodynamics is desired for these same reasons. These design requirements are often interrelated and self defeating. Mixing to enhance mass transport erodes plug-flow performance and may effect cell viability. Packed or immobilized biocatalytic systems, while favoring high cell density and plug-flow performance, may be mass transport limited in their substrate conversion. It is evident that efficient and commercializable liquid fuel production from synthesis gas will require the design of novel gas-phase bioreactors that combine high cell density with high product yield and high rates of substrate flux to the biocatalyst.

Development of such gas phase bioreactors may also have application in the removal of SO_x and NO_x constituents from coal combustion flue gases, since this will be an analogous process with many of the same types of process restraints and problems. The most common means of flue gas desulfurization is the use of disposable adsorbents such as limestone. This produces calcium sulfate as a waste product and does not affect NO_x removal. Regenerable adsorbents are becoming more widely used, but their regeneration creates concentrated NO_x and SO_x waste streams. Microorganisms such as

Desulfontomaculum nigrificans and *Pseudomonas denitrificans* have been shown to interact with SO_x and NO_x respectively [4, 5]

CRADA TASKS

While some research has been conducted on bioreactors for these systems, an efficient process capable of competing with conventional catalytic research has not been developed. This Cooperative Research and Development Agreement (CRADA) between Oak Ridge National Laboratory (ORNL) and Bioengineering Resources, Inc. (BRI) addressed the further investigation of optimal bacterial strains, growth media and kinetics for the biocatalytic conversion of coal synthesis gas to liquid fuel such as ethanol and the reduction of gaseous flue gas constituents. The primary emphasis was on the development of advanced bioreactor systems coupled with innovative biocatalytic systems that will provide increased productivity under controlled conditions. It was hoped that this would result in bioprocessing options that have both technical and economic feasibility, thus, ensuring early industrial use. Predictive mathematical models were formulated to accommodate hydrodynamics, mass transport, and conversion kinetics, and provide the data base for design and scale-up. The program was separated into four tasks: (1) optimization of **Biocatalytic Kinetics**; (2) development of **Well-mixed and Columnar Reactors**; (3) development of **Predictive Mathematical Models**; and (4) **Industrial Demonstration**. Research activities addressing both synthesis gas conversion and flue gas removal were conducted in parallel by BRI and ORNL respectively. Explanations of the individual tasks are found below.

Task 1 - Optimization of Biocatalytic Kinetics - Although several microbial consortia for syn-gas conversion and combustion gas removal have been identified, the preferred strains, media constituents and operating conditions have not been fully established. Indeed, the preferred, most economical products desired for flue gas removal need to be identified. The selection of a carbon source for the NO_x and SO_x reducing bacteria will be of paramount importance to the economic viability of the process. Small, laboratory-scale tests will be conducted using promising strains and combination of strains or syn-gas conversion and SO_x/NO_x removal in both shake flasks and stirred-tank bioreactors to determine optimal growth and conversion parameters.

Task 2 - Development of Well-mixed and Columnar Reactors - Both well-mixed and columnar reactors will be investigated for the two processes. Much of the initial work in biocatalytic synthesis gas conversion was conducted using well-mixed bioreactors such as CSTR's. Therefore, this task represents an important extension of these previous efforts with an emphasis upon optimizing operating conditions and providing scaleup information. Initial research with the production of methanol and acetate production from syn-gas has established that increased system pressure may increase conversion. The effects of substrate and system pressure will be of paramount

importance, and the effects of mass transport will be measured in investigations of both bioprocessing tasks.

Continuous fluidized and fixed-bed bioreactors have been shown to significantly enhance biological processes for several other applications. For instance, a mixed-culture conversion of carbon monoxide to methane, it was found that a trickle-bed reactor yielded higher conversions at a given gas loading rate than either a CSTR or packed-bubble column [3]. Complete conversion was achieved at gas loading rates of 0.4 h^{-1} . Similar advantages over CSTR's have been identified in carbon monoxide conversion to acetate by *P. productus* [6]. The possible plug-flow performance, enhanced mass transport, increased cell density, and ease of operation make columnar reactors attractive alternatives to well mixed reactors. Tests will be made on engineering bench-scale systems. Previous research results will form the basis for studying various techniques for microbial immobilization and the best approach for each application will be established. This will present new obstacles in cell immobilization since most of the systems studied are strictly anaerobic. The reactor concepts will be studied in both liquid-phase-continuous and gas-phase-continuous systems and will be evaluated over a wide range of operating conditions including substrate pressure. The aim will be to establish optimum operating conditions and to enable the prediction of hydrodynamic and mass transport effects for process scaleup.

Task 3 - Development of Predictive Mathematical Models - The development of mathematical models of reactor performance will be of utility in reactor design, operation, and scaleup. Predictive models that include hydrodynamics, mass transfer, and biochemical kinetics will be developed for both types of gas-phase processing systems. Modern numerical methods will be used in developing these predictive models.

Task 4 - Industrial Demonstration - Following feasibility assessment, bench-scale demonstration mathematical modeling and scaleup, an advanced bioreactor system will be designed, built, and operated at the industrial site to demonstrate at least one of the two bioprocessing applications at a scale in which true technical feasibility may be established. This system will be operated continuously for extended periods and the production rate and general operability will be assessed. Data from this task will form the basis of future scaleup.

RESULTS

Biological Conversion of SO_x in Flue Gases

Key impact parameters in flue gas treatment are the minimization of carbon and energy costs for the bioreactor and the optimization of reactor volumetric productivity through increased biomass density. To this end, we have investigated low cost carbon and energy sources as well as novel reactor designs utilizing the immobilization of the biocatalyst.

In laboratory studies of the reduction of SO_x , the principal nutrient for the biocatalyst is lactic acid. This carbon source would be far too expensive to use in a large scale process. We have investigated the use of low cost nutrients in order to improve the economic viability of a biological flue gas treatment process. Anaerobically digested-municipal sewage sludge (AD-MSS) medium has been used as sole carbon and energy source for the a mixed culture of sulfate reducing bacteria (SRB) and heterotrophs in the reduction of SO_x to hydrogen sulfide with subsequent chemical conversion to elemental sulfur. Municipal sewage sludge is readily available at a negative or near zero cost. Through the use of a mixed bacterial population, a more robust biocatalyst is achieved which is less sensitive to upsets in anaerobic conditions and which is better able to utilize a variety of carbon and energy sources. We have optimized the process of converting diffused air flotation sewage solids to AD-MSS media, minimizing the conversion time and use of added chemicals, and maximizing the yield of organic acids which serve as the carbon and energy sources for the biocatalyst. Figure 1 demonstrates the variety and yield of organic acids available in the AD-MSS media.

A successful reactor design for flue gas treatment must maximize volumetric productivity (mass of SO_x reduced per reactor volume per unit time) in order to decrease the capital cost of the reactor unit. We have investigated the use of immobilized cell reactors in order to maximize the biocatalyst density in the reactor. Gelatin beads used for cell immobilization proved too chemically fragile and were not able to withstand the high sulfite concentrations within the reactor. Porous polymeric beads proved more robust and have been utilized in reactor operation for more than 9 months (see Figure 2). Using this configuration, we have achieved a conversion rate of 16.5 mmol sulfite/h·L (32 kg/d·m³) with 100% conversion to H_2S .

AD-MSS Medium Preparation Continuous Process

Figure 1: Organic acids present in AD-MSS media. Sewage serves as an inexpensive source of organic acids for use as carbon and energy sources for sulfate-reducing bacteria which are capable of converting SO_x in flue gases.

Columnar Reactor with BIO-SEP™ Beads

Figure 2: Conversion of SO_x in an immobilized cell bioreactor.

Though municipal sewage digest is a readily available low-cost carbon source - the real cost of the medium depends on the location of the sewage and power plant and the transportation to bring the sewage back and forth from the sewage plant. Therefore the research focused on using synthesis gas as an alternative low-cost feedstock for SRB as they desulfurize flue gases, recycle flue gas desulfurization waste gypsum, and treat acid mine drainage waste waters. Synthesis gases are only sparingly soluble in the aqueous environments encountered in conventional bioreactor systems. Advanced bioreactor concepts are needed to increase synthesis gas mass transport and utilization.

A gas mixture containing 36% H₂, 47% CO, 10% CO₂, 5% CH₄ and balance N₂ as a model coal synthesis gas was utilized in these studies. This composition is typical of an oxygen blown, coal fed gasifier. Initially, with development of mixed SRB culture using the syn-gas as sole carbon and energy source, a stirred tank and trickle bed reactors were operated with syn-gas fed as gaseous feed. The mass transfer characteristics were determined under these conditions. Following this, the syn-gas has been fed as microbubbles to enhance the mass transport properties.

Utilization of syn-gas by mixed SRB culture developed from municipal sewage was investigated in a serum bottle containing minimal salt medium and SO₂ as terminal electron acceptor. The headspace of the bottle was then filled with the synthesis gas. The bottle was inoculated with mixed SRB culture and incubated at 30°C with shaking at 200 rpm. The syn-gas utilization is shown in Figure 3. Initially, a decrease in CO concentration was observed with no change in H₂ concentration. However, hydrogen sulfide was detected during this time in the head space of the serum bottle. This indicates that the CO was utilized by certain type of bacteria and produced H₂ as shown in the equation below.

With limited SO₂ reduction due to a possible CO inhibition at higher concentration, as seen in the Figure 3, hydrogen concentration declined only after the CO concentration was less than about 5% in the mixture. This suggests that the mixed culture developed from sewage solids would be able to use CO as the sole carbon and energy source and produce H₂. Kinetically, the CO utilization was much faster than hydrogen utilization by SRB cultures.

Figure 3: Utilization of synthesis gas as a carbon and energy source by sulfate reducing bacteria.

Following the development of culture in serum bottle, a 2-L chemostat was operated with mixed SRB culture and SO_2 gas as the terminal electron acceptor. Initially, the reactor was fed with syn-gas, minimal salt medium and SO_2 gas mixture containing 1% or 5% SO_2 , 5% CO_2 , and balance nitrogen. The effluent reactor medium was recirculated through a hollow fiber membrane to recycle the biomass into the reactor to achieve higher biocatalyst concentration in the reactor. At these conditions, the maximum productivity of the reactor was 1.2 mmol $\text{SO}_2/\text{h}\cdot\text{L}$ with 100% conversion of SO_2 into H_2S .

In an effort to develop a bioreactor for the above process with better mass transfer for syn-gas, a trickle bed reactor was operated with BIO-SEP beads (a porous bead of carbon black and plastic) as carrier medium for biocatalyst. Initially, as shown in Figure 4, the SO_2 feed rate was at 2.7 mmol/h·L. The SO_2 feed rate was then increased incrementally with no sulfite detected in the effluent. Presently, the reactor has been operated at 9.5 mmol $\text{SO}_2/\text{h}\cdot\text{L}$ with 98% conversion.

Figure 4: Trickle bed reactor with BIO-SEP beads using syn-gas as the carbon and energy source.

In an effort to improve the mass transfer properties of syn-gas, the CSTR reactor was then fed with syn-gas microbubbles as follows. Microbubbles are small, surfactant coated bubbles of gas that are generated by creating a gas-liquid interface in a high-shear zone. The bubbles are between 50 and 100 μm in diameter and the surfactant coating helps to prevent coalescence by electrostatic repulsion from the diffuse electric double layer around the bubble. In our work, the microbubble dispersions were generated using a spinning disk apparatus. This microbubble generator uses a high speed motor that spins a 4 cm disk at speeds above 4000 RPM. The bubble size measurements were performed using laser diffraction technique.

Initially, a 2 L chemostat was operated with mixed SRB culture with syn-gas fed as gaseous feed. The pH, temperature, and agitation rate were 6.8, 30°C, and 300 rpm, respectively. The reactor effluent was recirculated through a hollow fiber membrane to recycle the cells back into the reactor. At these conditions, the maximum productivity of the reactor was 1.2 mmol SO₂/h•L with 100% conversion of SO₂ into H₂S. At this time, the syn-gas was fed as microbubbles into the reactor. With syn-gas fed as microbubbles, the reactor was able to convert more SO₂ into H₂S reaching maximum productivity of 2.1 mmol SO₂/h•L in 33 h (Figure 5). The biomass concentration in the reactor prior to the microbubble operation was 5 g/L. The increase of productivity from 1.2 to 2.1 mmol/h•L within 33 h at the same biomass concentration of 5 g/L indicated that the mass transport was a limiting parameter in the above process. A summary of our work in various reactor configurations is given in Table 1.

Figure 5. Sulfite conversion in a CSTR with syn-gas as feedstock. With syn-gas fed as microbubbles, the reactor productivity was increased from 1.2 to 2.1 mmol/h.L in 33 h.

The mass transfer property of syn-gas was studied in three different reactor systems: serum bottle, CSTR and trickle bed. In serum bottle experiments, the syn-gas (specifically H_2 and CO) utilization was determined using different biocatalyst concentrations to confirm that the rate of depletion of syn-gas was not due to the biocatalyst concentration. In a mass transport limited system, the rate of depletion will not depend on biocatalyst concentration. In the CSTR, the mass transport property of syn-gas was determined by varying its flow rate. The mass transfer coefficients were $30.6\ h^{-1}$ for CO and $74.64\ h^{-1}$ for H_2 at an agitation rate of 300 rpm. With similar experiments in the trickle bed reactor, the mass transfer coefficients of syn-gas were $26.0\ h^{-1}$ for CO and $20.2\ h^{-1}$ for H_2 .

Table 1: Summary of SO_x Reactor Results in a Variety of Reactor Configurations

Reactor Type	Feedstock	SO ₂ /SO ₃ Throughput (mmol/h•L)	% SO ₂ Conversion	Medium Utilization (per mol of SO ₂)
CSTR with SRB Flocs	Sewage Digest	2.1	100	14.3 g of COD
Column with BIO-SEP Beads	Sewage Digest	16.5	100	7.3 g of COD
CSTR with Hollow Fiber Membrane	Syn-gas	1.23	100	1.8 mol H ₂ 2.3 mol CO
Trickle Bed with BIO-SEP Beads	Syn-gas	8.8	100	1.0 mol H ₂ 1.2 mol CO

Syn-Gas Conversion to Ethanol

Clostridium ljungdahlii as well as other BRI isolates converts synthesis gas to ethanol by the following reactions:

Acetic acid is produced as a by-product:

Initial research efforts focused on changing *C. ljungdahlii* from a predominantly acid-producing bacterium to an ethanol-producing bacterium. Nutrient composition and concentration, fermentation pH and the dissolved substrate concentrations were found to

be the most significant parameters that affected the ethanol production and the product ethanol acetic acid ratio.

A series of reactor systems was used to eliminate or at least minimize the production of the by-product acetic acid in the fermentation of synthesis gas to ethanol by *C. ljungdahlii*. In this arrangement, nutrient limited medium was fed to the first CSTR, with the effluent from this reactor used as feed for the second CSTR. The product from second reactor showed more than 20 g/L ethanol and 4 g/L acetic acid, for a product ratio of 5 g/g.

Further study used four isolates in addition to *C. ljungdahlii* identified at BRI. Three of the cultures were used in CSTR studies. Isolate C-01 was shown to produce 20-24 g/L ethanol and 3-4 g/L acetic acid in a single CSTR. The isolate O-52 was used in two-stage CSTR system. CO conversions of 90% and H₂ conversions of 80% were obtained with 25 g/L of ethanol and 3 g/L of acetic acid production. Following this study, the isolate O-52 was selected as the best bacterial strain for the conversion of synthesis gas to ethanol with regard to CO tolerance and culture stability in the presence of the products ethanol and acetate. Both single-stage and two-stage reactor systems were operated with isolate O-52 to obtain performance data for the systems. These systems were also operated with and without cell recycle. A summary of operating data for the two systems are shown in Tables 2 & 3.

Table 2: Results from the Single CSTR Reactor System (O-52). XRT = cell retention time, LRT = liquid retention time, GRT = gas retention time, ETOH = ethanol, HAC = acetic acid, Y = yield.

XRT (hr)	LRT (hr)	GRT (min)	ETOH (g/L)	HAC (g/L)	CELL S (g/L)	Y _{VB} (mol EtOH/L•d)	Conversions	
							% CO	% H ₂
46.4	23.2	12.5	20.4	4.4	3.8	0.46	96.3	81.2
43.2	17.3	9.7	21.1	3.5	4.9	0.64	86.7	49.9
43.2	17.3	9.2	20.5	5.1	4.6	0.62	89.4	64.5
43.2	17.3	7.5	22.2	3.7	5.0	0.67	81.8	42.1
49.4	17.3	9.2	21.1	4.4	4.6	0.64	85.3	52.1
46.0	16.1	8.4	20.8	5.1	4.5	0.67	85.2	61.4
54.3	16.3	6.8	23.4	5.7	4.7	0.75	84.7	57.7
54.3	16.3	7.2	19.0	4.4	4.0	0.61	83.1	55.2
54.3	16.3	7.4	21.9	5.5	5.0	0.70	86.6	66.7
55.6	16.7	6.4	23.5	4.9	5.6	0.73	83.3	53.1

Table 3: Results from the Two-Stage CSTR Reactor System (O-52). XRT = cell retention time, LRT = liquid retention time, GRT = gas retention time, ETOH = ethanol, HAC = acetic acid, Y = yield.

XRT (hr)	LRT (hr)	GRT (min)	ETOH (g/L)	HAC (g/L)	CELL S (g/L)	Y _{VE} (mol EtOH/L•d)	Conversion	
							% CO	% H ₂
173.0	43.0	14.0	33.0	4.0	6.5	0.40	90	60
47.5	47.5	17.0	27.0	4.0	2.8	0.30	90	61
62.0	29.2	12.0	28.5	4.0	4.6	0.50	89	65
84.0	20.5	9.0	24.5	4.0	5.3	0.64	87	62
43.0	43.0	19.5	27.0	1.9	3.0	0.33	95	55

A significant discovery in terms of minimizing acetic acid formation came when process water was recycled back into the reactor. Once acetic acid levels reached 5 g/L, little new acetic acid was produced (Table 4, Figures 6&7). Gross ethanol concentrations up to 27 g/L were attained with gross acetic acid concentrations of 5-7 g/L. Net ethanol concentrations were 20-25 g/L and net acetic acid production was 0-4 g/L.

Table 4: Results from the Single CSTR with Water Recycle (O-52). LRT = liquid retention time, GRT = gas retention time, ETOH = ethanol, HAC = acetic acid.

GRT (min)	LRT (hr)	% Gas Conversion		Total Products (g/L)		Net Products (g/L)	
		CO	H ₂	ETOH	HAc	ETOH	HAc
13.8	24	94	63	23.5	6.5	23	(-)
13	24	93	61.5	22.5	6.0	22	(-)
11.3	24	91	52	23	4.5	20	1.5
9.7	24	90	47	23	7.5	20.5	3

Figure 6: Gas conversion by isolate O-52 in the CSTR water recycle system.

Figure 7: Total product concentrations by isolate O-52 in the CSTR water recycle system.

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PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

P.T. Selvaraj, M.H. Little, and E.N. Kaufman, "Biodesulfurization of flue gases and other sulfite/sulfate waste streams using immobilized mixed sulfate reducing bacteria." 1997, *Biotechnology Progress*. (submitted for publication).

P.T. Selvaraj, M.D. Bredwell, M.H. Little, and E.N. Kaufman, "Biodesulfurization of flue gases using synthesis gas delivered as microbubbles." 1997, *American Chemical Society*. (in press).

P.T. Selvaraj, M.H. Little, and E.N. Kaufman, "Analysis of immobilized cell bioreactors for desulfurization of flue gases and sulfite/sulfate laden waste water." 1997, *Bioremediation*. (in press).

E.N. Kaufman and B.E. Dale, "Emerging Topics in Industrial Biotechnology." 1997, *Applied Biochemistry and Biotechnology*. 63: 625-626.

P.T. Selvaraj, G. B. Meyer, and E.N. Kaufman, "Microbial reduction of sulfur dioxide in immobilized, mixed cultures of sulfate reducing bacteria with sewage digest as carbon and energy source." 1996, *Applied Biochemistry and Biotechnology*. 57/58: 993-1002.

W. Weigand and E.N. Kaufman, "Bioprocessing Research Overview," 1995, *Appl. Biochem. and Biotech.* 51: 293.

PRESENTATIONS

"Microbial Reduction of Sulfur Dioxide in Mixed Cultures of Sulfate Reducing Bacteria" Presented at 17th Symposium on Biotechnology for Fuels and Chemicals, Vail, CO. May 1995.

"Immobilized-Cell Bioreactors for Microbial Reduction of Sulfur Dioxide" Presented at AIChE's 1995 Annual Meeting at Miami, FL, November, 1995.

"Analysis of Immobilized Cell Bioreactors for Desulfurization of Flue Gases and Sulfite/Sulfate-Laden Wastewater" Presented at the Third International Symposium of the International Society for Environmental Biotechnology, Boston, MA, July, 1996.

"Recycling of FGD Gypsum to Calcium Carbonate and Elemental Sulfur using Mixed Sulfate-Reducing Bacteria with Sewage or Syn-Gas as a Carbon Source" Presented at the Eighteenth Symposium on Biotechnology for Fuels and Chemical, Gatlinburg, TN, May 1996.

"Microbial Reduction of Sulfur Dioxide - Lower Cost Feedstocks and Optimized Reactor Configuration to Improve Economic Feasibility" Presented at the Eighteenth Symposium on Biotechnology for Fuels and Chemical, Gatlinburg, TN, May 1996.

"Bioreactors for Flue Gas Biodesulfurization using Coal Synthesis Gas as Feedstock" Presented at AIChE's 1996 Annual Meeting at Chicago, IL, November, 1996.

"Biodesulfurization of Flue Gases using Synthesis Gas Delivered as Microbubbles" Presented at American Chemical Society Spring 1997 Symposium at San Francisco, CA, April 1997.

"Conversion of Synthesis Gas to Liquid Fuels Using Microbubbles to Enhance Gas-Liquid Mass transfer" Presented at 19th Symposium on Biotechnology for Fuels and Chemical, Colorado Springs, CO, May 1997.

RELATED PROJECTS AND PUBLICATIONS

Work on this CRADA has led to the establishment of a host of interactions with industry. We have had discussions with Hoogovens Technical Services regarding collaborations in bringing biological flue gas desulfurization to market. Similar discussions have been held with the Tennessee Valley Authority. The reactor system for flue gas desulfurization has also proven to have utility for other processes such as the desulfurization of waste gypsum from limestone scrubbing of SO_x at coal power plants and in the treatment of acid mine drainage. We have written a proposal in this area to the Illinois Clean Coal Institute and have collaborated in efforts with the Bureau of Mines.

At the writing of this report, Hercules Inc. and Petro Star Inc. are in the process of signing Funds in CRADA's with us for work supporting the desulfurization of feedstocks specific to their industries.

E.N. Kaufman, M. H. Little, and P.T. Selvaraj, "A Biological Process for the Reclamation of FGD Gypsum Using Mixed Sulfate-Reducing Bacteria with Inexpensive Carbon Sources." 1997, *Applied Biochemistry and Biotechnology*. 63: 677-693.

E.N. Kaufman, M. H. Little, and P.T. Selvaraj, "Recycling of FGD Gypsum to Calcium Carbonate and Elemental Sulfur Using Mixed Sulfate Reducing Bacteria with Sewage Digest as a Carbon Source." 1996, *Journal of Chemical Technology and Biotechnology*. 66: 365-374.