DOE STI Product/Report No.	DOE/ER/83907-3
Project Title	"CO_2 Sequestration in Cell Biomass of *Chlorobium Thiosulfatophilum*"
Product Type and Reporting Period	Final Report for the Period July 2005 – September 2007
Date of Issuance or Publication	Date Issued/Published April 2009
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Originating Research Organization	Bioengineering Resources, Inc.
Sponsoring Organization	Prepared for the United States Department of Energy Office of Energy Research
DOE Program	Small Business Innovation Research
Award/Contract/Financial Number	Work Performed Under Grant No. DE-FG02-04ER83907

CO_2 SEQUESTRATION IN CELL BIOMASS OF *CHLOROBIUM THIOSULFATOPHILUM*

SBIR Phase II Grant No. DE-FG02-04ER83907

Final Report

July 15, 2005 - September 30, 2007

Submitted by

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April 2009

ABSTRACT

World carbon dioxide emissions from the combustion of fossil fuels have increased at a rate of about 3 percent per year during the last 40 years to over 24 billion tons today. While a number of methods have been proposed and are under study for dealing with the carbon dioxide problem, all have advantages as well as disadvantages which limit their application.

The anaerobic bacterium *Chlorobium thiosulfatophilum* uses hydrogen sulfide and carbon dioxide to produce elemental sulfur and cell biomass. The overall objective of this project is to develop a commercial process for the biological sequestration of carbon dioxide and simultaneous conversion of hydrogen sulfide to elemental sulfur. The Phase I study successfully demonstrated the technical feasibility of utilizing this bacterium for carbon dioxide sequestration and hydrogen sulfide conversion to elemental sulfur by utilizing the bacterium in continuous reactor studies.

Phase II studies involved an advanced research and development to develop the engineering and scale-up parameters for commercialization of the technology. Tasks include culture isolation and optimization studies, further continuous reactor studies, light delivery systems, high pressure studies, process scale-up, a market analysis and economic projections. A number of anaerobic and aerobic microorgansims, both non-photosynthetic and photosynthetic, were examined to find those with the fastest rates for detailed study to continuous culture experiments. *C. thiosulfatophilum* was selected for study to anaerobically produce sulfur and *Thiomicrospira crunogena* waws selected for study to produce sulfate non-photosynthetically. Optimal conditions for growth, H_2S and CO_2 comparison, supplying light and separating sulfur were defined.

The design and economic projections show that light supply for photosynthetic reactions is far too expensive, even when solar systems are considered. However, the aerobic non-photosynthetic reaction to produce sulfate with *T. crunogena* produces a reasonable return when treating a sour gas stream of 120 million SCFD containing 2.5 percent H_2S. In this case, the primary source of revenue is from desulfurization of the gas stream. While the technology has significant application in sequestering carbon dioxide in cell biomass or single cell proten (SCP), perhaps the most immediate application is in desulfurizing LGNG or other gas streams. This biological approach is a viable economical alternative to existing hydrogen sulfide removal technology, and is not sensitive to the presence of hydrocarbons which act as catalyst poisons.

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INTRODUCTION

Fossil fuels currently supply over 85 percent of the world's energy needs, and are expected to be the predominant source of energy well into the 21st century (Herzog *et al.,* 1997). While the world derives many benefits from the burning of fossil fuels, it is recognized that the increase in greenhouse gases is largely due to fossil fuel combustion, as well as changes in land use (Bolin *et al.,* 1986). Although deforestation and land exploitation have been responsible for rising CO_2 levels in the past, these contributions should be comparatively small in the future, since the rate of deforestation is expected to decline. Stationary industrial sources such as cement plants, ammonia plants and ethanol plants emit about one billion tons of CO_2 annually (Hagler Bailley Co., 1986). Except for the short period following the 1973 oil embargo, world CO_2 emissions from the combustion of fossil fuels have increased at a rate of about 3 percent per year during the last 40 years to over 24 billion tons (Post *et al.,* 1990). Hence, future trends in atmospheric CO_2 concentration will depend primarily upon fossil energy use (Smith, 1988).

Many methods have been proposed and are under study for dealing with the CO_2 problem, including CO_2 capture and storage, the use of oil, gas or biomass in place of coal, improvements in the efficiency of combustion, and conversion or sequestration of CO_2 into alternative products or forms. Such studies include CO_2 storage underground or in the ocean. Statoil is presently storing one million metric tons of CO_2 per year from Norwegian gas fields in an aquifier beneath the North Sea (Herzog *et al.,* 1997). Plans have been advanced by Exxon and Pertamina for a large aquifier storage project in the South China Sea. Separation of CO_2 from effluent gas streams, with subsequent reuse of the CO_2, is being considered as a mitigation option. Conversion technologies such as the production of methanol from CO_2 are being examined. Finally, the use of alternative feedstocks, such as biomass, for power production is being considered. Each of these scenarios has advantages and disadvantages and would be more expensive than current practice, which will probably dictate the preferable application. It is clear, however, that implementation of many of these options will be required to solve the vast problem of CO_2 accumulation.

Microorganisms are also able to sequester CO_2, either through its use as a substrate in forming a product (CO_2 to methane by methanogens or CO_2 to ethanol by *Clostridium ljungdahlii*, as examples) or by incorporating the CO_2 into cell biomass (algae, for example). The formation of methane or ethanol from CO_2 requires a supply of H_2, the production of which produces CO_2 as a by-product, unless the H_2 is produced from biomass. In order to be a viable practical candidate in this latter category, the organism must have fast rates and assimilate a significant quantity of carbon in the biomass. One such bacterium that satisfies these criteria is *Chlorobium thiosulfatophilum*, an anaerobic photosynthetic bacterium which uses CO_2 and H_2S to produce biomass and elemental sulfur by the equation:

$$2H_2S + CO_2 \rightarrow 2S + CH_2O + H_2O \quad (1)$$

In analyzing the stoichiometry, *C. thiosulfatophilum* assimilates 1.5 pounds of CO_2 per pound of biomass (CH_2O) produced.

The bacterium not only sequesters CO_2 into biomass, but also simultaneously converts H_2S to elemental sulfur for reuse. Sulfur is deposited outside the cell membrane where it settles and is collected as a finely divided powder (Ackerson *et al.,* 1991). Hydrogen sulfide is available at most all refineries, and at many gas plants and chemical plants. The gas is separated and converted into elemental sulfur with Claus, or similar technology, at about 150 plants in the U.S. (Ober, 2003). Therefore, processes based upon Equation (1) could be used at these locations to assimilate CO_2 produced by fossil fuel combustion processes at those, or nearby, locations.

From the stoichiometry of Equation (1), it is noted that about 0.65 pounds of CO_2 are consumed per pound of sulfur produced. This nation now produces about 10 million tons of sulfur annually from H_2S (Buckingham and Ober, 2002). Utilization of this technology could therefore result in a reduction of CO_2 emissions by 6.5 million tons per year. The economics of these processes should be attractive, since the technology would replace Claus facilities and produce a valuable by-product in protein as an animal feed.

High concentrations of H_2S (15 percent) enhance the reaction rate, so the technology applies to a broad range of H_2S concentrations found in sources such as various refinery gas streams and low quality natural gas (LQNG) (Cork and Ma, 1982). The reaction is irreversible (extracellular sulfur is not utilized), so there are no equilibrium constraints; therefore, high concentrations can be achieved. Others have found that some species of sulfur bacteria utilize intracellular sulfur to produce sulfate when H_2S is deficient (Cork and Ma, 1982). BRI has developed technology to avoid such secondary metabolism by insuring adequate mass transfer of gaseous substrates. Furthermore, the presence of other gases, such as N_2 or hydrocarbons, has no adverse effects on the performance of the bacterium.

The principal objective of this Phase II program was to perform advanced process development research to move the technology for biological CO_2 and H_2S conversion from the laboratory to the commercial scale. This objective was accomplished by the completion of seven tasks, which are briefly summarized below.

Task 1. Culture Isolation and Optimization. To ensure that the optimal biological system is developed, other organisms will continue to be examined. The best strains will be optimized to obtain the highest possible yields and rates of elemental sulfur and SCP from H_2S and CO_2 under various operating conditions. Growth and rate parameters will include pH, temperature, nutrients concentration and agitation rate, which will be optimized for the various compositions of H_2S in industrial gases.

Task 2. Continuous Bioreactor Studies. The CSTR with sulfur recovery by gravity separation was identified in Phase I as having the best potential for application to elemental sulfur and SCP production from H_2S and CO_2. The CSTR will be operated with sulfur recovery and cell recycle, while testing the best ways to deliver light (if necessary) to the reactor, the best methods of sulfur and cell recovery and to obtain operating data under optimized culture conditions.

Task 3. Photosynthetic Requirements. The light requirements will be defined for the photosynthetic bacterium *C. thiosulfatophilum* including desired wavelengths and intensity. The use of fiber optics to deliver light to the reactor will be investigated.

Task 4. Advanced Bioreactor Concepts. Advanced concepts such as high pressure operation will be used in an effort to improve the rate of mass transfer of H_2S and CO_2 from the gas phase to the liquid phase for subsequent biological conversion.

Task 5. Scale-up Development. Scale-up parameters will be developed and correlated in order to permit the design of a prototype unit in Phase III. In addition, preliminary designs of commercial units of various sizes will be prepared as the next step toward commercialization (combined with Task 7).

Task 6. Market Analysis. A market analysis will be prepared to identify and develop potential markets for SCP. A Phase III field demonstration will produce sufficient quantities of SCP for feeding trials.

Task 7. Process Economic Analysis. Economic projections of the technology will be prepared to guide the research program and define high cost areas for more intensive study.

ACCOMPLISHMENTS

Sixteen potential strains were identified for converting CO_2 and H_2S to elemental sulfur without requiring light as the source of energy. Three of these strains (*Thiomicrospira kuenenii* sp. nov. (DSM 12350), *Thiomicrospira frisia* sp. nov. (DSM 12351) and *Thiomicrospira chilensis* sp. nov. (DSM 12352)) were obtained from DSM (German culture collection), and *Thiomicrospira crunogena* (ATCC 35932) was ordered from the American Type Culture Collection. Experiments were performed with *T. crunogena*, first under a laminar flow hood and later in a 2L continuous stirred tank reactor, where a number of important variables were studied. The reactor systems were operated aerobically at 26°C, with gentle agitation, on modified ATCC medium #1422, *Thiomicrospira* medium. No light was provided to the culture. It was found that the CO_2 to sulfide uptake rate was much higher for *T. crunogena* in comparison to *C. thiosulfatophilum*.

A number of continuous bioreactor experiments were performed with *C. thiosulfatophilum* to better understand the important variables (sulfur removal from the culture, sulfur/sulfate production, light intensity, reactor cooling during light delivery, cell recycle combined with sulfur settling and H_2S/CO_2 conversion) in converting CO_2 and H_2S to cell biomass and elemental sulfur. These variables were studied in reactor systems employing a tungsten light source, both with and without sulfur removal. One of the biggest problems with *C. thiosulfatophilum* is the accumulation of sulfur on the walls of the reactor vessel when using an external light source. A double magnet system (one outside the reactor and one inside the reactor) was used to help rid the reactor walls of cell biomass by acting as a scraper of cell debris. This double magnet system was used both in the CSTR connected to the sulfur settler and the CSTR with no settler. Cleaning resulted in the production of less sulfate, and the gas conversions improved slightly. Another reactor alternative that was employed involved the use of a light source which could be operated external to the reactor (such as a condenser or flat window in a flow loop) for easier cleaning. In comparing light delivery systems, it was found that the flat window arrangement gave higher CO_2 and H_2S uptakes and cell concentrations.

Continuous bioreactor studies were also performed with *T. crunogena* (non-photosynthetic) with various sulfur sources such as Na_2S, Na_2SO_3 and thiosulfate. Na_2SO_3 proved to be the best sulfur source for the bacterium. However, operation of the *T. crunogena* system was more difficult than the *C. thiosulfatophilum* system, and thus the *C. thiosulfatophilum* system is preferred despite the need for external light.

Finally, a near infrared light source was designed for use with *C. thiosulfatophilum* in the CSTR. Multiple 1 W LED lights were used as the light source, which provided more than adequate light to the culture to maintain cell growth. An arrangement of ten 1 W LEDs appeared to be optimum for the laboratory reactor. Key variables such as culture circulation rate, culture volume and time in light vs. time in dark were studied with these light delivery systems. The best and most economic

method of light delivery was a flat panel light design with cyclic light addition of 50 to 55 seconds followed by about 13 minutes of darkness. CO_2 utilization was maximized in the *C. thiosulfatophilum* system by employing a more effective light source, cell recycle and sulfur recovery in a single reactor system. The best arrangement was to employ the cell recycle system after sulfur recovery, because of the tendency to accumulate sulfur in the cell recycle system.

In an effort to improve sulfur and cell separation in the sulfur settler and minimize the cell loss, a mild amount of turbulence was added to the sulfur settler by bubbling helium into the settler. Results indicate that a small amount of turbulence in the settler reduces the amount of cells lost due to settling without inhibiting the collection of sulfur. At the same time, culture performance was not affected. A reactor was also used to test the effect of increased nutrition and the effects of acetylene on the sulfur to sulfate ratio. Higher acetylene contact did not inhibit the production of sulfate, but may have hurt cell growth.

A secondary use of the *C. thiosulfatophilum* bacterial cells could be as a single cell protein source for livestock or poultry feed. Analyses of protein, fat, and amino acid content performed on the bacteria show that the bacterial cells are rich in protein and contain significant levels of many of the amino acids found in animal feed supplements.

TASK 1. CULTURE ISOLATION AND OPTIMIZATION

Summary. Sixteen potential alternatives to *C. thiosulfatophilum* were identified for converting CO_2 and H_2S to elemental sulfur without requiring light as the source of energy. Three of these strains (*Thiomicrospira kuenenii* sp. nov. (DSM 12350), *Thiomicrospira frisia* sp. nov. (DSM 12351) and *Thiomicrospira chilensis* sp. nov. (DSM 12352)) were obtained from DSM (German culture collection), and *Thiomicrospira crunogena* (ATCC 35932) from the American Type Culture Collection. Experiments were performed with *T. crunogena*, first under a laminar flow hood and later in a 2L continuous stirred tank reactor, where a number of important variables were studied. The reactor systems were operated aerobically at 26°C, with gentle agitation, on modified ATCC medium #1422, *Thiomicrospira* medium. No light was provided to the culture. It was found that the CO_2 to sulfide uptake rate was much higher for *T. crunogena* in comparison to *C. thiosulfatophilum*.

In addition to *C. thiosulfatophilum*, a number of other bacteria have been noted in the literature, also able to oxidize H_2S and fix CO_2. Many of these strains do not require light for growth, which could potentially result in a significant energy savings when employing the strains in bioreactors. A listing of these strains by type is shown below.

Aerobic, free-living rods or ovoids

Thiomicrospira kuenenii sp. nov. (DSM 12350), operates at pH 6.0 and 29-33.5°C, and is chemolithoautotrophic

Thiomicrospira frisia sp. nov. (DSM 12351), operates at pH 6.5 and 32-35°C, and is

chemolithoautotrophic

Thiomicrospira chilensis sp. nov. (DSM 12352), operates at pH 7.0 and 32-37°C

Thiomicrospira pelophila (DSM 1534)

Thiomicrospira crunogena (ATCC 35932)

Thiomonas

Thiovulum

Thiobacillus

Candidatus Arcobacter sulfidicus

Filamentous forms
Thioploca produces mats, is a facultative chemolithoautotroph, can tolerate up to 10% air, stores sulfur internally, and has a doubling time of 69-139 days

Thioploca chileae

Thioploca araucae

Beggiatoa is aerobic, develops inclusions of sulfur, and *Beggiatoa alba* produces only elemental sulfur (no sulfate)

Thiothrix operates at 25-30°C and deposits sulfur internally

Thermothrix thiopara (ATCC 29244) is aerobic, deposits sulfur external to the cell, and operates at 40-80°C, with an optimum of 73°C

Photolithotrophic Forms
Rhodospirillum rubrum

Rhodobacter sphaeroides

Selection of Bacteria for CSTR Studies. Batch experiments were performed to determine which aerobic organism (*T. kuenenii*, *T. frisia*, *T. chilensis* or *T. crunogena*) exhibited the best growth characteristics for continued study in a CSTR. Each batch bottle (150 mL, total volume) contained 50 mL of DSMZ Medium #142 (*Thiomicrospira pelophila* medium). The headspace (100 mL) was filled with 60% CO_2 and 40% air at a pressure of 7 psig. The pH was adjusted to 8.0 with 20% KOH. The bottles were inoculated with 2.5 mL of active culture for a 5% inoculation size. The batch bottles were then kept in a shaker-incubator maintained at 30°C. The progress of each fermentation was monitored using GC analysis of the bottle headspace to determine O_2

usage and protein concentration (using the Bradford Protein Assay) to determine cell growth. Carbon dioxide uptake was not measured directly due to the high solubility of CO_2 in the liquid phase. However, a high cell growth and high O_2 utilization were used as good indirect indicators of CO_2 uptake by the bacteria, since CO_2 was the only carbon source present in the medium.

As is noted in Table 1, oxygen consumption by *T. crunogena* far exceeded oxygen consumption by the other organisms. Within 24 hours, one batch bottle of *T. crunogena* consumed 75 percent of the available oxygen. A second bottle of *T. crunogena* consumed 79 percent of the available oxygen within 48 hours. Both *T. crunogena* batch bottles consumed most of the available oxygen within 120 hours of incubation, so the headspace in each bottle was replenished and the culture continued to consume oxygen. Each of the other cultures consumed only small amounts of oxygen. *T. kuenenii* and *T. frisia* both consumed about 20 percent of the oxygen within 170 hours. *T. chilensis* only consumed 5 percent of the oxygen in 146 hours.

T. crunogena also outgrew each of the other cultures, as can be seen in the protein assays of Table 2. An increase in protein is a direct correlation to cell growth. Within 120 hours of inoculation, *T. crunogena* showed a protein measurement of 32 and 28 mg/L. All of the other cultures had protein measurements of 2-3 mg/L. By 170 hr, one bottle of *T. crunogena* showed a protein measurement of 39 mg/L. The next highest protein measurement at 170 hr was 6.6 mg/L for the *T. frisia* culture.

Among the four organisms tested, *T. crunogena* outperformed all others in measured cell growth as well as oxygen metabolism over a seven day period. These batch bottle test results, along with information from literature studies (Table 3), indicate that *T. crunogena* is the best aerobic culture for continued CSTR study.

Table 1. Oxygen Consumption (%) in the Aerobic Batch Bottle Cultures to Determine a Suitable Culture for CSTR Study

Run Time (hr)	*T. kuenenii*	*T. frisia*	*T. chilensis*	*T. cunogena* (Bottle 1)	*T. crunogena* (Bottle 2)	Control
24	-1.78	-0.23	1.42	75.82	1.38	1.25
48	-1.68	0.10	1.64	89.25	79.64	1.40
120	7.34	8.11	4.30	95.09	83.41	1.33
146	21.35	13.97	5.32	13.02*	9.49*	1.69
170	20.54	21.07	5.61	84.13	40.62	1.87

* The *T. crunogena* batch bottles were regassed between the 120 and 146 hr sampling

Table 2. Protein Analysis of the Aerobic Batch Bottle Cultures to Determine a Suitable Culture for CSTR Study

Run Time (hr)	*T. kuenenii*	*T. frisia*	*T. chilensis*	*T. cunogena* (Bottle 1)	*T. crunogena* (Bottle 2)	Control
120	2.62	3.36	2.09	32.05	27.90	1.0
170	3.84	6.60	2.27	38.74	NA	3.23

Table 3. Known Information from a Literature Search - A Side by Side Comparison of the Thiomicrospira Aerobic Cultures

	T. crunogena	*T. kuenenii*	*T. frisia*	*T. chilensis*
Max Growth Rate (hr^{-1})	0.80	0.35	0.45	0.40
Max CO_2 Uptake Rate – RuBisCO Activity (nmol/min per mg protein)	97.2 nmol	9.75	12.65	8.2
pH Range	5.0 – 8.5	4.0 – 7.5	4.2 – 8.5	5.3 – 8.5
Optimal pH	7.5 – 8.0	6.0	6.5	7.0
Temp Range (°C)	4 – 38.5	3.5 - 42	3.5 - 39	3.5 - 42
Optimal Temp (°C)	28 - 32	29 - 33.5	32 - 35	32 - 37
Formation of Sulfur (y/n)	yes	no	no	yes
Na^+ Conc Range (mM)	>45	100 - 640	100 - 1240	100 – 1240
Optimal Na^+ Conc (mM)	Nd	470	470	470

Thiomicrospira crunogena (ATCC 35932) was obtained from the American Type Culture Collection as non-photosynthetic alternatives to *C. thiosulfatophilum*. In addition, *Thiomicrospira kuenenii* sp. nov. (DSM 12350), *Thiomicrospira frisia* sp. nov. (DSM 12351) and *Thiomicrospira chilensis* sp. nov. (DSM 12352) were obtained from DSM (German culture collection). *Thiomicrospira crunogena* is aerobic, and is present as a free-living rod or ovoid. *Thiomicrospira kuenenii* sp. nov. (DSM 12350) is aerobic, operates at pH 6.0 and 29-33.5°C, and is chemolithoautotrophic. *Thiomicrospira frisia* sp. nov. (DSM 12351) is also aerobic, operates at pH 6.5 and 32-35°C, and is chemolithoautotrophic. Finally, *Thiomicrospira chilensis* sp. nov. (DSM 12352) is an aerobe that operates at pH 7.0 and 32-37°C.

A comparison of the strains based on information from the literature (Dobrinski *et al.,* 2005; Ruby and Jannasch, 1982; Wirsen *et al.,* 1998; Brinkhoff *et al.,* 1999a, 1999b) is shown in Table 4. *T. crunogena* was selected for further CSTR study since it has highest CO_2 uptake rate, a rate that is nearly ten times the rates of the other strains.

Table 4. Comparison of *Thiomicrospira* Strains

Strain	*T. crunogena*	*T. kuenenlii*	*T. frisia*	*T. chilensis*
Max growth rate (hr^{--1})	0.8	0.35	0.45	0.4
Max CO_2 uptake rate – Rubis CO activity	97.2 nmol m^{-1} (mg protein)$^{-1}$	9.75 nmol C fixed (mg protein)$^{-1}$ min^{-1}	12.65 nmol C fixed (mg protein)$^{-1}$ min^{-1}	8.2 nmol C fixed (mg protein)$^{-1}$ min^{-1}
pH range	5.0 – 8.5	4.0 – 7.5	4.2 – 8.5	5.3 – 8.5
pH – optimal	7.5 – 8.0	6	6.5	7
Temp range, °C	4 – 38.5	3.5 – 42	3.5 - 39	3.5 – 42
Optimal Temp, °C	28 - 32	29 – 33.5	32 - 35	32 – 37
Formation of sulfur from thiosulfate	Yes	No	No	Yes

TASK 2. CONTINUOUS BIOREACTOR STUDIES

Task 2A. Studies with *C. thiosulfatophilum*

Summary. A number of continuous bioreactor experiments were performed with *C. thiosulfatophilum* to better understand the important variables (sulfur removal from the culture, sulfur/sulfate production, light intensity, reactor cooling during light delivery, cell recycle combined with sulfur settling and H_2S/CO_2 conversion) in converting CO_2 and H_2S to cell biomass and elemental sulfur. These variables were studied in reactor systems employing a tungsten light source, both with and without sulfur removal. One problem with *C. thiosulfatophilum* is that it will accumulate on the walls of the reactor vessel when using an external light source. A double magnet system (one outside the reactor and one inside the reactor) was used to help rid the reactor walls of cell biomass by acting as a scraper of cell debris. Cleaning resulted in the production of less sulfate, and the gas conversions improved slightly. Another reactor alternative that was employed involved the use of a light source which could be operated external to the reactor (such as a condenser or flat window in a flow loop) for easier cleaning. In comparing light delivery systems, it was found that the flat window arrangement gave higher CO_2 and H_2S uptakes and cell concentrations.

Analytical Procedures. Analytical procedures have been standardized for the operation of continuous reactors using *C. thiosulfatophilum* with sulfur recovery. These procedures are briefly outlined below.

Gas Sampling. Gas sampling for the green sulfur bacterium was performed on a Perkin Elmer 8500 gas chromatograph. The gas was tested for H_2S, N_2, and CO_2 concentrations. The carrier gas was helium with a flow rate of 35ml/min. The column was 6 feet long containing 80/100 mesh Chromosorb from Supelco, Inc. The column oven temperature was kept at 80°C for 1 min, and then a temperature ramp of 20°C/min

was used for 3 min to reach a final temperature of 140°C. Total run time was 4 min. The sample size used was 0.1 ml.

Cell Density. Cell density was measured indirectly using a methanol extraction of chlorophyll from the cells. The optical density (OD) of the methanol/chlorophyll solution was read at 670 nm using a Spectronic 20 Genesys spectrophotometer. A standard curve of chlorophyll extraction vs. cell density was used to calculate the cell density of the sample. The cell density was determined using dry cell weight experiments.

$SO_4^=$. Sulfate measurement was performed using the barium precipitate procedure. Anhydrous $BaCl_2$ was mixed with fermentation broth (minus the cells/sulfur) to form a suspension of the insoluble $BaSO_4$ precipitate. The OD of the $BaSO_4$ suspension was read at 420 nm using a Spectronic 20 Genesys spectrophotometer. A standard curve of OD vs $SO_4^=$ concentration was used to calculate the $SO_4^=$ concentration.

Sulfur. Sulfur measurements were performed in three stages. First, an acetone extraction of the sulfur precipitate in the culture sample was used to separate the sulfur from the cells. Next, the acetone was allowed to evaporate, leaving the sulfur behind. The sulfur was then dissolved in chloroform for an OD reading at 290 nm using a Shimadzu UV mini 1240 spectrophotometer. A standard curve of OD vs. sulfur concentration was used to calculate the sulfur concentration.

pH. The pH of the culture was determined using an Orion pH/ISE meter model 710A with a Cole-Parmer pH electrode (#5990-45 JJ3).

Sulfide. Sulfide measurement was performed using the ion-selective electrode method. A silver/sulfide ion-selective electrode was used to measure the mV readings of the culture sample. A standard curve of mV vs. sulfide concentration was then used to calculate sulfide concentration. A UB-10 UltraBasic pH/mV meter and a $Ag/S^=$ redox probe (Ag/S 7506) from Denver Instruments were used to take the mV readings.

Initial CSTR Studies. Two CSTRs were operated to first grow *C. thiosulfatophilum* to an acceptable level with good H_2S/CO_2 uptake, reach a steady state at that level, and then start adjusting parameters (temperature, agitation rate, pH, cell retention time (XRT), liquid retention time (LRT), light intensity, with/without cell recycle, gas retention time (GRT), and $\%H_2S$ in the feed) to optimize system performance. Figure 1 shows a schematic of the proposed system with cell recycle and sulfur recovery. The reactors were started in batch mode on medium containing 0.87 g/L K_2HPO_4, 1.42 g/l KH_2PO_4, 0.74 g/L NH_4Cl, 7.4 g/l NaCl, 1.8 g/L $MgSO_4 \cdot 7H_2O$, 0.05 g/L $CaCl_2 \cdot 2H_2O$, 0.01 g/L $FeCl_2 \cdot 4H_2O$, 0.0625 ml/L of 85% H_3PO_4, 0.5 mg/L $ZnSO_4 \cdot 7H_2O$, 0.15 mg/L $MnCl_2 \cdot 4H_2O$, 1.5 mg/L H_3BO_3, 1 mg/L $CoCl_2 \cdot 6H_2O$, 0.1 mg/L $CuCl_2 \cdot 2H_2O$, 0.2 mg/L $NiCl_2 \cdot 6H_2O$, 0.15 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.05 mg/L Na_2SeO_3, 0.5 mg/L $Na_2WO_4 \cdot 2H_2O$ and 5 µg/L of vitamin B12. The pH set point was 7.0 and 10% NaOH was used for pH control. The feed gas was a blend of CO_2 and 2.5% H_2S (balance N_2). A 60 W tungsten bulb was

used as the light source, held external to the bioreactor. Temperature was kept at approximately 30°C using a cold finger to balance the heat generated by the light source.

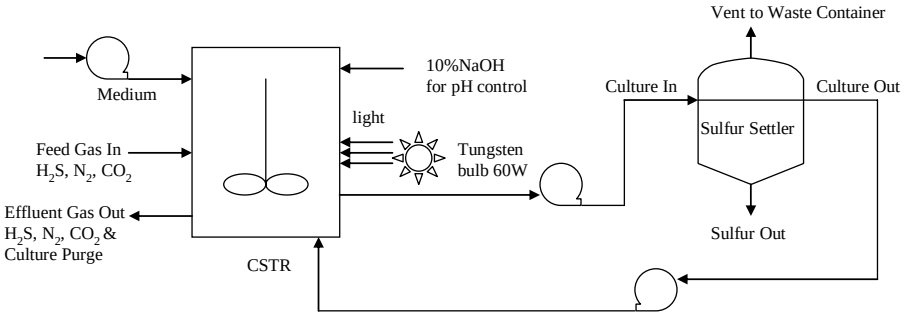


Figure 1. Schematic of CSTR with Cell Recycle and Sulfur Recovery

As a straight through CSTR with no sulfur settler, the cell density reached and was maintained at about 0.4 g/L. The H_2S and CO_2 uptake rates were maintained at 0.025 mmol/min and 0.013 mmol/min, respectively. The sulfate concentration was about 1000 ppm. The sulfur concentration reached 250 ppm, but dropped to 100-150 ppm due to sulfide toxicity. The cell retention time (XRT) (and liquid retention time (LRT)) was maintained at 50 to 60 hours, and the gas retention time (GRT) was 40 to 45 hours. Once the sulfur settler was installed, the sulfur level in the reactor dropped quickly to 0 to 30 ppm. The sulfate concentration remained at around 1000 ppm, but the readings varied widely. The cell density was only 0.15 g/L, due in part to increasing the total culture volume from 1.7 to 2.7 L and due also to an increase in medium flow. The H_2S and CO_2 uptake rates increased to 0.035 mmol/min and 0.017 mmol/min, respectively, as the GRT dropped to about 32 hours. The H_2S in the feed gas was raised to 1.9 percent and remained there consistently. Figures 2 and 3 show gas conversion and gas uptake rate for one of the reactors. As is noted in Figure 2, the H_2S conversion averaged 90 percent.

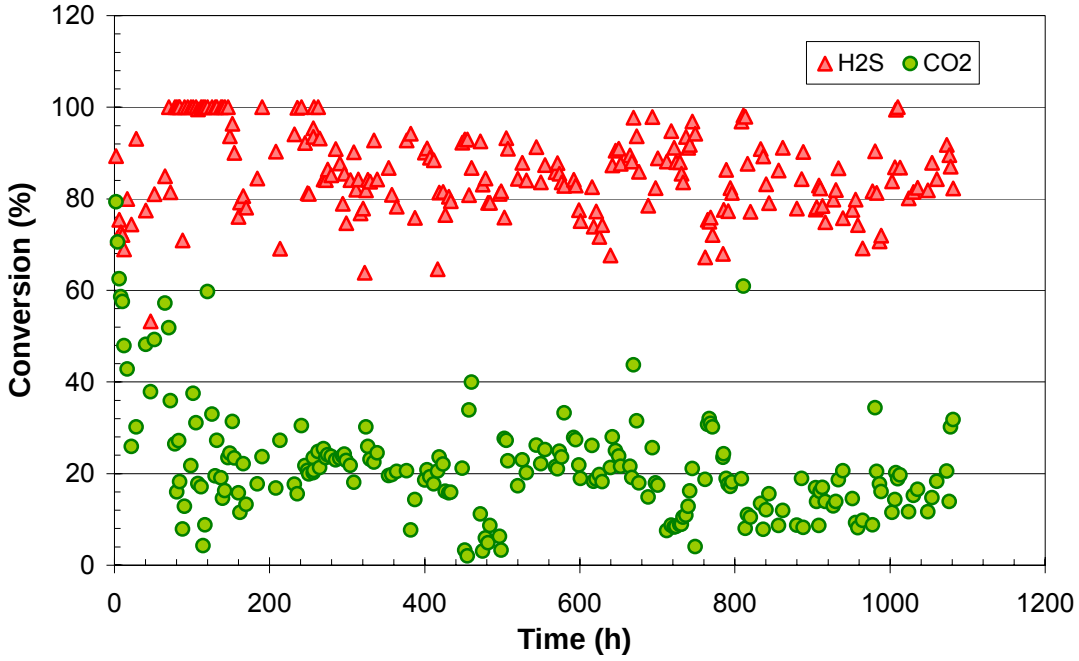


Figure 2. Gas Conversions in the Chlorobium Thiosulfatophilum Reactor E7, a Straight Through CSTR used in H_2S and CO_2 Uptake Studies

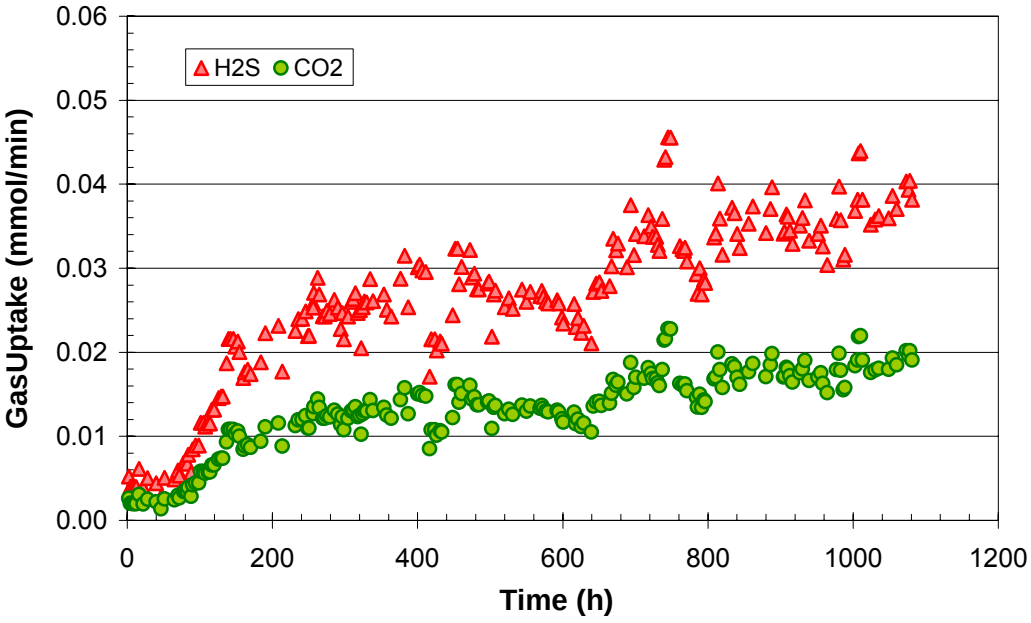


Figure 3. Substrate Uptake in the Chlorobium Thiosulfatophilum Reactor E7, a Straight Through CSTR used in H_2S and CO_2 Uptake Studies - CO_2 Uptake Is Calculated Based H_2S Uptake

Initial Parametric Studies. A number of continuous bioreactor experiments were performed over the reporting period to better understand the important variables in converting CO_2 and H_2S to cell biomass and elemental sulfur. Important variables

include sulfur removal from the culture and H_2S feed rate. Carbon dioxide is in abundance and non-inhibiting. Response variables are cell production, H_2S/CO_2 conversion, sulfur/sulfate production.

Initial Reactor Setup. Two 1.7 liter CSTRs were operated with identical conditions using *C. thiosulfatophilum* (one with sulfur removal by gravity through a 1 liter separatory funnel, 700 ml working volume, and one without sulfur settling) to determine the effects of sulfur settling on reactor performance. The reactors were fed medium containing 0.87 g/L K_2HPO_4, 1.42 g/l KH_2PO_4, 0.74 g/L NH_4Cl, 7.4 g/l NaCl, 1.8 g/L $MgSO_4 \cdot 7H_2O$, 0.05 g/L $CaCl_2 \cdot 2H_2O$, 0.01 g/L $FeCl_2 \cdot 4H_2O$, 0.0625 ml/L of 85% H_3PO_4, 0.5 mg/L $ZnSO_4 \cdot 7H_2O$, 0.15 mg/L $MnCl_2 \cdot 4H_2O$, 1.5 mg/L H_3BO_3, 1 mg/L $CoCl_2 \cdot 6H_2O$, 0.1 mg/L $CuCl_2 \cdot 2H_2O$, 0.2 mg/L $NiCl_2 \cdot 6H_2O$, 0.15 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.05 mg/L Na_2SeO_3, 0.5 mg/L $Na_2WO_4 \cdot 2H_2O$ and 0.0201g/L of vitamin B12. The pH set point was 7.2 and 10 percent NaOH was used for pH control. The feed gas was a blend of CO_2 and 2.5 percent H_2S (balance N_2). A 60W tungsten bulb was used as the light source, held external to the bioreactor. The temperature was kept at approximately 30°C using a cold finger to balance the heat generated by the light source. Sulfur settler samples were removed regularly from the bottom of the settler. The H_2S conversion was maintained at 70 percent, or greater in both reactors, and the CO_2 conversion was maintained at around 25 percent. The liquid retention time (LRT = liquid flowrate / liquid volume) in both reactors was 42 hours. However, culture flowed to the settler at 30ml/min giving a settler LRT of 30 minutes based on a settler volume of 900 ml. Thus, the LRT in the reactor with the settler was 37 hours.

Table 5 shows a comparison of results from the two reactors. The cell concentration was higher in the reactor without the sulfur settler, while the H_2S and CO_2 conversions, as well as sulfide concentrations and sulfate concentrations were higher in the reactor with sulfur settling. As expected, the elemental sulfur concentration was greatly reduced in the reactor with the sulfur settler, which removed the sulfur. Some sulfate and cells were also removed by the settler.

Table 5. Reactor Performance With and Without Sulfur Settling.
Initial Experiment

Variable	With Settler	Without Settler
LRT	42	37
Cell Concentration (OD)	0.17	0.29
CO_2 Delivered, mmol/min	0.512	0.652
CO_2 Conversion, %	26	20
CO_2 Uptake, mmol/min	0.1331	0.1304
H_2S Delivered, mmol/min	0.04	0.03
H_2S Conversion, %	79	66
H2S Uptake, mmol/min	0.0316	0.0198
Sulfide Concentration, ppm	18.8	12.2
Sulfur Concentration, ppm	1.8	91
Sulfate Concentration, ppm	191	135

Light Source: 1-60 W bulb, Reactor Volume: 1.7 L, Temperature: 30°C, same gas composition

The experiment discussed from Table 5 was repeated, but with the medium flow rate increased to reduce the LRT to 33 hours for both with and without the settler. The H_2S feed rate was maintained the same for the two reactors. As in the previous experiment, the H_2S conversion, sulfide concentrations and sulfate concentrations were higher in the reactor with the sulfur settler (see Table 6). The cell concentration and the elemental sulfur concentration was higher in the reactor without the sulfur settler. Again, sulfur, some sulfate and some cells are removed by the settler.

Table 6. Reactor Performance With and Without Sulfur Settling. Equal LRT (33 hr)

Variable	With Settler	Without Settler
LRT	33	33
Cell Concentration (OD)	0.16	0.24
CO_2 Delivered, mmol/min	0.512	0.652
CO_2 Conversion, %	21	21
CO_2 Uptake, mmol/min	0.1075	0.1369
H_2S Delivered, mmol/min	0.04	0.04
H_2S Conversion, %	67	60
H_2S Uptake, mmol/min	0.0268	0.0240
Sulfide Concentration, ppm	41	34
Sulfur Concentration, ppm	8.5	71
Sulfate Concentration, ppm	241	66

Light Source: 1-60 W bulb, Reactor Volume: 1.7 L, Temperature: 30°C, same gas composition

The culture flow rate to the sulfur settler was then increased to 60 ml/minute to lower the LRT in the settler to 15 minutes. The H_2S flow rate was also increased 50 percent. By doing so, the cell concentration, H_2S uptake (although H_2S conversion reduced), and sulfide concentration in the reactor increased; while the CO_2 conversion, sulfur concentration and sulfate concentration decreased. These results are summarized in Table 7.

Table 7. Reactor Performance With Sulfur Settling. Parameter Studies

Variable	Baseline	Increased Settler LRT
LRT	33	34
Cell Concentration (OD)	0.16	0.20
CO_2 Delivered, mmol/min	0.512	0.515
CO_2 Conversion, %	21	14
CO_2 Uptake, mmol/min	0.1075	0.0721
H_2S Delivered, mmol/min	0.04	0.06
H_2S Conversion, %	67	59
H_2S Uptake, mmol/min	0.0268	0.0354
Sulfide Concentration, ppm	41	44
Sulfur Concentration, ppm	8.5	7.7
Sulfate Concentration, ppm	241	108

Light Source: 1-60 W bulb, Reactor Volume: 1.7 L, Temperature: 30°C, same gas composition

In another experiment (Table 8) the H_2S feed rate, and the medium flow rate was decreased to give a reactor LRT of about 47 hr. The cell concentration, H_2S conversion and sulfur concentration increased, while the H_2S uptake, CO_2 conversion and sulfide concentration decreased. Sulfate concentration results were not reliable due to problems with the assay.

Table 8. Reactor Performance Without Sulfur Settling. Parameter Studies

Variable	Baseline	Increased Reactor LRT/ Decreased H_2S Rate
LRT	33	47
Cell Concentration (OD)	0.24	0.34
CO_2 Delivered, mmol/min	0.652	0.652
CO_2 Conversion, %	21	18
CO_2 Uptake, mmol/min	0.0137	0.1193
H_2S Delivered, mmol/min	0.04	0.02
H_2S Conversion, %	60	76
H_2S Uptake, mmol/min	0.024	0.0152
Sulfide Concentration, ppm	34	14
Sulfur Concentration, ppm	71	85
Sulfate Concentration, ppm	66	n.a.

Light Source: 1 60 W bulb, Reactor Volume: 1.7 L, Temperature: 30°C, same gas composition

The CO_2 feed rate and resulting CO_2 uptake rate were also studied in the reactor with sulfur settling (Table 9). In this experiment, the CO_2 feed rate was reduced by 50 percent while maintaining a constant H_2S feed rate by increasing the N_2 feed rate to replace the reduced CO_2 flow. The H_2S conversion declined, but only by 15 percent of its original value. The sulfur/sulfate ratios were affected, as observed by an increase in sulfate concentration and a decrease in sulfur concentration. Interestingly, the cell concentration increased by a third but the H_2S uptake was reduced by 20 percent.

Table 9. Reactor Performance With Sulfur Settling. Decreased CO_2 Feed Rate

Variable	Baseline	Decreased CO_2 Rate
%CO_2 in Feed Gas	23	14
Cell Concentration (OD)	0.15	0.25
CO_2 Delivered, mmol/min	0.700	0.376
CO_2 Conversion, %	13	17
CO_2 Uptake, mmol/min	0.091	0.0642
H_2S Delivered, mmol/min	0.068	0.063
H_2S Conversion, %	73	62
H_2S Uptake, mmol/min	0.050	0.0391
Sulfide Concentration, ppm	0	0
Sulfur Concentration, ppm	21	15
Sulfate Concentration, ppm	69	99

Light Source: 1 60 W bulb, Reactor Volume: 1.7 L, Temperature: 30°C, LRT: 34 hr

In a final gas study, the H_2S feed rate was decreased in order to achieve high H_2S conversion. As shown in Table 10, a 100 percent H_2S conversion was attained in the 1.7 liter reactor without sulfur settling when the H_2S delivery rate is reduced to 0.019 mmol/min. The cell concentration increased while the CO_2 conversion decreased, and sulfate became the major product in the reactor.

Table 10. Reactor Performance Without Sulfur Settling. Achieving High H_2S Conversion

Variable	Baseline	Decreased H_2S Rate
Cell Concentration (OD)	0.20	0.60
CO_2 Delivered, mmol/min	0.452	0.445
CO_2 Conversion, %	27	13
CO_2 Uptake, mmol/min	0.122	0.0578
H_2S Delivered, mmol/min	0.027	0.019
H_2S Conversion, %	72	100
H_2S Uptake, mmol/min	0.0194	0.019
Sulfide Concentration, ppm	0	0
Sulfur Concentration, ppm	78	65
Sulfate Concentration, ppm	45	209

Light Source: 1 60 W bulb, Reactor Volume: 1.7 L, Temperature: 30°C, LRT: 35 hr. same gas composition

Since the H_2S uptake was not reduced with reduced H_2S in the gas, it can be seen that the excess dissolved H_2S inhibits sulfate production. This also shows that there is a limit on dissolved H_2S that inhibits cell growth.

Product recovery is necessary and a settling device seems to work. Cell concentration in the reactor is reduced when using the cell separator because some cells settle with the sulfur. Increasing the flow rate through the settler and back to the reactor actually increased the cell density in the reactor and improved the H_2S uptake. Increasing the LRT in the reactor by reducing medium flow rate did increase the cell concentration because the cell purge rate was reduced, but the H_2S uptake also reduced. Interestingly, decreasing the carbon dioxide flow rate by 50 percent resulted in an increased cell concentration in the reactor of about 67 percent and an increase in H_2S uptake of about 20 percent. Another interesting result was that reduced H_2S feed rate resulted in a three-fold increase in cell concentration. The H_2S uptake remained about the same, indicating that the H_2S supply was not being utilized and perhaps inhibiting cell growth.

Operation with a Tungsten Light Source, No Sulfur Removal. The studies with *C. thiosulfatophilum* that used an external light source without sulfur removal employed a 1.65 L CSTR with no cell recycle loop. The culture was maintained in the sulfate production mode as much as possible to maximize cell growth and CO_2 uptake. The feed gas was a blend of CO_2 and a gas mixture containing 97.5% N_2 and 2.5% H_2S. The light source was a single 100 W tungsten bulb, positioned just outside the reactor wall. External cooling using a simple fan was provided as needed to maintain the

reactor temperature at 30°C. A section of reactor wall, located just in front of the external light source was scrubbed twice daily, in part to re-suspend some of the cells and sulfur back into the fermentation broth; but more importantly, to keep a section of the reactor wall clear for the light source. Scrubbing was accomplished by the movement of two magnets, one on the inside and one on the outside of the reactor.

As was noted above, the strategy to operate this reactor without sulfur recovery was to maximize CO_2 uptake by operating in the sulfate production mode. This is quite different from maximizing the conversion of H_2S to elemental sulfur. When producing elemental sulfur as the product, the target CO_2 to H_2S uptake ratio is 0.5 (Equation 1), and when producing sulfate as the product, the target uptake ratio is 2.0 (Equation 2).

$$2H_2S + CO_2 \rightarrow 2S + CH_2O + H_2O \quad (1)$$

$$H_2S + 2CO_2 + 2H_2O \rightarrow H_2SO_4 + 2CH_2O \quad (2)$$

Three major operating variables were studied: the cell and liquid retention time, the H_2S feed rate and the CO_2 feed rate. The agitation rate was maintained at 700 rpm, the temperature was held at 30°C, the pH was set at 7.0 and the light intensity was maintained constant with the aid of the single 100 W bulb. However, sulfur production was not entirely eliminated. When light is limiting or the sulfide concentration is too high in the liquid phase, sulfide oxidation can stop at elemental sulfur and thus sulfur accumulates. A single 100 W bulb as the light source is not sufficient to totally eliminate sulfur production.

Effect of Liquid Retention Time. Table 11 shows the effect of liquid retention time (LRT) on the average gas uptake rates and conversions, cell production and product formation by *C. thiosulfatophilum* in the straight through (no cell recycle loop) CSTR with external tungsten light and no sulfur recovery. The CO_2 and H_2S uptake rates include modifications for dissolved gas carried out by the culture purge as described later in the report. Also, there were difficulties in measuring sulfide during the early stages of this experimentation, such that 0.00 ppm sulfide (as indicated in some of the tables below) do not really indicate that there was no sulfide in the liquid.

Table 11. Effects of Liquid Retention Time on Gas Uptake by *C. thiosulfatophilum*

LRT (hr)	CO_2 Conversion (%)	H_2S Conversion (%)	CO_2 Uptake (mmol/min)	H_2S Uptake (mmol/min)	CO_2/H_2S Uptake (Predicted)
32	34	97	0.0290	0.0242	0.816
40	36	84	0.0397	0.0225	1.417
46	44	96	0.0399	0.0280	1.246

Table 11 (continued). Effects of Liquid Retention Time on Gas Uptake by *C. thiosulfatophilum*

LRT (hr)	CO_2/H_2S Uptake (Actual)	Cells (g/L)	Sulfide (g/L)	Sulfur (g/L)	Sulfate (g/L)
32	1.198	0.749	5.93	190.7	150
40	1.764	0.319	11.0	51.5	78
46	1.425	0.831	3.17	128.7	339

H_2S feed rate: 0.0335 mmole/min, CO_2 feed rate: 0.0856 mmole/min

As noted in the table, the CO_2 and H_2S uptake rates generally increased with liquid retention time. The lower H_2S uptake rate at the 40 hour liquid retention time likely resulted from the poor condition of the culture. At the 40 hour LRT, the H_2S conversion was only 84 percent and the cell concentration was only 0.319 g/L. Except for the 40 hour LRT data which could have been caused by poor culture condition, the CO_2 to H_2S uptake ratio favored sulfate production, approaching 2.0 for the longer liquid and cell retention time. At longer cell retention times, more cells are present inside the fermenter. Thus, the sulfide concentration will be lower in the liquid phase thus favoring sulfate production. Not only is the sulfate to sulfur ratio increased, but the CO_2 uptake rate automatically increases as more sulfate is produced. This is especially obvious when observing the 32 and 46 hour LRT data. The sulfide concentration increased from 3.17 to 5.93 ppm and the sulfur concentration also increased from 129 to 191 ppm when the cell retention time was reduced from 46 to 32 hours. On the other hand, the sulfate concentration fell from 339 to 150 ppm, and the CO_2/H_2S uptake ratio fell from 1.425 to 1.198.

Effect of CO_2 Feed Rate. The CO_2 feed rate was varied from 0.0856 to 1.1357 mmole/min while holding the LRT and H_2S feed rate constant. Four experiments were performed, as is noted in Table 12:

- An experiment where the CO_2 feed rate was changed from 0.171 to 0.142 mmol/min, while holding the LRT at 32 hours and the H_2S flow rate at 0.021 mmol/min.
- An experiment where the CO_2 feed rate was changed from 0.142 to 0.114 mmol/min, while holding the LRT at 32 hours and the H_2S flow rate at 0.0146 mmol/min.
- An experiment where the CO_2 feed rate was changed from 0.426 to 1.136 mmol/min, while holding the LRT at 32 hours and the H_2S flow rate at 0.0168 mmol/min.
- An experiment where the CO_2 feed rate was changed from 0.981 to 0.085 mmol/min, while holding the LRT at 46 hours and the H_2S flow rate at 0.025 mmol/min.

The consistent observation from these four pairs of experiments is that only a slight increase in the H_2S uptake is observed when the CO_2 feed rate is increased, even when employing a very high ratio of CO_2/H_2S. In general, there is no significant advantage in further increasing the CO_2 feed rate once enough CO_2 is supplied to the culture. The

CO_2 conversion will increase (due to more CO_2 being dissolved in the liquid phase and being carried out by the purge liquid due to a higher percentage of CO_2 in the gas outlet stream), but the actual CO_2 uptake rate will not increase.

Note that the CO_2 uptake is 0 for a 1.136 mmole/min CO_2 feed rate. This is because the CO_2 concentration in the feed gas is so high that conversion estimation is difficult by using gas chromatography. With this high CO_2 feed rate, there was a significant number of 'less than zero' CO_2 conversion data points, even before the correction for the dissolved CO_2 lost. GC accuracy is insufficient to correctly calculate the differences between the inlet and outlet gas at these high CO_2 feed rates. This accuracy problem also underestimates CO_2 conversions for the 0.426 mmole/min CO_2 run and results in a much lower actual CO_2/H_2S ratio than predicted.

Table 12. Effects of CO_2 Feed Rate on Gas Uptake by *C. thiosulfatophilum*

CO_2 Feed Rate (mmol/min)	CO_2 Conversion (%)	H_2S Conversion (%)	CO_2 Uptake (mmol/min)	H_2S Uptake (mmol/min)	CO_2/H_2S Uptake (Predicted)
0.171[a]	30	99	0.0164	0.0206	1.073
0.142[a]	30	92	0.0117	0.0193	1.156
0.142[b]	36	100	0.0160	0.0149	0.944
0.114[b]	48	100	0.0347	0.0143	1.196
0.426[c]	24	88	0.0176	0.0213	1.837
1.136[c]	8	99	0	0.0184	1.668
0.981[d,e]	9	99	0.0246	0.0248	1.635
0.085[d,e]	37	94	0.0315	0.0232	1.466

CO_2 Feed Rate (mmol/min)	CO_2/H_2S Uptake (Actual)	Cells (g/L)	Sulfide (g/L)	Sulfur (g/L)	Sulfate (g/L)
0.171[a]	0.796	0.380	0	91.9	164
0.142[a]	0.606	0.372	0	53.3	114
0.142[b]	1.074	0.438	0	82.7	101
0.114[b]	2.426	0.430	0	94.1	240
0.426[c]	0.826	0.356	7.8	7.6	163
1.136[c]	0	0.327	0.01	43.5	429
0.981[d,e]	0.992	0.636	0	54.2	493
0.085[d,e]	1.358	0.853	1.7	93.9	424

LRT: 32 hr; [a]H_2S feed rate: 0.021 mmol/min; [b]H_2S feed rate: 0.0146 mmol/min; [c]H_2S feed rate: 0.0168 mmol/min; [d]H_2S feed rate: 0.025 mmol/min; [e]LRT increased to 46 hr

Effect of H_2S Feed Rate. The CO_2 feed rate was varied from 0.0168 to 0.0451 mmole/min while holding the LRT and H_2S feed rate constant. Two experiments were performed, as is noted in Table 13:

- An experiment where the H_2S feed rate was changed from 0.0168 to 0.0251 mmol/min, while holding the LRT at 32 hour and the CO_2 flow rate at 1.136 mmol/min.
- An experiment where the H_2S feed rate was changed from 0.0335 to 0.0451 mmol/min, while holding the LRT at 32 hour and the CO_2 flow rate at 0.0856 mmol/min.

In general, an increase in the H_2S feed rate increases the H_2S and CO_2 uptake rates, the cell concentration, and the CO_2 to H_2S uptake ratio as long as the CO_2 feed rate to H_2S feed rate ratio is somewhat above the theoretical CO_2 to H_2S uptake ratio for full sulfate production, 2.0. The excess CO_2 is required to overcome the dissolved CO_2 lost in the liquid purge. Once the H_2S feed rate is higher than this critical ratio, sulfide and sulfur begin to accumulate, and the CO_2 uptake rate, as well as the CO_2 to H_2S uptake ratio, reduce somewhat in response to the accumulation of sulfide.

Table 13. Effects of H_2S Feed Rate on Gas Uptake by *C. thiosulfatophilum*

H_2S Feed Rate (mmol/min)	CO_2 Conversion (%)	H_2S Conversion (%)	CO_2 Uptake (mmol/min)	H_2S Uptake (mmol/min)	CO_2/H_2S Uptake (Predicted)
0.0168[a]	8	99	0	0.0184	1.668
0.0251[a]	4	100	0.0010	0.0238	1.842
0.0335[b]	34	97	0.0290	0.0242	0.816
0.0358[b]	38	88	0.0355	0.0283	1.505
0.0369[b]	42	94	0.0516	0.0298	1.505
0.0392[b]	24	86	0.0479	0.0257	1.831
0.0415[b]	41	81	0.0508	0.0259	1.521
0.0427[b]	45	95	0.0564	0.0324	1.230
0.0451[b]	45	92	0.0671	0.0337	1.160

H_2S Feed Rate (mmol/min)	CO_2/H_2S Uptake (Actual)	Cells (g/L)	Sulfide (g/L)	Sulfur (g/L)	Sulfate (g/L)
0.0168[a]	0	0.327	0.01	43.5	429
0.0251[a]	0.042	0.421	0	20.2	515
0.0335[b]	1.198	0.749	5.93	190.7	150
0.0358[b]	1.254	0.299	12.27	9.7	59
0.0369[b]	1.732	0.676	1.11	72.2	450
0.0392[b]	1.864	0.255	12.50	5.4	85
0.0415[b]	1.961	0.320	14.90	17.3	57
0.0427[b]	1.741	0.472	7.88	67.8	136
0.0451[b]	1.991	0.465	14.64	64.7	144

LRT: 32 hr; [a]CO_2 feed rate: 1.136 mmol/min; [b]CO_2 feed rate: 0.0856 mmol/min

Operation with a Tungsten Light Source, and with Sulfur Removal. The studies with *C. thiosulfatophilum* that used an external light source with sulfur removal

employed a 1.7 L straight through CSTR. The feed gas was a blend of CO_2 and a gas mixture containing 97.5 percent N_2 and 2.5 percent H_2S. The light source was either a single 95 W or 100 W tungsten lamp, positioned just outside the reactor wall. External cooling using a simple fan was provided as needed to maintain the reactor temperature at 30°C. A section of reactor wall, located just in front of the external light source was scrubbed twice daily in order to re-suspend some of the cells and sulfur back into the fermentation broth and, more importantly, to keep a section of the reactor wall clear for the light source. Scrubbing was accomplished by the movement of two magnets, one on the inside and one on the outside of the reactor.

Effect of Sulfur Removal on Culture Performance at High Dissolved H_2S Levels (Experiment 1). An experiment was set up to determine how the removal of sulfur from the system using a sulfur settler affected culture performance at high dissolved H_2S concentrations. Culture was pumped into the sulfur settler at a rate of 60 ml/min. A second pump was used to pump the culture from the settler back to the reactor. The return pump speed was set slightly higher than the delivery pump to prevent overfilling of the settler. The culture was kept in sulfur production mode by using a reduced H_2S conversion. The cell retention time based on reactor volume only was 34 hr, and the LRT in the settler (900 ml volume) was 15 minutes. The gas retention time based on the reactor volume alone was 20 to 25 minutes. Sulfur was removed from the settler by drawing the sulfur sludge from the bottom of the settler twice per day.

Results from this experiment are shown as Experiment 1 in Table 14. The cell concentration was 0.079 g/L, the H_2S conversion was 69 percent and the CO_2 conversion was 13 percent. The CO_2 uptake rate was 0.0145 mmol/min and the H_2S uptake was 0.044 mmol/min. The sulfur concentration in the reactor was 17 ppm and the sulfate concentration was 67 ppm. The CO_2/H_2S ratio was 0.33, compared with the predicted CO_2/H_2S ratio of 1.34.

Table 14. Comparison of Steady State Culture Parameters Reached at Different Sulfur Settler Liquid Retention Times

Experiment	1	2	3[b]
Reactor Run Time (hr)	488	607	381
Settler Liquid Retention Time (min)	15	15	10
Cell Concentration (g/L)	0.079	1.09	0.695
H_2S Conversion (%)	69	89	84
CO_2 Conversion (%)	13[d]	11[d]	14[d]
Sulfur in Reactor (ppm)	17	29	21
Sulfate (ppm)	67	195	30↑150[c]
Sulfide (ppm)	na	8	11
Liquid Retention Time, LRT (hr)	51.8	109.5	106.7
Gas Retention Time, GRT(min)	19.5	22.9	25.0
Cell Retention Time, XRT (hr)	34	72	69.7
Cell Make Rate (g/day)	0.097	0.63	0.40

Table 14 (cont.). Comparison of Steady State Culture Parameters Reached at Different Sulfur Settler Liquid Retention Times

H_2Si Flow Rate (mmol/min)	0.074	0.071	0.064
CO_2i Flow Rate (mmol/min)	0.606	0.209	0.209
Culture Temperature (ºC)	29.7	29.8	30.4
Culture pH	7.26	7.06	7.03
Specific CO_2 Uptake (mmol/g•min)	0.123	0.01710	0.042
Specific H_2S Uptake (mmol/g•min)	0.39	0.035	0.038
Average CO_2 Uptake (mmol/min)	0.0145	0.028	0.0456
Average H_2S Uptake (mmol/min)	0.044	0.061	0.044
CO_2/H_2S Ratio	0.329	0.459	1.036
Predicted CO_2/H_2 Ratio	1.34	0.554	0.527

[a]Calculations did not include the sulfur collected from the settler.
[b]Culture was not at steady state.
[c]No average was taken. Concentration rose continuously throughout the indicated time period.
[d]Negative numbers were not included in the calculations

Changing Culture Conditions, Constant Settler Retention Time. An experiment was designed to observe changes in overall culture conditions when the LRT in the sulfur settler was held at 15 minutes, but other changes were made to the culture conditions. The H_2S conversions were held at a higher level than in Experiment 1. The overall liquid retention time of the whole system was increased to 110 hours and the cell retention time based on reactor volume only was increased to 72 hours. The gas retention time based on the reactor volume alone was approximately 23 minutes. In operating the settler system, sulfur was removed from the settler by changing out the settler approximately once per week with a new, clean settler filled with medium. Sulfur was then separated from the culture in the removed settler using a series of water washes. Once the sulfur was separated, it was dried and weighed. The overall average mass of sulfur collected per day was then added to the overall sulfur measurement used in the predicted CO_2/H_2S uptake ratio based on product concentrations.

Results from this experiment are shown as Experiment 2 in Table 14. The cell concentration was 1.09 g/L, the H_2S conversion was 89 percent and the CO_2 conversion was 11 percent. The CO_2 uptake rate was 0.028 mmol/min. and the H_2S uptake was 0.061 mmol/min. The sulfur concentration in the reactor was 29 ppm, the sulfate concentration was 195 ppm and the sulfide concentration was 8 ppm. The CO_2/H_2S ratio was 0.46, compared with the predicted CO_2/H_2S ratio of 0.55.

There were some big differences observed in the culture parameters in Experiments 1 and 2. When the Cell Retention Time (XRT) was increased to 72 hours and the LRT was raised to 110 hours in Experiment 2, the cell density increased nearly 14 times from 0.079 to 1.09 g/L. The sulfate concentration also rose considerably from 67 to 195 ppm. The sulfur concentration in the fermentation broth rose somewhat from 17 to 29 ppm. The H_2S uptake increased nearly 50 percent from 0.044 to 0.061 mmol/min., but the CO_2 uptake also increased from 0.0145 to 0.028 mmol/min. With the

large increase in cell density in the system without a similar increase in H_2S uptake, the amount of H_2S available per gram of cells dropped significantly as can be seen when comparing the specific H_2S uptake rates (0.39 mmol/min•gram in Experiment 1 and 0.035 mmol/min•gram for Experiment 2). This difference in specific uptake rates explains the large increase in sulfate production.

Reducing the Liquid Retention Time (LRT) in the Settler. The purpose of this experiment was to observe the change in overall culture conditions when the LRT in the sulfur settler was reduced to 10 minutes. The H_2S conversions, the overall liquid retention time of the system (110 hours) and the cell retention time based on reactor volume (72 hours) only were comparable to Experiment 2. Once again, the gas retention time based on the reactor volume alone was approximately 23 minutes. In operating the settler system, sulfur was removed from the settler by changing out the settler approximately once per week with a new, clean settler filled with medium. Sulfur was then separated from the culture in the removed settler using a series of water washes. Once the sulfur was separated, it was dried and weighed. The overall average mass of sulfur collected per day was then added to the overall sulfur measurement used in the predicted CO_2/H_2S uptake ratio based on product concentrations.

Results from this experiment are shown as Experiment 3 in Table 14. The cell concentration was 0.70 g/L, the H_2S conversion was 84 percent and the CO_2 conversion was 14 percent. The CO_2 uptake rate was 0.046 mmol/min. and the H_2S uptake was 0.044 mmol/min. The sulfur concentration in the reactor was 21 ppm, the sulfate concentration was 30 to150 ppm with a steady increase, and the sulfide concentration was 11 ppm. The CO_2/H_2S ratio was 1.04, compared with the predicted CO_2/H_2S ratio of 0.53.

Sulfur collection from the settler during Experiments 1 and 2 is shown in Table 15. The average sulfur collected rate for Experiment 2 was 0.687 mg/min. and the average for Experiment 3 was 0.685 mg/min.

In comparing Experiments 2 and 3, the major change to the fermenter between the two experiments was a decrease in the LRT in the sulfur settler from 15 to 10 minutes. The most significant change that was observed in reactor performance was a drop in the sulfur concentration from approximately 30 ppm to less than 10 ppm, and an increase in the sulfate level from about 30 ppm to a high of approximately 150 ppm. A comparison of the parameters listed in Table 11 shows very similar specific H_2S uptake rates, but a 100 percent increase in the specific CO_2 uptake rate. The cell density was lower for Experiment 3 by 36 percent, but this is most likely due to the lower H_2S feed rate. The increasing sulfate concentrations match well with the increased CO_2 uptake rate, which seems to indicate that a lower LRT in the settler improves CO_2 uptake. However, the culture instability experienced later in Experiment 3 makes this condition impractical.

Table 15. Sulfur Collection During Experiments 2 and 3

Settler Changes / Sulfur Analysis	Hours Collected	Sulfur Collected (mg)	Sulfur Collection Rate (mg/min)
Experiment 2			
Settler Change 2.1	162	No Data	No Data
Settler Change 2.2	168	5023	0.498
Settler Change 2.3	171	7463	0.727
Settler Change 2.4	169	7774	0.771
Experiment 3			
Settler Change 3.1	169	7593	0.744
Settler Change 3.2	192	8087	0.701
Settler Change 3.3	169	7774	0.771
Settler Change 3.4	166	7593	0.756
Settler Change 3.5	169	5961	0.590
Settler Change 3.6	170	6548	0.645
Settler Change 3.7	211	No Data	No Data
Settler Change 3.8	123	5713	0.771
Settler Change 3.9	174	10201	0.877
Settler Change 3.10	212	8573	0.746

TASK 2B. CONTINUOUS BIOREACTOR STUDIES WITH *T. CRUNOGENA*

Summary. Continuous bioreactor studies were also performed with *T. crunogena* with various sulfur sources such as Na_2S, Na_2SO_3 and thiosulfate. Na_2SO_3 proved to be the best sulfur source for the bacterium. However, operation of the *T. crunogena* system was more difficult than the *C. thiosulfatophilum* system, and thus the *C. thiosulfatophilum* system is preferred despite the need for external light.

T. crunogena was inoculated into a 2 L straight through CSTR (no cell recycle) from batch bottles, and then operated in a CSTR with CO_2 and H_2S as substrates for more than 4000 hours. The temperature was 30°C, the pH was 8.0, and the liquid and cell retention was 70 hours. The original medium formulation used sodium bicarbonate as the carbon source and thiosulfate as the energy source. However, the medium formulation was changed to use sodium sulfide (Na_2S) as the sulfur source because of its stronger reducing power. Carbon dioxide was used as the carbon source after about 650 hours from inoculation. Air was added to the reactor using a mass flow controller.

Medium Formulation for *T. crunogena* (weights and volumes of components per 3 L of medium)

Medium A

150.60 g NaCl
4.86 g NH_4Cl
7.43 g $MgCl_2 \cdot 6H_2O$
1.74 g $CaCl_2 \cdot 2H_2O$
2.52 g KH_2PO_4

1.20 mL Trace Metal Solution.
Medium B
Weight of component per 1 L volume
2.59 g Na_2S (60%)

Trace Metal Solution (weights of components per 1 L of medium)
50.00 g EDTA
22.00 g $ZnSO_4 \cdot 7H_2O$
5.54 g $CaCl_2$
5.06 g $MnCl_2 \cdot 4H_2O$
4.99 g $FeSO_4 \cdot 7H_2O$
1.10 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$
1.57 g CuSO4·5H2O
1.61 g $CoCl_2 \cdot 6H_2O$
Adjusted pH to 6.0 with KOH

The purpose of the reactor studies was to determine if *T. crunogena* will uptake CO_2 at a rate similar or better than *C. thiosulfatophilum*. Throughout the reporting period, changes were made to the agitation rate, the operating pH, the CO_2 flow rate, the O_2 flow rate, and the sulfide flow rate to determine the effects of these variables on culture growth and CO_2 uptake.

Effect of Agitation. A study was performed to assess the effect of agitation rate on gas (CO_2 and O_2) uptake and product concentration. The air feed rate was set at 27.3 ml/min. and the CO_2 flow rate was set at 5 ml/min., and the Na_2S flow rate was 0.002 mmole/min. The agitation rate was varied from 125 to 500 rpm (see Table 16). Although the pH was not very stable, the oxygen and carbon dioxide uptake rates generally increased with increased agitation rate, until an agitation rate of 500 rpm was reached, when dissolved oxygen accumulated in the liquid. The agitation was subsequently held at 400 rpm for the balance of the experimental study, the agitation rate yielding the highest CO_2 uptake and specific CO_2 uptake rates.

Table 16. Effect of Agitation Rate on the Performance of *T. crunogena*

Agitation Rate (rpm)	O_2 Uptake (mmole/m)	CO_2 Uptake (mmole/m)	Cell O.D.	Sulfur (ppm)	Sulfate (ppm)	Sulfide (ppm)
125	0.0173	0.0938	0.123	3.11	802	0
225	0.0211	0.0518	0.166	7.38	771	0.137
275	0.0264	0.0469	0.151	0.0	551	0
400	0.0210	0.1092	0.173	28.53?	570	0.07
500	0.0105	0.0876	0.214	3.84	506	0.011

Effect of pH. The effect of pH on gas uptake and product concentration was studied at a CO_2 feed rate of 10 ml/min. and an air rate at 25 ml/min. PH was studied in three ranges: pH 5.1 to 6, pH 6.5 and pH 7.8 to 8.0. Average results for these pH ranges are shown in Table 17. As is noted in the table, a pH range of 7.8 to 8.0 gave a

slightly better culture activity, maximizing both the CO_2 uptake rate and the sulfate concentration. However, the cell density (optical density, O.D.) measurement was not as high in this pH range, perhaps because more base solution was required to maintain this pH level and thus the resulting culture purge rate was higher.

Table 17. Effect of pH on the Performance of *T. crunogena*

pH Range	CO_2 Uptake (mmole/min)	Cell O.D.	Sulfide (ppm)	Sulfur (ppm)	Sulfate (ppm)
5.1-6.0	0.133	0.167	0	7.88	462
6.5	0.158	0.149	0.005	11.88	505
7.8-8.0	0.158	0.127	0	14.56	589

Effect of CO_2, Air and Sulfide Feed Rates. The air and CO_2 feed rates were initially set at 27.3 ml/min. and 10 ml/min., and the sulfide feed rate was initially set at 0.00219 mmole/min. After 760 hours at these conditions, the CO_2 flow rate was doubled. After another 56 hours, the Na_2S flow rate was nearly doubled to 0.00377 mmole/min and after an additional 1150 hours, the air feed rate was reduced from 27.3 to 11.3 ml/min. The Na_2S feed rate was further increased to 0.088 mmole/min after 400 additional hours, and the CO_2 feed rate was increased to 43 ml/min. after 350 additional hours.

Average CO_2 and sulfide uptake rates, cell optical densities, and sulfur and sulfate concentrations for these experiments are shown in Table 18. In general, an increase in the CO_2 feed rate will increase the CO_2 uptake rate. In increasing the CO_2 feed rate from 10 to 20 ml/min., the CO_2 uptake rate increased from 0.146 to 0.220 mmole/min, and the O_2 uptake rate nearly doubled from 0.021 to 0.041. However, this result is limited only to low CO_2 feed rates. When increasing the CO_2 feed rate from 20 to 42 ml/min., the CO_2 uptake also increased from 0.476 to 0.870 mmole/min. However, there was no obvious increase in the O_2 uptake rate in this case. There was no observed increase in the cell O.D. in either case, likely because the higher CO_2 feed rate will cause a higher culture purge rate due to more base solution being used, and thus a dilution of the cell concentration.

Table 18. Effect of CO_2, Air and Sulfide Feed Rates on the Performance of *T. crunogena*

Air (ml/ min)	CO_2 (ml/ min)	Na_2S Feed and Uptake (mmole/ min)	CO_2 uptake (mmole /min)	O_2 Uptake (mmole/ min)	CO_2/Na_2S mole/mole	Cell O.D.	Sulfur (ppm)	Sulfate (ppm)
27.3	10	0.00533	0.146	0.021	66.8	0.121	10.92	580
27.3	20	0.00533	0.220	0.041	100.5	0.103	27.15	558
27.3	20	0.00920	0.309	0.071	82.0	0.132	9.51	778
11.3	20	0.0104	0.378	0.064	100.6	0.127	0.12	727
11.3	20	0.0232	0.476	0.132	58.0	0.274	11.09	1620
11.3	42	0.0209	0.870	0.134	105.9	0.229	0	1431

An increase in the sulfide feed rate also resulted in an increase in the CO_2 uptake rate. The CO_2 uptake rate was increased from 0.220 to 0.309 mmole/hr when the sulfide feed rate was increased from 0.00219 to 0.00376 mmole/min, and the CO_2 uptake rate was increased from 0.378 to 0.476 mmole/min when the sulfide feed rate was increased from 0.00376 to 0.00821 mmole/min. Unlike the previous study, the cell O.D. also increased with increased sulfide feed, from 0.103 to 0.132 and from 0.127 to 0.274, respectively. Similarly, the O_2 uptake also increased from 0.047 to 0.071 and from 0.064 to 0.132 mmole/min, respectively. As expected, the sulfate concentration also increased with the increase in sulfide feed rate.

A decrease in the air feed rate from 27.3 to 11.3 ml/minute increased the CO_2 uptake rate from 0.309 to 0.378 mmole/hr. The O_2 uptake rate fell slightly from 0.071 to 0.064 mmole/hr and the cell O.D. fell slightly from 0.132 to 0.127. Decreasing the O_2 feed rate decreases the dissolved O_2 rate and therefore a reduction in the rate at which sulfide can direct react with O_2.

Analytical Procedures. Analytical procedures in using *C. thiosulfatophlum* were described earlier. However, in using *T. crunogena*, certain modifications to these analytical procures were necessary. Gas sampling for *T. crunogena* was performed on a SRI 310 gas chromatograph. The gas was analyzed for O_2, N_2, and CO_2. The carrier gas was helium with a flow rate of 30mL/min. The gas chromatograph was equipped with a 6-foot column containing silica gel accompanied by a 3-foot column containing a molecular sieve, both purchased from SRI Inc. The column oven temperature was held at 40°C for 1 minute, and then a temperature ramp of 10°C/min. was used for 9 minutes to reach a final temperature of 130°C. The total run time was 10 minutes. The sample size used was 1.0 mL. The cell density of *T. crunogena* was measured by optical density (OD) measurements of the bacterial culture at 580 nm using a Spectronic 20 Genesys spectrophotometer. A standard curve of cell density (from dry cell weight) vs. optical density was prepared. Beginning in September 2006, the sulfide measurements for all bacteria were performed using a methylene blue procedure. Sulfides react completely with excess dimethyl-p-phenylenediamine in the presence of ferric chloride to produce the intensely colored methylene blue compound. The OD of the methylene blue solution was read at 664 nm using a Spectronic 20 Genesys spectrophotometer. A standard curve of OD vs. methylene blue concentration was used to calculate the S^{2-} concentration.

Under most circumstances, all sulfide fed to the reactor is completely consumed by the culture. In general, no sulfide is detected in most of the liquid samples and no H_2S is detected in the gas, even when using Drager tubes, which can detect sulfide at concentrations as low as 1 ppm. Therefore, the sulfide consumption rate is essentially the same as the sulfide feed rate under most reactor conditions.

However, a significant amount of CO_2 is dissolved in the liquid and is carried out of the reactor by culture purge. Since the CSTR was operated at pH levels as high as 8.0, dissolved CO_2, present as HCO_3^-, will be appreciable and base usage at pH 8.0 will

be significant as well. A correction for the presence of dissolved CO_2 has been included in the CO_2 uptake rate. However, during this reporting period, base usage was not tracked and the culture purge rate was assumed to be the same as medium feed rate. These assumptions/shortcomings could underestimate the amount of CO_2 being carried out of the reactor by culture purge and overestimate the CO_2 uptake rate by the culture. Henceforth, base usage is tracked and used to correct the culture purge rate. In this report, base usage is estimated by a sodium balance. There are two inlet sodium sources for pH adjustment: Na_2S in Medium B and 10% NaOH, 2.5 mmole/ml, in the base solution. The inlet sodium rate should be equal to the outlet rate, which is equal to the culture purge rate, in ml/min, times the sum of the HCO_3^- concentration and two times the CO_3^{2-} concentration.

Assuming a base usage rate of X ml/min, the medium B rate in ml/min*2*sulfide concentration in mmole/ml + 2.5 mmole/ml *X ml/min = (Medium A+ Medium B + X) ml /min * (HCO_3^- + 2*CO_3^{2-}) mmole/ml. The Medium A and Medium B flow rates, and the sulfide concentrations are all known, and the HCO_3^- and CO_3^{2-} concentrations can be estimated from the dissolved CO_2 concentration, pKa_1, pKa_2, and the culture pH. The dissolved CO_2 concentration can be estimated from CO_2 concentration in the outlet gas, the CO_2 solubility at the reactor temperature and the assumption of an ideal solution. The base usage rate, X ml/min, will become the only unknown and can be solved for. The base usage rate was estimated to be about 5 percent of the total medium flow rate at a low CO_2 supply rate and about 20 to 30 percent of the total medium flow rate at a high CO_2 feed rate. This ratio should not be ignored in estimation of amount of CO_2 carried out by culture purge.

Correction in Gas Uptake. Previously, H_2S and CO_2 uptake were based on the difference between inlet and outlet GC measurements with N_2 as inert tracer gas. However, this method has some inaccuracies. Since both H_2S and CO_2 are highly soluble acid gases, significant gas are trapped in the liquid phase at high pH. Therefore, the actual gas uptake should be corrected for the amount of gas lost to the liquid purge.

To estimate the amount of trapped gases, both first and second dissociation constants of CO_2 and H_2S must be estimated. The liquid concentrations of H_2CO_3, HCO_3^-, and CO_3^{2-} can be calculated based on solubility, the measured liquid pH and the dissociation constants. [H_2CO_3] can be calculated from its solubility, [HCO_3^-] = [H_2CO_3]*K_{a1}/[H^+], and [CO_3^{2-}] = [HCO_3^-]*K_{a2}/[H^+]. Similarly, the concentrations of H_2S, HS^-, and S^{2-} can be determined. The concentrations of dissolved acid gases, H_2CO_3 and H_2S can be estimated based on solubility and the outlet gas concentration measured by GC. Therefore, each of the dissociated gas concentrations and the total trapped CO_2 and H_2S concentration in the liquid can be calculated, and the sum of all forms of dissociation can be found. The actual culture gas uptake will then equal the apparent uptake, estimated from the GC, minus the removed dissolved gas, that is, the culture purge rate times total trapped gas concentration.

As an example, at t = 10922.5 hr in Reactor S2, the reactor pH was 7.00 and the temperature was 29.5°C. Based on GC measurement, the H_2S and CO_2 uptake rates are 0.0304 and 0.0672 mmole/min. The pK_{a1} and pK_{a2} for H_2S are 6.562 and 17.072, the dissolved H_2S, HS^-, and S^{2-} concentrations are 0.0000771, 0.00027 and 0.0 gmole/L, and the total dissolved sulfide concentration = 0.00034 gmole/L (or mmole/ml). With purge rate (= medium rate) of 0.84 ml/min, the revised H_2S uptake is reduced from 0.0304 to 0.0301 mmole/min (about 1%). At same time, the CO_2 uptake rate based on GC measurement was 0.0672 mmole/min, the pK_{a1} and pK_{a2} for CO_2 are 6.12859 and 9.47535, the dissolved CO_2, HCO_3^-, and CO_3^{2-} concentrations are 0.0023272, 0.01731, 0.00006 gmole/L, and the total dissolved CO_2 concentration = 0.01969 gmole/L (or mmole/ml). With 0.84 ml/min culture purge, the actual CO_2 uptake rate is reduced from 0.0672 to 0.0507 mmole/min, a 24.6% reduction. The CO_2 to H_2S uptake ratio is reduced from 2.21 to 1.684. Compared to the prediction based on product ratio, the CO_2 to H_2S uptake is 1.755 (within 5 percent). Note that 1.684 is within 5 percent of the predicted, as opposed to 2.21 which is within only 25 percent of predicted.

In general, the correction is larger for CO_2 uptake than H_2S uptake. Usually, the H_2S conversion is high in the reactor, and therefore the dissolved H_2S concentration will be very small and may be even zero if 100 percent conversion is achieved. Therefore, the correction may be small or not even exist for H_2S. However, the CO_2 concentration in the outlet gas is high. Therefore, a higher dissolved CO_2 concentration is expected. Also, CO_2 has a lower dissociation constant than H_2S, which means there is more dissociated CO_2 than dissociated H_2S in the liquid.

The dissociation constants for CO_2 and H_2S can be estimated from the following equations and Table 19. Dissociation constants are a function of absolute temperature (T) and salinity (S). These relations can be expressed in the form of following equations:

$$pK_a = pK_a^0 + f_1 * S^{0.5} + f_2 * S$$
$$pK_a^0 = a_0 + a_1/T + a_2*T$$
$$f_1 = b_0 + b_1/T$$
$$f_2 = c_0 + c_1/T$$

Table 19. Estimating the Dissociation Constants of H_2S and CO_2

	a_0	a_1	a_2	b_1	b_2	c_1	c_2
pK_{a1} for H_2S (Note1)	-98.08	5765.4	15.0455	-0.1498	0.0	0.0119	0.0
pK_{a2} for H_2S	36.631	-0.0646	0.0	0.0	0.0	0.0	0.0
pK_{a1} for CO_2	-14.84	3404.71	0.03278	-0.0231	-14.346	6.9188×10^{-4}	0.429955
pK_{a2} for CO_2	-6.498	2902.39	0.02379	-0.4459	41.2405	0.0284743	-2.55895

Note 1: "The dissociation of hydrogen sulfide in seawater", Limnol. Oceanogr., 33(2), 269-274, 1988. Salinity assumed as 10.

Comparing the H_2S and CO_2 Uptake Rates to Predictions from Product Concentrations. In previous reports, the calculated gas uptake rates did not consider any dissolved gas removed by liquid purge, such that the gas uptakes were artificially high, especially CO_2. After correction for dissolved gas and acid gas dissociation in the

liquid, the true gas uptake rates can be estimated. Based on the true gas uptake rates, the ratio of CO_2 uptake to H_2S uptake is now in a more reasonable range. For H_2S conversion to sulfur, ½ mole of CO_2 will be converted per mole of H_2S uptake. For sulfate production, 2 moles of CO_2 will be consumed per mole of H_2S uptake. The CO_2 to H_2S uptake ratios are much closer to the predictions from the sulfur/sulfate ratio, especially when using average gas uptake rates. However, individual data points still show some scatter. Variations in temperature and pH, along with gas GC measurement limitations, all contribute to scatter in the gas uptake values.

Parametric Studies. Experiments were performed to determine if *T. crunogena* could use sulfide and sulfite as sulfur sources instead of the preferred sulfur source thiosulfate. *T. crunogena* is an aerobe that optimally uses thiosulfate and CO_2 for growth, and does not require light. CO_2 sequestration with *T. crunogena* would be a more viable option if the bacteria were found to be capable of using one of these alternate sulfur sources, and especially sulfite, along with its capability to function without light. The medium formulation for these studies was presented earlier in this report. Prior to March, 2007 the cell density of *T. crunogena* was estimated by OD measurements at 580nm using a Spectronic 20 Genesys spectrophotometer. Beginning in March, the Bradford protein assay was implemented for cell density measurements. This protein assay uses Coomassie brilliant blue G-250 dye, which binds to proteins such as arginine, tryptophan, tyrosine, histidine and phenylalanine. The anionic dyed protein is then read at 595 nm using the Spectronic 20. A standard curve of OD vs. protein concentration was used to calculate the amount of protein concentration in the sample. *T. crunogena* was used in a 2 L straight through CSTR (no cell recycle), and operated on CO_2 and sulfur compounds as substrates. The temperature was 30°C, the pH was 8.0, the agitation rate was 400 rpm and the liquid and cell retention was about 70 hr. CO_2 was the carbon source for growth, fed to the reactor as a mixture of air and CO_2.

Sodium Sulfide as a Sulfur Source. Attempts were made to use Na_2S as the source of sulfur by running the CSTR at a sulfide feed concentration of 0.008 mmol/min. Although the oxygen uptake initially averaged 0.09mmol/min., the CO_2 uptake increased to 1.0 to 1.1mmol/min and the cell density initially increased to 0.43 with Na_2S as the feed, these levels could not be maintained perhaps partially due to a feed gas delivery problem. Sulfate was the only product seen in the reactor. In viewing the culture under the microscope, the morphology did not match the known morphology of *T. crunogena*, and there were at least two (and possibly three) different cultures seen. These issues, along with the results from earlier sulfide feed trials from the last reporting period, suggest that *T. crunogena* cannot, or finds it difficult, to use sulfide as its sulfur source.

Sodium Sulfite as a Sulfur Source. The sulfur source was next changed to 24 g/L sodium sulfite (Na_2SO_3), a compound that is a prevalent industrial waste. Because of culture difficulties in the previous study with Na_2S, a fresh inoculum of *T. crunogena,* grown in batch bottles from a freeze-dried stock, was used in these experiments. Also, the Na_2SO_3 was oversupplied to ensure that sufficient substrate was available to the culture and was not merely chemically reacted with oxygen. For most of this

experiment, the culture did not grow. The cell density remained extremely low at <0.03g/L, and the only product measured was sulfate, which occurred as a result of a chemical reaction between oxygen and sulfite, rather than any activity from the culture. Even the measured oxygen uptake was shown to be from the chemical reaction. Thus, *T. crunogena* cannot grow using sulfite as its sulfur source.

Chemical Conversion of Sulfite to Sulfate. In changing the sulfur source to sodium sulfite, there was a strong suspicion that there was a chemical reaction between oxygen and sodium sulfite that formed sulfate. A similar reaction was seen and tested during the last reporting period between oxygen and sulfide. In order to verify this reaction, the CSTR was restarted using similar conditions of pH, temperature, liquid volume, medium components, concentrations and feed rate, and feed gas flow rates, but no culture was added. Gas samples and sulfate measurements were taken regularly to follow gas use and the chemical reaction to form sulfate.

For the first chemical reactor test, the CO_2 feed rate was held at 0.93mmol/min, and the O_2 feed rate was initially 0.1mmol/min. Within 47 hours, the sulfate concentration in the reactor was 2000 ppm, and within 300 hours, the sulfate level reached 15000 ppm. The sulfate concentration continued to increase until the end of the experiment. As Na_2SO_3 was added to the reactor, oxygen was reacting to form sulfate. Given enough time, the sulfate level in the reactor would have reached a steady state of 19050 ppm, if all of the sulfite had been converted.

Other parameters were also changed during the test. First, the oxygen feed rate was reduced to 0.08 mmol/min. for 50 hours. This drop in the O_2 feed rate had a small affect on the reaction by slowing down the production of sulfate. When the O_2 feed rate decreased, the measured sulfate concentration remained at about 5780 ppm, despite the fact that sulfite was still being added at 6.25 mg/min. Oxygen had to be the limiting factor. The medium flow rate was next increased, which dramatically increased the sulfate concentration. With increased oxygen and the increased sulfite concentration and feed rate, the sulfate concentration naturally increased more rapidly until reaching a steady state concentration. Another interesting result from this experiment was the conversions calculated for both CO_2 and O_2. The CO_2 conversion was relatively constant at 80 to 97 percent. Since CO_2 does not participate in the formation of sulfate, the CO_2 uptake consisted entirely of dissolved CO_2 leaving the system in the liquid. The oxygen conversion was initially about 30 percent, but this increased quickly once sulfite addition was increased from 0.25 to 0.75ml/min. By the end of the experiment, the oxygen conversion was 75 percent, all due to the conversion of sulfite to sulfate. Oxygen is only sparingly soluble in an aqueous solution.

In a second chemical reaction test, medium containing sulfite was added to the system all at once to speed up the experiment instead of waiting for the sulfite to bleed into the system with flow rate. The CO_2 conversion remained constant at 80 to 96 percent, as in Test #1. Again, this was due to CO_2 solubility rather than chemical reaction. The oxygen conversion began high at 73 to 84 percent, but soon the oxygen conversion dropped to 27 percent and then remained 14 to 20 percent for the balance

of the test. This was an indication that all of the sulfite that was initially in the reactor had finally been converted to sulfate within 24 hours from the start of testing. The continuing low oxygen conversion was due to the conversion of the low level of sulfite coming in with the medium flow. The average sulfate concentration in the reactor during this test was 3490 ppm. The first sulfate analysis was done about 20 hours after the test was started, so most of the sulfite that was going to react had already done so. However, if all the sulfite initially in the reactor (18 g/L) had changed to sulfate, the sulfate concentration should have been 6095 ppm, not 3490 ppm. Only 57 percent of the sulfite had reacted. One theory to explain this incomplete conversion is that the reaction between oxygen and sulfite to make sulfate, under the reactor-set conditions, has an equilibrium that leaves about 40 percent of the sulfite unconverted.

In a third chemical reaction test, sulfite was again added to the system all at once to speed up the experiment instead of waiting for the sulfite to bleed into the system with medium flow. The oxygen conversion was initially high at 90 percent until about 24 hours after the start of Test #3, when it dropped and stayed between 20 and 30 percent for the remainder of the test. Again, this was most likely caused by the initial conversion of all the sulfite that was in the reactor at the start of the test. After about 24 hours, all the convertible sulfite had converted, and O_2 was only needed to convert the sulfite in the medium flow. The CO_2 conversion was again initially high at near 100 percent, but this time it dropped steadily to about 65 percent. Because this test was run for 146 hours, the liquid in the reactor was probably saturated with CO_2, causing the overall uptake to drop once enough time had past. The sulfate measurements did not give a clear trend or stable concentration. The initial sulfate concentration was 4700 to 5000 ppm, and during the last half of the test, the sulfate concentration was 3700 to 7800 ppm. The average of all sulfate analyses was 5490 ppm. As in Test #2, if all of the sulfite had been converted to sulfate, the sulfate concentration should have been 9140 ppm. This 60 percent conversion to sulfate is very close to the 57 percent conversion seen in Test #2, indicating that there must be an equilibrium in the sulfite to sulfate conversion.

In a fourth chemical reaction test, the sulfite was once again added to the system one time. The CO_2 and oxygen feed rates were kept the same at 0.93 and 0.1 mmol/min., respectively. The oxygen conversion was very high initially at 68 to 77 percent. After 83 hours, the oxygen conversion dropped and held at 47 to 66 percent. This was not like the results of Tests #2 and #3. In both of the previous tests, the oxygen conversion dropped after about 24 hours to 20 to 30 percent, where it remained. Test #4 had a higher initial sulfite concentration and a higher concentration of sulfite in the medium than in Tests #2 and #3. It is likely that the O_2 conversion remained high, because the feed rate of sulfite more closely matched the conversion rate to sulfate based on the 0.1mmol/min O_2 flow rate. This time it took about 72 hours to reach a steady concentration of sulfate in the reactor (11594 ppm). If all sulfite were converted to sulfate, the concentration at steady state should have been 19048 ppm. As before, 61 percent of the sulfite was converted.

Tests #1 - #4 showed, without a doubt, that there is a chemical reaction between sulfite and oxygen under the conditions favorable to *T. crunogena*. All of the tests showed that there is an apparent equilibrium that will convert about 60 percent of the sulfite to sulfate. The tests also indicated that the oxygen feed rate is the limiting factor, so increasing the oxygen flow to the culture will only cause a faster conversion of sulfite to sulfate. However, due to the apparent equilibrium, not all of the sulfite will convert to sulfate. Thus, there should be some sulfite left over for the bacteria to use.

Thiosulfate as a Sulfur Source. After the failed attempts to grow *T. crunogena* on sulfate or sulfite, the sulfur source was changed to sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$). It has been well established that *T. crunogena* prefers thiosulfate as its sulfur source. The purpose of this experiment was to grow the culture on a preferred sulfur source in order to perform CO_2 uptake studies. The reactor was initially run in batch mode using *T. crunogena* grown in batch bottles from stock culture as the inoculum, and with 5g/L $Na_2S_2O_3 \cdot 5H_2O$ added to the medium as the sulfur source.

Initially the culture grew quickly and very well. The cell density reached nearly 0.7g/L within 48 hours and the oxygen uptake peaked at 0.05 mmol/min.; causing the oxygen conversion to hit 80 percent. Unfortunately, this was short-lived. Within 100 hours of inoculation, the oxygen uptake had reduced significantly to 0.02mmol/min. Within 300 hours, the cell density had dropped to <0.1 g/L where it remained for the rest of the experiment. The cause for this failure has been speculated to be the failure to turn on medium flows soon enough. However, once medium flow was established, the culture did not recover. The reason for the overall failure is unknown and most likely due to the lack of experience growing *T. crunogena*. Several additional attempts were made to grow *T. crunogena* using thiosulfate as the sulfur source, but all attempts failed.

TASK 3. PHOTOSYNTHETIC REQUIREMENTS

Summary. Finally, a near infrared light source was designed for use with *C. thiosulfatophilum* in the CSTR. Multiple 1 W LED lights were used as the light source, which provided more than adequate light to the culture to maintain cell growth. An arrangement of ten 1 W LEDs appeared to be optimum for the laboratory reactor. Key variables such as culture circulation rate, culture volume and time in light vs. time in dark were studied with these light delivery systems. The best and most economic method of light delivery was a flat panel light design with cyclic light addition of 50 to 55 seconds followed by about 13 minutes of darkness. CO_2 utilization was maximized in the *C. thiosulfatophilum* system by employing a more effective light source, cell recycle and sulfur recovery in a single reactor system. The best arrangement was to employ the cell recycle system after sulfur recovery, because of the tendency to accumulate sulfur in the cell recycle system.

A number of experiments were performed to improve light availability to *C. thiosulfatophilum* in or external to the CSTRs. Methods included the use of a higher intensity light, cleaning the walls of the reactor of culture build-up to allow better light

passage and adding cooling to a loop containing the light source. Experiments were conducted on infrared light sources to reduce photosynthetic power (cost) requirement.

In the first experiment, the 60 W bulb was replaced with a 95 W bulb, and the sulfur settler was covered with foil to prevent light from affecting the growth of the bacteria when it was present in the settler. The LRT was held at 34 hours, and the culture flow rate to the settler was 60 ml/min., thus yielding a 15 minute LRT in the settler. Results from this experiment are shown in Table 20. As is noted, the cell concentration, H_2S conversion, sulfur concentration and sulfate concentration increased, while the CO_2 conversion, sulfide concentration decreased.

Table 20. Effects of Light Intensity on Reactor Performance With Sulfur Settling

Variable	Baseline	Increased Light Intensity
Light Source (W)	60	95
Cell Concentration (OD)	0.20	0.26
CO_2 Delivered, mmol/min	0.515	0.518
CO_2 Conversion, %	14	13
CO_2 Uptake, mmol/min	0.0721	0.0673
H_2S Delivered, mmol/min	0.06	0.06
H_2S Conversion, %	59	66
H_2S Uptake, mmol/min	0.0354	0.0396
Sulfide Concentration, ppm	44	25
Sulfur Concentration, ppm	8	12
Sulfate Concentration, ppm	108	111

Reactor Volume: 1.7 L, Temperature: 30°C, LRT: 34 hr, same gas composition

The experiment was repeated, but without sulfur settling. Once again, the 60 W bulb was replaced with a 95 W bulb. The LRT was different for the two systems. Results from this experiment are shown in Table 21. As is noted, the cell concentration, H_2S uptake, sulfur concentration and sulfide concentration increased, while the H_2S and CO_2 conversions decreased.

Table 21. Effects of Light Intensity on Reactor Performance Without Sulfur Settling

Variable	Baseline	Increased Light Intensity
Light Source (W)	60	95
LRT (hr)	47	33
Cell Concentration (OD)	0.34	0.39
CO_2 Delivered, mmol/min	0.682	0.484
CO_2 Conversion, %	18	14
CO_2 Uptake, mmol/min	0.1173	0.0677
H_2S Delivered, mmol/min	0.02	0.04
H_2S Conversion, %	76	58
H_2S Uptake, mmol/min	0.0152	0.0232
Sulfide Concentration, ppm	14	24
Sulfur Concentration, ppm	85	89
Sulfate Concentration, ppm	n.a.	70

Reactor Volume: 1.7 L, Temperature: 30°C, same gas composition

In operating a reactor with *C. thiosulfatophilum* and a light source that was external to the reactor, but shining on the glass reactor wall; cell mass (and sulfur) accumulates on the walls of the reactor, especially the wall facing the external light source. This layer of cells and sulfur on the glass wall inhibits light transmission to the cells in the mixed reactor. A double magnet system (one outside the reactor and one inside the reactor) was used to help rid the reactor walls of cell biomass by acting as a scraper of cell debris. This double magnet system was used both in the CSTR connected to the sulfur settler and the CSTR with no settler. Cleaning the reactor wall once per day removes debris (cells, sulfur) that inhibit light transmission to the cellular biomass in the reactor. Tables 22 and 23 show data from the reactors with and without magnet cleaning systems. Both systems used a 95 W light source, and had LRTs of 34 to 35 hours. Cleaning resulted in less sulfate production, and slightly improved gas conversions. In the experiment with the sulfur settler, H_2S and CO_2 uptake increased 36 and 46 percent respectively. Whereas in the same experiment, but without sulfur settling (Table 20), H_2S and CO_2 uptake increased only 12 and 10 percent.

Table 22. Effects of Reactor Cleaning on Reactor Performance With Sulfur Settling

Variable	Baseline	With Cleaning
Cell Concentration (OD)	0.28	0.15
CO_2 Delivered, mmol/min	0.518	0.700
CO_2 Conversion, %	12	13
CO_2 Uptake, mmol/min	0.0621	0.0910
H_2S Delivered, mmol/min	0.055	0.068
H_2S Conversion, %	66	73
H_2S Uptake, mmol/min	0.0363	0.0496
Sulfide Concentration, ppm	20	0
Sulfur Concentration, ppm	12	21
Sulfate Concentration, ppm	144	69

Light Source: 95 W, Reactor Volume: 1.7 L, Temperature: 30°C, LRT: 34 hr, same gas composition, Settler LRT: 15 min

Table 23. Effects of Reactor Cleaning on Reactor Performance Without Sulfur Settling

Variable	Baseline	With Cleaning
Cell Concentration (OD)	0.45	0.20
CO_2 Delivered, mmol/min	0.484	0.452
CO_2 Conversion, %	23	27
CO_2 Uptake, mmol/min	0.111	0.122
H_2S Delivered, mmol/min	0.027	0.027
H_2S Conversion, %	64	72
H_2S Uptake, mmol/min	0.0173	0.0194
Sulfide Concentration, ppm	56	0
Sulfur Concentration, ppm	91	78
Sulfate Concentration, ppm	70	45

Light Source: 95 W, Reactor Volume: 1.7 L, Temperature: 30°C, LRT: 35 hr, same gas composition

Modifications were made to the light delivery system to improve delivery efficiency and to minimize culture deposition on the reactor walls. In the first modification, a 700 ml CSTR was set up to have a working volume of 550 ml. The volume of the external condenser was about 25ml and the flow rate was 200ml/min. Sulfur was not settled from the CSTR system. The condenser was about 12 inches tall with the spiral tube on the inside of the glass jacket shell. Two 95 W light bulbs shined on the condenser, one to cover the upper 6 inches of the condenser and the second to cover the remaining surface. Culture flowed through the inner loop of the condenser and cooling water was present in the jacket to offset any temperature rise. Results from this set-up, with and without water cooling, are shown in Table 24. Water cooling attenuated both the growth and gas conversions. H_2S flowrate was not able to be increased.

Table 24. Use of an External Light Contactor with and without Cooling

Variable	Without Cooling	With Cooling
Cell Concentration (OD)	0.47	0.16
CO_2 Delivered, mmol/min	0.504	0.525
CO_2 Conversion, %	12	19
CO_2 Uptake, mmol/min	0.0604	0.0997
H_2S Delivered, mmol/min	0.005	0.001
H_2S Conversion, %	76	69
H_2S Uptake, mmol/min	0.0038	0.0007
Sulfide Concentration, ppm	26	6
Sulfur Concentration, ppm	31	14
Sulfate Concentration, ppm	459	151

Light Source: 95 W, Reactor Volume: 0.7 L, Temperature: 30°C, LRT: 40 hr, same gas composition

Since deposits formed in the light loop and the loop could not be cleaned with the magnet system, the loop had to be removed every 2 to 3 weeks. This frequent change over resulted in poor culture performance each time the loop was replaced. To remedy this situation, a piece of “flat” glassware was custom designed by a glassblower that consisted of a 5 inch x 7 inch rectangle with ports on the top and bottom, at opposite ends and diagonal, to flow culture through from end to diagonal end and to shine light on both the top and bottom glass surfaces. It was hoped that this arrangement would increase the surface area of the culture being exposed to the light while minimizing shut down time for cleaning. Table 25 shows results from this light delivery device, when compared to the previous light loop design. With this new system, H_2S uptake increased more than three-fold, the cell concentration nearly doubled, indicating that the light for growth was effectively reaching the culture.

During the baseline experiment reported in Table 24 (without cooling) the circulation rate to the light contactor was 200ml/min., whereas in the experiment reported in Table 25, the circulation rate was increased to 400ml/min. The increased circulation rate permitted the H_2S uptake to increase three-fold, the CO_2 uptake rate increased two-fold and the cell concentration increased 21 percent. This verifies that

the culture has a light saturation point beyond which more light to an individual cell will not enhance performance. This also verifies that performance of the reactor can be improved by exposing more of the cell biomass to the light as seen by comparing the H_2S uptake of the two baseline experiments in Tables 24 and 25.

Table 25. Use of a New External Light Contactor

Variable	Baseline	New Light Delivery System
Cell Concentration (OD)	0.57	0.90
CO_2 Delivered, mmol/min	0.504	0.504
CO_2 Conversion, %	25	19
CO_2 Uptake, mmol/min	0.1260	0.0957
H_2S Delivered, mmol/min	0.014	0.063
H_2S Conversion, %	87	73
H_2S Uptake, mmol/min	0.0122	0.0460
Sulfide Concentration, ppm	n.a.	n.a.
Sulfur Concentration, ppm	21	51
Sulfate Concentration, ppm	285	351

Light Source: 95 W, Reactor Volume: 0.7 L, Temperature: 30°C, LRT: 40 hr, same gas composition

The photosynthetic reaction of the cell biomass is light limited, so any improvements to the amount of light delivered to the reactor improves performance. Increased light to individual cells does not improve performance. Increased wattage/intensity did improve performance. Maintaining transmittance into the reactor by periodically cleaning debris on the reactor wall, also improved performance. Increasing the surface area and thereby, the number of cells exposed to the light; improves performance as does increasing the liquid circulation rate to an external light contactor. So, clearly the reaction is limited by the light intensity, but also the light distribution throughout the cell population.

Near Infrared Light Source Design. A near infrared light source was designed for use with *C. thiosulfatophilum* in the CSTR. The device (shown in the appendix) operates normally at a current of 600 mA, generating a voltage of approximately 9 volts across the device. The voltage-current curve of a diode is exponential, making it much more stable to control diode current than diode voltage. In addition, the voltage across the device varies with temperature, making it all the more important to control the current in the device.

After reviewing many design options, including sophisticated feedback, the design settled on a very simple design that drops the voltage from a 12V plug-in power supply module down to 9V using a series resistor. This design does not offer precise current control, but does offer the advantages of high reliability, simple design and low component count. Adding a series fuse to the design assures that the device will not be operated at excessive voltage and current, thereby protecting the high-cost infrared source device at the expense of a low cost fuse element. For ease of configuration, a standard power connector is provided for the modular power supply to the black box.

The circuit from the 12V supply to the device is a 5 ohm power resistor network in series with a 1A fuse. Three wires come from the black box to the device assembly. Two wires run the infrared source, and a third wire provides 12V directly to a fan that runs any time the infrared source is on. The power supplied to the device is 9 volts times 600 mA, giving 5.4 watts. With a total radiated power of 1 watt, that leaves 4.4 watts dissipated as heat. This makes it necessary to use a heat sink and assure a flow of air across the device, which the fan accomplishes.

It was important to assess the potential hazards to human health from exposure to the light source. The radiant intensity of the device is 450 mW/steradian. The power received through a human pupil at a reference distance of one foot is calculated as follows:

The human pupil radius is 0.150 inches.
The angle from the center to the edge of a cone is $\tan^{-1}(0.15/12) = 0.72°$
corresponding to $2\pi[1-\cos(0.72°)] = 0.00049$ steradians.
Therefore, the power through the pupil of 0.00049*450 mW or 0.22 mW.

Note that standing further away than one foot from the source significantly reduces the exposure power according to an inverse square law relationship. Comparing the 0.22 mW with published safety standards indicates this is an acceptable exposure. There is a considerable body of findings and standards on the subject of laser safety. Much less information exists for diffused sources appropriate to this application, but it appears the exposure presented is acceptable.

In any case, it was deemed prudent to avoid prolonged exposure, prevent close-in exposure, and mitigate unknowing, accidental exposure through the use of appropriate warning signage. The device does have significant visible radiation, making it easy to tell when it is on. However, the intensity of light that is not visible is much greater. Since there is significant visible red light from the device, it is easy to tell when the light is turned on, making it mostly unnecessary to use a detector card. Also, the detector cards require charging with strong light before use. The cards work fine, but the charging requirement makes them more inconvenient to use.

Alternative Methods of Providing Light to the Reactor. In addition to providing tungsten light through the reactor wall for the growth of *C. thiosulfatophilum*, light may also be delivered to the culture through a lighted external loop. A condenser type apparatus and a flat glass window are two alternatives that were described above. The following experiments show comparisons of these external light delivery systems.

Comparison of the Condenser Light Window to the Flat Glass Window in the External Light Loop Reactor. An experiment was performed to compare culture performance using two different light window configurations within the culture light loop using the same light source. The bioreactor was a 1L straight-through CSTR. The feed gas was a blend of pure CO_2 and a mixture containing 97.5% N_2 and 2.5% H_2S. The

light source was a culture loop in which culture was circulated from the reactor, through a "window" and then back into the fermenter. Aluminum foil covered the reactor to block any other external light source to the reactor. The culture was maintained in the sulfate production mode as much as possible to maximize cell growth and CO_2 uptake.

For the first 2500 hours of operation, the light window was a 4 cm dia. x 38 cm distillation condensing coil, and the light source was two 95 W bulbs. One bulb was placed at the top of the condenser, and the other bulb was placed 180º from the first bulb and at the bottom of the condenser. Initially the culture was circulated through the condenser at approximately 200 ml/min flowing from the bottom to the top. After 1700 hours in this configuration, the circulation rate was doubled to approximately 400 ml/min., where it remained for the next 850 hours. For the last 400 hours of operation, the light window was a 12cm x 20cm x 2cm flat glass window, again with a light source of two 95 watt bulbs. One bulb was placed on either side of the flat glass. Culture was circulated through the condenser at about 400ml/min. initially flowing from the bottom to the top. After 125 hours, the circulation rate was increased.

Results from the window configuration study are shown in Table 26. As expected, most of the product was present as sulfate, so the culture was in high CO_2 uptake mode (two times the sulfide uptake rate). The average H_2S uptake rate when using the condenser light window was 0.0105 mmol/min. and the average CO_2 uptake rate was 0.0532 mmol/min. For the flat light window, the average H_2S and CO_2 uptake rates were 0.0433 and 0.0989 mmole/min., respectively. Obviously, the flat light window configuration is much better than condenser light configuration in providing light to the culture. Also, the CO_2 to H_2S uptake ratio during operation with the flat configuration, 2.28, was much closer to the predicted ratio, slightly below 2.0. The correction for dissolved gases in liquid phase was also included in the cylinder light window calculation. However, the pH for calculation was based on pH controller values, instead of actual liquid sample values. This might have led to the unreasonably high CO_2 to H_2S uptake ratio of 5.07. The maximum cell concentration during the experiment with the condenser light window was about 0.1 g/L. After the change to the more efficient flat light window, the maximum cell concentration increased to about 0.5 g/L.

Table 26. Effect of Window Configuration in the Light Loop on the Performance of *C. thiosulfatophilum*

Window Configuration	H_2S Uptake (mmole/min.)	CO_2 Uptake (mmole/min.)	Cell Conc. (g/L)
Condenser	0.0105	0.0532	0.1
Flat Window	0.0433	0.0989	0.5

Use of an LED Source with the Flat Glass Window. An experiment was performed for 1400 hours using a 1 W light emitting diode (LED) with a wavelength of 760 nm as the light source on the flat glass window in the external light loop reactor. The purpose of this experiment was to determine how the culture performs with a light source closer to the exact wavelength required by the bacteria. If so, could a lower wattage source then be used, therefore lowering the power requirement for the reactor?

Also, knowing the exact wattage with the exact wavelength required by the bacteria, calculations can be made to determine light requirement per gram of cells in the system.

Once again, the bioreactor was a 1L straight-through CSTR. The feed gas was a blend of pure CO_2 and a mixture containing 97.5% N_2 and 2.5% H_2S. The light source was a culture loop in which culture was circulated from the reactor, through a "window" and then back into the fermenter. Aluminum foil covered the reactor to block any other light source other than through the light loop window. The light window was the flat glass window, and the light source was one 1 W LED at 760nm. The culture circulation rate was 490ml/min. through the light loop.

Results from the LED study in comparison to the two 95 W bulbs, both shining on the flat plate, are shown in Table 27. When compared to the two 95 W light bulbs experiment, the 1 W LED required only 0.5 percent of the energy, but gave 12.7 percent of the average H_2S uptake. Thus, the LED light efficiency was about 25 times the normal light bulb. The maximum cell concentration for the LED was about 0.15 g/L, or 30 percent of the cell concentration from the two 95 W bulbs. Thus, the cell growth efficiency was about 60 times the normal light bulb.

Table 27. Effect of Light Source in the Flat Window Light Loop on the Performance of *C. thiosulfatophilum*

Light Source	H_2S Uptake (mmole/min.)	CO_2 Uptake (mmole/min.)	Cell Conc. (g/L)
1 W LED	0.0055	0.0104	0.15
Two 95 W Bulbs	0.0433	0.0989	0.5

Use of Multiple LED Sources with the Flat Glass Window. An experiment was performed using ten 1 W LEDs with a wavelength of 760 nm as the light source on the flat glass window in the external light loop reactor. The purpose of this experiment was to determine the maximum CO_2 uptake and cell density that could be achieved using ten LEDs as compared to only one LED. Once this information is known, exact light requirements for the system can then be calculated.

Once again, the reactor was a 1L straight-through CSTR. The feed gas was a blend of pure CO_2 and a mixture containing 97.5% N_2 and 2.5% H_2S. The light source was a culture loop in which culture was circulated from the reactor, through a "window" and then back into the fermenter. Aluminum foil covered the reactor to block any other light source other than through the light loop window. The light window was the flat glass window, and the light source was ten 1 W LED at 760nm. The culture circulation rate was 490ml/min through the light loop.

Results from the LED intensity study are shown in Table 28. The average specific CO_2 and H_2S uptake rates for the ten 1 W LEDs were 0.0874 and 0.0486 mmol/min per gram of cells, respectively, for a ratio of 1.798 to 1 for CO_2 : H_2S uptake. The ten LEDs gave a maximum H_2S uptake that was about 3.89 times the uptake of 1 LED. The maximum cell concentration was also the same as the two 95 W bulbs, 0.5

g/L, or 3.33 times 1 LED. This information indicates that, with ten 1 W L.E.Ds, light is already over supplied. There is some other factor limiting CO_2 uptake.

Table 28. Effect of Light LED Intensity in the Flat Window Light Loop on the Performance of *C. thiosulfatophilum*

Number of 1W LEDs	H_2S Uptake (mmole/m)	CO_2 Uptake (mmole/m)	Cell Conc. (g/L)
1	0.0055	0.0104	0.15
10	0.0874	0.0486	0.5
Two 95 W Bulbs	0.0433	0.0989	0.5

Operation with an External Light Loop. A CSTR without cell recycle was used to study the effect of an external light loop on culture performance. At the beginning of the reporting period, the experiments were a continuation of experiments previously performed with the flat glass, light exposure cell (dimensions of 12 cm x 20 cm x 2 cm). The light source was ten 1-W LEDs, with a light wavelength of 760 nm. The dimensions of the light source were the same as the flat glass window, 12 cm x 20 cm. Later in the reporting period, after the flat glass window was damaged by pressure build up, a cylindrical light shining device, or window, was used.

During all of the experiments, the reactor design had two components, the light loop containing a "window" for light transfer and the light source, and a CSTR for temperature control, pH adjustments, medium and feed gas additions, and agitation. Culture was continuously pumped from the reactor into the bottom of the light "window", and then back into the CSTR at varying flow rates. When the light source was on the light loop, the CSTR was covered in aluminum foil to prevent ambient light from increasing the light addition. In the CSTR, the temperature was maintained at 29-30°C. The culture volume was initially held at approximately 450 ml, and the agitation rate was held at 300-500 rpm. The feed gas was a blend of pure CO_2 and a 2.5% H_2S:97.5% N_2 mixture. The medium feed was the Green Sulfur Bacteria medium, a mixture of salts, trace metals, and vitamin B_{12} that was described previously. The initial medium feed rate was 290 ml/day. This rate was later increased to 580 ml/min to compensate for a larger light-loop culture volume.

Circulation Rate Studies. Table 29 shows the effect of culture circulation rate on the performance of *C. thiosulfatophilum* when using the flat glass window for light transfer. The use of the faster circulation rate increased the average cell concentration from 0.614 to 0.738 g/L. The H_2S uptake increased slightly from 0.0459 to 0.0467 mmole/min, a 7.7% increase. However, the CO_2 uptake fell significantly from 0.0576 to 0.0273 mmole/min. The product ratio also confirmed the reduction in CO_2 uptake, with the average sulfur concentration increasing from 23 to 75 ppm, and the average sulfate concentration falling from 260 to 123 ppm. Only 0.5 moles of CO_2 are consumed when one mole of sulfur is produced by *C. thiosulfatophilum*, but 2 moles of CO_2 are consumed when one mole sulfate produced. The actual and predicted CO_2/H_2S uptake ratios were reduced from 1.26 and 1.72 to 0.639 and 1.18, respectively.

In addition to the increase in the culture circulation rate, the H_2S feed rate was also slightly increased in the experiment from 0.0679 to 0.0731 mmole/min. As a result, the CO_2 uptake was significantly reduced. An increase in the H_2S feed rate might result in this kind of product shift due to a higher dissolved sulfide concentration in the fermenter. However, the average sulfide concentration actually fell from 7.6 to 2.4 ppm, indicating the shift was not from increasing the supply of H_2S. The increased circulation rate might have reduced the effectiveness of the light supply by reducing the length of time the culture is exposed to light. Even though the light that was provided was exactly the same for the two experiments, the faster circulation rate shortened the time that individual cells had to absorb the light each time the cells passed through the light window. With a higher circulation rate, the total number of times the cells passed through the light window also increases. So, with the shorter exposure time, the overall exposure time should be the same since the light supply is the same. However, the results from the two experiments indicate that there is a minimum exposure time needed by the culture in order for the light source to be effective. The low sulfide concentration might indicate that the minimum light exposure time for sulfate production is much longer than for sulfur production.

Table 29. Effect of Culture Circulation Rate on *C. thiosulfatophilum* When Using the Flat Glass Window for Light Transfer

Circulation Rate, ml/min	**472**	**574**
Reactor Run Time, hr	1109	194
Fermenter/Total Volume, ml	450/800	450/800
Cell Concentration, g/L	0.614	0.738
H_2S Conversion, %	88.37	86.65
CO_2 Conversion, %	19.47	9.680
Sulfur, ppm	23.2	75.0
Sulfate, ppm	260	123
Sulfide, ppm	7.61	2.42
Liquid Retention Time,hr	67.0	66.5
Gas Retention Time, min	11.2	10.3
H_2S Feed Rate, mmole/m	0.0679	0.0731
CO_2 Feed Rate, mmole/min	0.223	0.235
Specific CO_2 Uptake Rate, mmole/g min	0.124	0.0498
Specific H_2S Uptake Rate, mmole/g min	0.0646	0.532
Average CO_2 Uptake Rate, mmole/m	0.0576	0.0273
Average H_2S Uptake Rate, mmole/m	0.0459	0.0467
CO_2/H_2S Ratio	1.26	0.639
Predicted CO_2/H_2S Ratio	1.72	1.18

Culture Volume Studies. Table 30 shows the effects of culture volume on the performance of *C. thiosulfatophilum* as the culture level in the CSTR was increased from 450 to 600 ml, thereby reducing the frequency of light exposure from 1.33 to 1.60 times/min. by increasing the length of time the culture spent in the dark. The culture circulation through the light loop was held at 575-600ml/min. The average H_2S supply

rate was reduced from 0.0731 mmole/min to 0.0638 mmole/min, a 13 percent reduction. These actions enhanced the average CO_2 uptake rate from 0.0273 to 0.0619 mmole/min and slightly lowered the H_2S uptake rate from 0.0467 to 0.0423 mmole/min even though culture circulation rate did not change. Both the actual and predicted CO_2/H_2S uptake ratios agreed with this shift, increasing to 1.52 and 1.59, respectively. The sulfur concentration fell from 75 to 25 ppm while the sulfate concentration increased from 123 to 193 ppm. It is not clear why the CO_2 uptake increased as much as it did. The culture circulation was kept as high as 600 ml/min, so only the exposure time was reduced. Perhaps the reduction of 13% in the H_2S feed rate might also have had some effect.

Table 30. Effect of Culture Volume on *C. thiosulfatophilum* When Using the Flat Glass Window for Light Transfer

Fermenter/Total Volume, ml	**450/800**	**600/950**
Reactor Run Time, hr	194	480
Circulation Rate, ml/m	574	600
Cell Concentration, g/L	0.738	0.752
H_2S Conversion, %	86.65	85.23
CO_2 Conversion, %	9.680	12.12
Sulfur, ppm	75.0	25.4
Sulfate, ppm	123	192
Sulfide, ppm	2.42	5.38
Liquid Retention Time,hr	66.5	78.2
Gas Retention Time, min	10.3	14.3
H_2S Feed Rate, mmole/m	0.0731	0.0638
CO_2 Feed Rate, mmole/min	0.235	0.235
Specific CO_2 Uptake Rate, mmole/g min	0.0498	0.0893
Specific H_2S Uptake Rate, mmole/g min	0.532	0.0545
Average CO_2 Uptake Rate, mmole/m	0.0273	0.0619
Average H_2S Uptake Rate, mmole/m	0.0467	0.0423
CO_2/H_2S Ratio	0.639	1.52
Predicted CO_2/H_2S Ratio	1.18	1.59

Further Reductions in the Culture Circulation Rate. In another experiment, the culture circulation rate was further reduced by half, from 600 to 293 ml/min (see Table 31). The fermenter volume was still kept high, at 600 ml, giving a total culture volume of 950 ml. However, the H_2S feed rate was increased from 0.0638 to 0.0784 mmole/min, a 23 percent increase. As a result the cell concentration fell from 0.753 to 0.687 g/L. The average CO_2 uptake rate fell from 0.0619 to 0.0505 mmole/min, while the average H_2S uptake rate increased from 0.0423 to 0.0525 mmole/min. Accompanying the higher H_2S uptake and lower CO_2 uptake, the average sulfur concentration slightly increased from 25 to 35 ppm, and the sulfate concentration fell from 193 to 132 ppm. The actual and predicted CO_2/H_2S uptake ratios also confirmed the shift in products. This time, a 23 percent increase in the H_2S feed rate probably helped the product shift since the sulfide concentration also increased slightly. The intensity of the product shift was not as large as the shift seen in the second

experiment. Cutting the circulation rate should have helped increase the CO_2 uptake, but the increase in the H_2S feed rate may have caused the CO_2 uptake to drop.

Table 31. Effect of a Further Reduction in Culture Circulation Rate on *C. thiosulfatophilum* When Using the Flat Glass Window for Light Transfer

Circulation Rate, ml/m	**600**	**293**
Reactor Run Time, hr	480	223
Fermenter/Total Volume, ml	600/950	600/950
Cell Concentration, g/L	0.752	0.687
H_2S Conversion, %	85.23	87.78
CO_2 Conversion, %	12.12	17.39
Sulfur, ppm	25.4	35.0
Sulfate, ppm	192	132
Sulfide, ppm	5.38	5.68
Liquid Retention Time,hr	78.2	81.0
Gas Retention Time, min	14.3	11.4
H_2S Feed Rate, mmole/min	0.0638	0.0784
CO_2 Feed Rate, mmole/min	0.235	0.235
Specific CO_2 Uptake Rate, mmole/g min	0.0893	0.0734
Specific H_2S Uptake Rate, mmole/g min	0.0545	0.0784
Average CO_2 Uptake Rate, mmole/min	0.0619	0.0505
Average H_2S Uptake Rate, mmole/min	0.0423	0.0525
CO_2/H_2S Ratio	1.52	1.00
Predicted CO_2/H_2S Ratio	1.59	1.45

System Redesign. Soon after the conclusion of the last experiment in Table 31, the flat glass window was broken due to pressure buildup in the reactor. Subsequent experiments were carried out using a borosilicate, glass column with dimensions of 25 mm ID x 450 mm effective length. The column had three hose connections, one at the bottom of the column, and two at the top, one on top of the other (Figure 4). Culture was pumped into the bottom hose connection, and then it overflowed back into the reactor through the lower of the two top ports. The upper top port was connected to the CSTR head plate as a pressure relief point to prevent pressure buildup in the column.

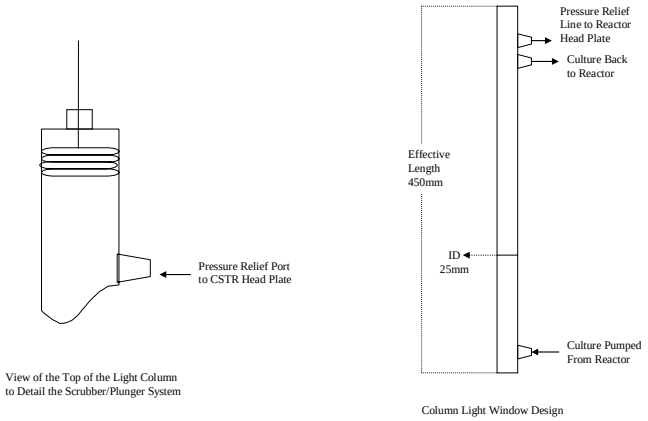


Figure 4. Design of the Column Light Window and Scrubber/Plunger System

The light source was changed to eight 1-W 760 nm LED setup with two rows of four LEDs each. The LEDs were mounted in a rectangular aluminum box, open at the top and bottom (Figure 5). A scrubber/plunger system was installed inside the column to clean the cell/sulfur/debris buildup normally seen in the *C. thiosulfatophilum* reactors. The scrubber consisted of several layers of flexible, thin Teflon® sheets, stacked and held together with nuts on a 1/8-inch stainless steel rod which was threaded on the bottom. The rod fit through a bore-through 1/8-inch Swagelok® to ¼ inch NPT connector. The rod was sealed against gas leaks, in or out, using a Teflon® ferrule and nut. The seal on the rod could be loosened when needed, so that the scrubber could be plunged the length of the column to remove buildup. Once the column was scrubbed, the scrubber was positioned at the top of the column and the rod was resealed.

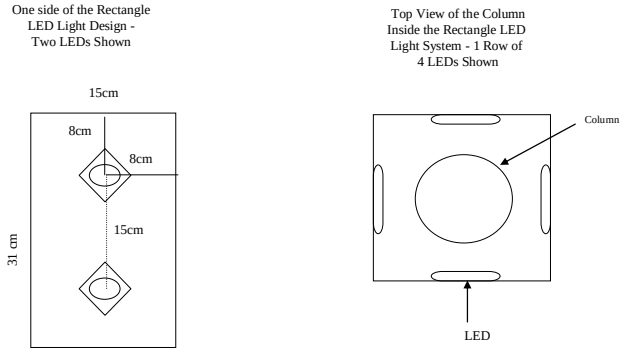


Figure 5. Design and Dimension of the L.E.D. Light Source System

Between April 25 and June 12, 2007, many attempts were made to start the new reactor setup with the column light window. Several combinations of tungsten bulbs and/or LEDs as the light source were tried, with and without culture circulation through the column. Inoculation was tried as a continuous stream of culture and as several high doses of culture all at once. Modifications were made to the pressure relief port system on the column and the plunger/scrubber system to prevent possible air contamination. However, none of these modifications permitted a successful startup. The culture would sustain or even improve on gas uptake as long as it was continuously inoculated, but when inoculation was stopped, the gas uptake and cell density steadily dropped. On June 12, 2007, the CSTR was changed from a 0.75 L capacity to a 1.8 L capacity vessel.

With the new CSTR in place, gas uptake was easily maintained as long as a tungsten bulb was used for the light source. However, when the tungsten bulb was turned off and the LEDs were the only light source, the cell density and gas uptake declined. Different culture circulation rates (300, 150, 450, and 276 ml/min) through the light column were tried. The culture volume in the reactor was also reduced from 1700 ml to 1100 ml to reduce the length of time the culture stayed in the dark. The reactor was inoculated many times to restart the culture, but regardless of the operating parameters, the culture would die out whenever the LEDs were the only light source.

Improved External Light Source. The column "window" and eight LED system was further tested in an effort to determine why the culture would not grow. For all experiments, the reactor was run as a straight through CSTR. The reactor/light source design had two components: the light loop containing a "window" for light transfer and the light source, and a CSTR for temperature control, pH adjustments, medium and feed gas additions, and agitation. Culture was continuously pumped from the reactor into the bottom of the light "window", and then back into the CSTR. When the light source was on the light loop, the CSTR was covered in aluminum foil to prevent ambient light from increasing the light addition. In the CSTR, the temperature was maintained at 29-30°C. The culture volume was held at approximately 800 mL, and the agitation rate was held at 300 rpm. The feed gas was a blend of pure CO_2 and a mixture of 2.5% H_2S and 97.5% N_2. The medium feed was the Green Sulfur Bacteria medium, a mixture of salts, trace metals, and vitamin B_{12} that was described previously. The medium feed rate was about 430 ml/day.

Further attempts were made to grow the culture. Culture circulation rates through the external light loop of 150 mL/min and then 75 mL/min were tried, but the culture did not survive when the only light source was the eight 1-watt LED setup. When the 100 watt tungsten bulb was used, aimed at the CSTR, the grew. These were the same results seen in all previous attempts.

The next approach was to move the LED light box close to the column so that the column was no longer centered between the eight LEDs. The column was pushed nearer to one corner of the aluminum box so that four LEDs were very close (see Figure 6). It was thought that perhaps this configuration would provide better light penetration into the culture. The culture flow rate was held at 75 mL/min. Unfortunately this attempt failed as well.

One possibility for the difficulties was that is it necessary for the light source and H_2S supply to be in close proximity. To test this, the eight LED system was replaced by a 100-watt tungsten bulb directed at the column "window". No other culture conditions were changed. With this arrangement, the culture was able to maintain without losing cell density, thus proving that the separation of light and sulfide was not the issue.

The eight 1-watt LED system essentially provided two points of light contact with the culture. All previous, and successful, attempts to grow the culture with an external light loop had used light sources (tungsten bulb or ten LEDs) that provided a much larger light contact area for the culture. It was possible that all the difficulties with the current design were due to the need for a larger light delivery area. To test this, the eight LED system was exchanged for the ten 1-watt LED setup used in previous experiments with the flat glass "window", while keeping the column light exposure device. The ten LED light source was positioned so that maximum spread of the LEDs on the column was achieved (see Figure 7). This time the culture grew, and increased uptake of H_2S and CO_2 were seen with gas flow rate increases.

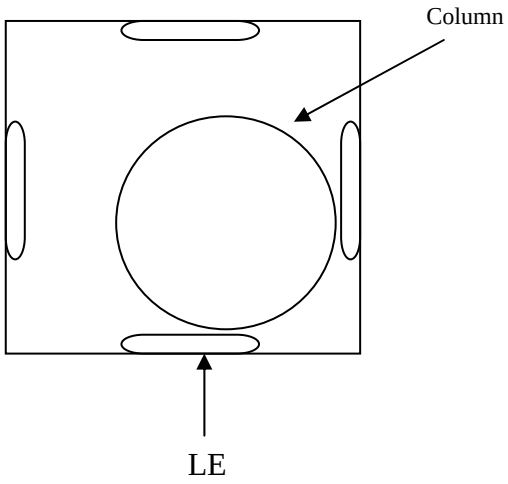
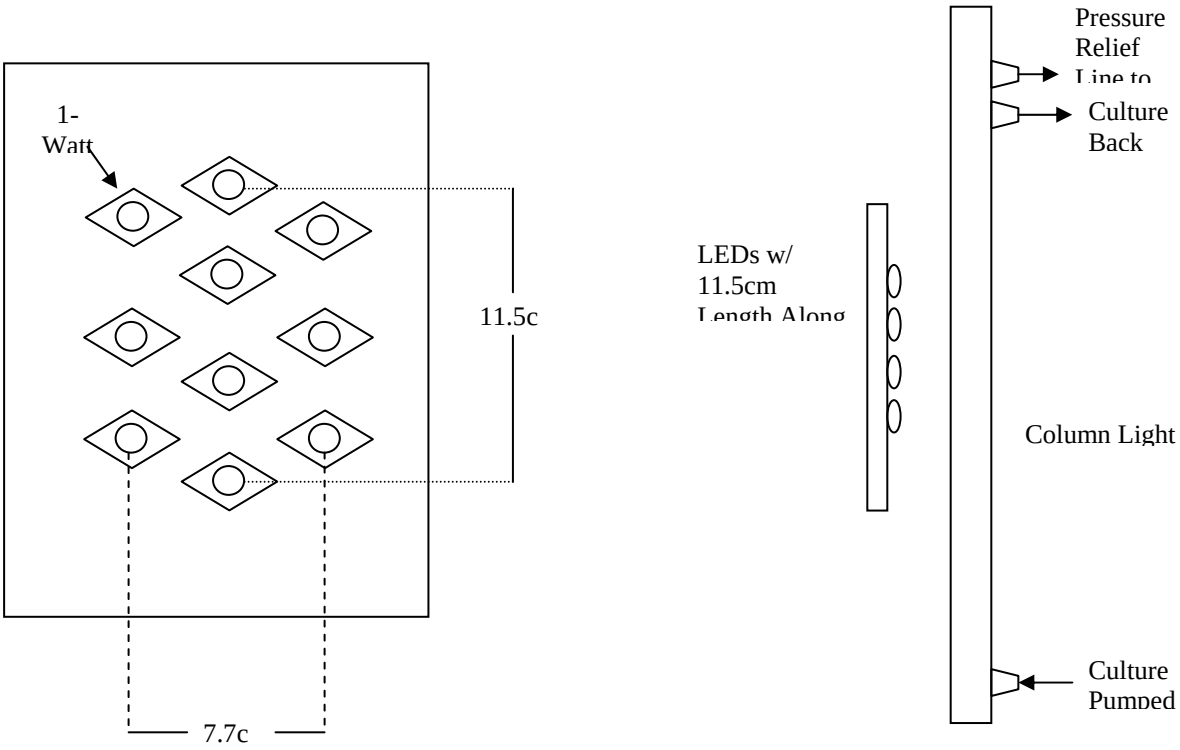


Figure 6. Position of Column with Four of Eight LEDs Moved for Maximum Light Penetration—Top View



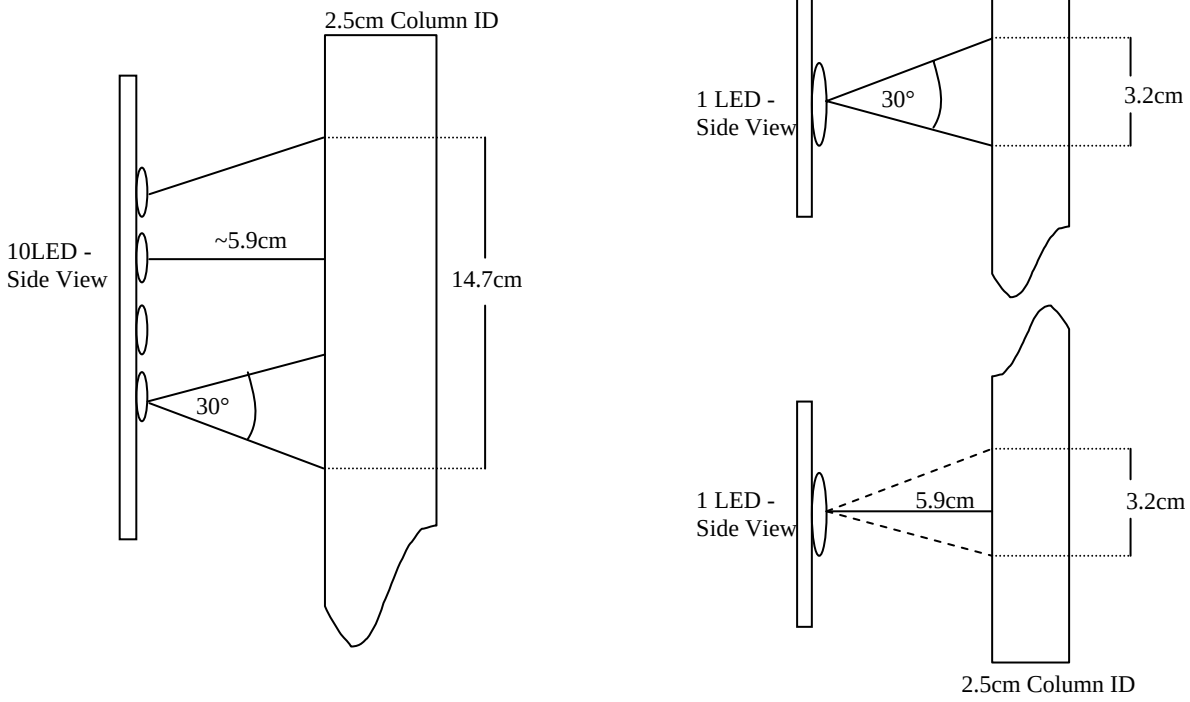
Ten 1-Watt LED Design

Figure 7. Orientation of the Ten 1-W LED Light Source and Column "Window"

Most of the experiments used the column light "window" and eight LED light source design. All attempts to grow the culture using this design failed despite changes in culture flow rate to the column, culture volume in the reactor and gas flow rate changes. The only time the culture could be maintained was when the light source was changed to a tungsten bulb either on the CSTR or column "window". Since the culture

was sustainable with the tungsten bulb on the column as well as reactor, it can be said that the separation of light source and sulfide source is not a problem. This also indicates that the problem was the eight LED setup. The eight LEDs were configured so that the light was delivered in two concentrated points of 4 watts each. This proved to be insufficient light delivery when the culture flow rate past those points of light was 75 mL/min or higher. However, once the light source was changed to the ten LED setup previously used with the flat glass "window", the light delivery area was greatly increased from two point two an area of roughly 89cm^2 (11.5cm x 7.7cm, see Figure 8, left). This light delivery setup proved to be successful, so either the light source must be spread out to allow more time for photon uptake or a 25% increase in light wattage was necessary. Since the culture not only maintained with the ten LED setup but also withstood gas flow rate increases, this indicates that all culture growth difficulties were due to the light source configuration.

At a culture flow rate of 75 mL/min, when the light source was changed from the eight LED to the ten LED setup, the time the culture was in the light increased from two points of 12.4 seconds of contact each to 54.9 seconds of continuous light contact. This was a 120% increase in overall time in the light. The time in light was calculated using the length of the column in the light path (14.7 cm for the ten LEDs and 6.32 cm for the eight LEDs), culture volume in the light path was calculated using column ID and the light path length (68.72cm^3 for the ten LEDs and 31.04 cm^3 for the eight LEDs) and culture flow rate of 75 ml/min. There are two possibilities as to why to eight LED light source did not work. The first possibility is that a total light contact of more than 24.8 seconds is needed by the culture. The second possibility is that the culture requires continuous contact longer than 12.4 seconds to achieve the full necessary photon loading, so even if there are two light exposures adding up to 24.8 seconds, each exposure time may have been insufficient to provide the photons needed before the culture left the light exposure area.



Dimensions for the 10-LED and Column Setup

Dimensions for the 8-LED and Column Setup

Figure 8. Dimensions Used to Calculate the Culture Time in the Light for the Eight and Ten LED Light Sources with the Column Window

The ten LED light source proved to be adequate for culture survival. With a CSTR volume of 800 ml, a light column culture volume of 177 ml, and approximately 50 ml of culture in the circulation tubing, the total approximate culture volume in the system was 1027 ml. A circulation rate of 75 ml/minute gives a 13.69 minute turnover rate of culture through the system. For the ten LED setup, the culture was in the light for 55 seconds and in the dark 766.6 seconds or 12.78 minutes. The culture was able to survive when the light contact was only 6.7 percent of the time. The other successful use of an external light exposure device and LED light source was with the flat glass design. Table 32 shows the light contact time and the time the culture was in the dark while using the flat glass – LED setup. The culture times in the light were very similar to the only successful experiment using the column light "window" and LED light source – 55 seconds. The times in the dark using the flat glass "window" were very short, by comparison, due to the fast culture circulation rates and low CSTR volume. The successful column – LED experiment indicates that a sufficient time in the light is more critical than a short period of darkness. A more economic method of light delivery would be a cyclic light addition of 50 to 55 seconds followed by 13 minutes of darkness.

Table 32. Time in the Light and Dark for the Culture in the CSTR Using the Flat Glass Sunning Device

Culture Volume in Reactor (ml)	Culture Volume in the Light (ml)[b]	Culture Volume in the Dark (ml)[a]	Culture FR (ml/min.)	Time in Light (sec.)	Time in Dark (sec.)
400	480	450	490	58.8	55.1
475	480	525	580	49.6	54.3
600	480	650	580	49.6	67.2
600	480	650	605	47.6	64.5
600	480	650	293	98.3	133

a. This includes 50ml of volume for the culture in the circulation tubing.
b. The flat glass window had dimensions of 12 x 20 x 2cm.

Sulfur Settling Systems with and Without Cell Recycle. A CSTR was used to study the effect of a sulfur settler system on the performance of *C. thiosulfatophilum*, as well as the operation of a cell recycle system with permeate purge. The sulfur settler was changed weekly. The sulfur was separated from the culture broth and cells, then dried and weighed. The weight of recovered sulfur was used to determine sulfur production in mg/minute, based on the total time the settler was in place. The reactor volume was kept at 1.7 L and the medium feed rate was about 600 ml/day. The temperature was 30°C, and the pH was maintained at 7.0. A solution of 10 percent NaOH was used for pH control. The light source consisted of one 100-W tungsten bulb aimed directly at the fermenter. Agitation was held at 500-550 rpm. The feed gas was a blend of pure CO_2 and a 2.5%H_2S in N_2 mixture. The medium feed was the Green Sulfur Bacteria medium, a mixture of salts, trace metals, and vitamin B_{12}. Initially the system was operated with only a sulfur settler, but a cell recycle system with permeate purge was added later. During the study, the H_2S feed rates were continuously adjusted to the maximum the culture could handle as indicated by a comfortable, apparent H_2S conversion of 80 percent or above.

Liquid Retention Time in Settler (No Cell Recycle). Settler liquid retention time experiments were conducted with a 15 minute (Experiment 1) settler liquid retention time and a 20 minute liquid retention time (Experiment 2). All culture conditions and parameters were as listed above. Culture was pumped into the sulfur settler at a rate of approximately 60 ml/min, and culture was pumped out of the settler at a slightly higher flow rate to prevent the overflow of culture out of the settler. The volume of culture in the settler at any one time was about 1L. The settler was not run completely full, leaving about 100ml of headspace. The settler headspace was connected to the effluent, waste system to prevent any air contamination. Results from this experiment (Experiment 1) are shown in Table 33.

Table 33. Comparison of Different Liquid Retention Times in the Sulfur Settler

Experiment	1	2
Reactor Run Time (hr)	357	1426
Settler Liquid Retention Time (min)	15	20
Cell Concentration (g/L)	0.502	0.565
H_2S Conversion (%)	89	86
CO_2 Conversion (%)	13 [b]	15 [b]
Sulfur in Reactor (ppm)	14	25
Sulfur Collected in Settler (mg/min)	0.785	0.901
Sulfur in Purge (mg/min)	0.00588	0.0105
Sulfate (ppm)	259	158
Sulfide (ppm)	6	18
Liquid Retention Time, LRT (hr)	70.7[c]	69.9[c]
Gas Retention Time, GRT(min)	29.2	28.0
Cell Retention Time, XRT (hr)	70.7[c]	69.9[c]
Cell Make Rate (g/day)	0.290	0.333
H_2S Feed Rate (mmol/min)	0.051	0.053
CO_2 Feed Rate (mmol/min)	0.368	0.368
Culture Temperature (ºC)	29.7	29.3
Culture pH	7.03	7.02
Specific CO_2 Uptake (mmol/g•min)	0.063	0.055
Specific H_2S Uptake (mmol/g•min)	0.047	0.045
Average CO_2 Uptake (mmol/min)	0.042	0.040
Average H_2S Uptake (mmol/min)	0.036	0.036
CO_2/H_2S Ratio	1.32[b]	1.37[b]
Predicted CO_2/H_2 Ratio	0.564	0.549
Predicted CO_2/H_2 Ratio excluded Sulfur in Settler	1.79[a]	1.53[a]

[a]Calculations did not include the sulfur collected from the settler.
[b]Negative numbers were not included in the calculations
[c] Calculations did not include the liquid volume in the sulfur settler.

The average sulfur removal rate in settler was 0.785 mg/min, and the sulfur removal rate through the culture purge was 0.0059 mg/min. Therefore, 99.3% of all of the sulfur produced was collected in the settler. This indicates that a sulfur settler operating with a 15 minute liquid retention time was quite capable of separating the sulfur from the fermentation broth. Not only was the sulfur concentrated in the settler, but the sulfur was effectively removed from the fermentation broth. This removal from the fermentation broth helps to avoid any operational complications in the reactor and enhances CO_2 uptake. The average H_2S uptake was 0.036 mmole/min, or 1.152 mg/min of sulfur put into the system. The elemental sulfur collected in settler was 0.785 mg/min, and the elemental sulfur from the culture purge was 0.006 mg/min. The sulfur production rate from the culture purge, as sulfate and sulfide, was 0.035 and 0.002 mg/min, respectively. The measured sulfur removed from the system was 0.828

mg/min, or 71.9% of what the system consumed. Considering the difficulty involved in purifying the sulfur collected in the settler, and the somewhat erratic nature of the sulfur, sulfate and sulfide measurements from the culture, this sulfur balance is deemed acceptable.

The average predicted CO_2/H_2S ratio was 0.564 when the sulfur collected in the settler was included in calculations. This value was much smaller than the 1.32 ratio calculated from the actual gas uptake numbers. When the sulfur collected in the settler was excluded from the prediction calculations, the predicted ratio increased to 1.79, which is much closer to the actual gas uptake ratio of 1.32.

The liquid retention time in the sulfur settler was next increased to 20 minutes. Culture was pumped into the sulfur settler at a rate of approximately 42 ml/min. All other reactor conditions and parameters remained the same. The results from Experiment 2 are also shown in Table 33.

The average sulfur collection rate in the settler for Experiment 2 was 0.901 mg/min, and the average sulfur collection rate from the culture purge was 0.0105 mg/min. Therefore, 98.8% of all sulfur produced was collected by the settler. As was seen in previous experiments, most of the sulfur was trapped inside the settler. When the settler liquid retention time was increased from 15 to 20 minutes, there were measurable changes in the culture condition. The sulfur production rate increased from 0.785 to 0.901 mg/min, and the average sulfate concentration in the reactor decreased from 259 to 158ppm. The sulfur concentration in the culture purge slightly increased from 14 to 25 ppm. The average H_2S feed rate was able to be only slightly increased from 0.051 to 0.053 mmol/min. However, the average H_2S uptake rate remained 0.036 mmol/min. The average CO_2 uptake rate was slightly reduced from 0.042 to 0.040 mmol/min as a result of a higher sulfur production rate. Remember that the culture will consume only 0.5 mole CO_2 when one mole of sulfur is produced, but the culture will consume two moles CO_2 for each mole of sulfate produced. The same increases and decreases in the specific gas uptakes were also seen. The cell concentration increased from 0.502 to 0.565 g/L, a 12.5% increase, when the settler culture feed rate was decreased from 60 to 42 ml/min. A lower culture feed rate could result in fewer cells lost during settler operation. Along with an increase in sulfur production, this is another advantage of operating the settler liquid retention time in the 20 min range.

The sulfur balance for Experiment 2 was even better in than in Experiment 1. The average H_2S uptake rate was 0.036 mmole/min or 1.15 mg/min sulfur consumed, or added to the system. The elemental sulfur collected in the settler was 0.911 mg/min. The sulfur collected from the culture purge as elemental sulfur, sulfate and sulfide were 0.010, 0.021, and 0.007 mg/min, respectively. The measured sulfur removed from the system was 0.939 mg/min, 81.5% of the consumed sulfur. As mentioned before, due to the difficulties associated with sulfur purification and product measurements, the higher sulfur balance in Experiment 2 should not be used to judge which settler liquid retention time is better.

The 20 min sulfur settler liquid retention time produced more sulfur and produced more cells which helped the stability of reactor operation. Therefore, a 20 minute liquid retention time was maintained through the rest of the reporting period for subsequent experiments.

Cell Recycle Filter (Between the Reactor and Sulfur Settler). An experiment was set up to observe changes in culture performance when a cell recycle system with permeate purge was installed. A 0.2 µm hollow fiber was installed in the culture circulation loop from the reactor to the settler. Thus, culture flow travels through the hollow fiber before entering the sulfur settler. The permeate flow rate was set at 0.2 ml/min and the medium flow rate was increased from 0.40 ml/min to 0.60 ml/min to maintain the 0.4 ml/min culture purge rate used in Experiments 1 and 2 above. All other culture conditions and parameters remained the same. Results from Experiment 3 are shown in Table 34.

The addition of a cell recycle system with permeate purge significantly enhanced the cell concentration. The average cell density increased from 0.565 to 0.837 g/L, a 48 percent increase. The average H_2S gas feed rate was able to be increased from 0.053 to 0.074 mmol/min., a 40 percent increase, most likely due to the increase in cell density. The CO_2 feed rate was not increased due to the fact that most of the available CO_2 was still not utilized and the conversions remained low. Both the H_2S and CO_2 average uptakes increased from 0.036 and 0.040 mmol/min. to 0.059 and 0.081 mmol/min., respectively. The average H_2S uptake increased by 64 percent and the average CO_2 uptake increased 102.5 percent. The higher CO_2 uptake increase indicates more cell generation, since more CO_2 uptake is required for cell growth.

The installation of the cell recycle system did not adversely affect the efficiency of sulfur removal in the settler. There was still 98.7 percent of the sulfur recovered by the settler, when comparing the sulfur collected in the settler verses the sulfur removed in the culture purge stream. However, the sulfur collected in settler did not increase in proportion to the increase in H_2S uptake—the sulfur in the settler only increased 5.7 percent compared to a 40 percent increase in the H_2S uptake. The sulfur balance of the system was much poorer compared to when there was no cell recycle system. The H_2S uptake rate was 0.059 mmol/min. or 1.89 mg/min. sulfur consumed or added to the system. The sulfur collected in settler was 0.952 mg/min. The sulfur in culture purge and permeate stream was 0.0126, 0.0184, and 0.0024 mg/min as elemental sulfur, sulfate, and sulfide, respectively. The accountable sulfur removed was 0.985 mg/min., 52.2 percent of the consumed sulfur. The sulfur balance seen in Experiment 4, another cell recycle run, was also low at only 44.7 percent. Compared to the sulfur balances in Experiments 1 and 2 (71 and 81 percent, respectively), the sulfur balance in Experiments 3 and 4 were about half of what should be expected. It is highly possible that the hollow fiber filter used for cell recycle, trapped the elemental sulfur and caused the sulfur balance to be low.

Table 34. Comparison Different Hollow Fiber Positions While at a Constant Liquid Retention Time in the Sulfur Settler

Experiment	3	4
Reactor Run Time (hr)	834	558
Settler Liquid Retention Time (min)	20	20
Cell Concentration (g/L)	.830	1.385
H_2S Conversion (%)	84	83
CO_2 Conversion (%)	13[d]	13[d]
Sulfur in Reactor (ppm)	20	19
Sulfur Collected in Settler (mg/min)	0.904	0.934
Sulfur in Purge & Permeate (mg/min)	0.0126	0.0076
Sulfate (ppm)	81	108
Sulfide (ppm)	3	6
Liquid Retention Time, LRT (hr)	47.2[C]	70.8[C]
Gas Retention Time, GRT(min)	20.8	18.9
Cell Retention Time, XRT (hr)	70.9[C]	141.7[C]
Cell Make Rate (g/day)	0.48	0.399
H_2S Feed Rate (mmol/min)	0.075	0.074
CO_2 Feed Rate (mmol/min)	0.375	0.383
Culture Temperature (ºC)	29.2	29.6
Culture pH	7.13	7.07
Specific CO_2 Uptake (mmol/g•min)	0.034	0.059
Specific H_2S Uptake (mmol/g•min)	0.051	0.038
Average CO_2 Uptake (mmol/min)	0.041	0.104
Average H_2S Uptake (mmol/min)	0.061	0.067
CO_2/H_2S Ratio	1.34	1.57
Predicted CO_2/H_2 Ratio	0.530	0.522
Predicted CO_2/H_2S Ratio Excluded Sulfur in Settler	1.38[a]	1.55[a]

[a]Calculations did not include the sulfur collected from the settler.
[b]Negative numbers were not included in the calculations
[c] Calculations did not include the liquid volume in the sulfur settler.

The cell recycle system significantly enhanced the performance of the reactor system. The average H_2S and CO_2 uptake rates were increased by 64 and 102.5 percent, respectively. However, the collected sulfur did not increase proportionally. Elemental sulfur might have been trapped in the hollow fiber, such that an additional sulfur recovery procedure from hollow fiber cleaning might be required.

At the end of Experiment 3, the medium flow rate was decreased to 0.40ml/minute. This reduced the culture purge rate to 0.20 ml/min, increasing the cell retention time to 142 hours. This medium flow rate changed further increased the liquid retention time to 70hr. This change occurred about eight days before the start of Experiment 4, and caused the expected results of increasing cell density, gas uptake and sulfate concentrations prior to the start of Experiment 4.

Cell Recycle Filter (Located at Return to the Reactor from Settler). An experiment was designed to observe changes when the position of the hollow fiber filter was changed. Initially, the hollow fiber was installed in the culture circulation loop so that culture was pumped through the hollow fiber before it entered the settler. In Experiment 4, the position of the hollow fiber was moved so that the culture from the settler was pumped through the hollow fiber before re-entering the reactor. The permeate flow rate remained at 0.2 ml/minute and the medium flow rate remained at 0.40 ml/minute. The culture purge rate remained at 0.2 ml/minute. All other culture conditions and parameters remained the same. Results from Experiment 4 are shown in Table 34.

In comparison to Experiment 3, there were actually two changes in the Experiment 4 run. Not only was the position of the hollow fiber changed, but the medium flow rate and culture purge rate were reduced. The reason for the change in hollow fiber position was to reduce the amount of sulfur trapped in the hollow fiber membrane. This change was not expected to influence culture behavior. The medium flow rate was reduced from 0.6 to 0.4 ml/min. and culture purge rate was reduced from 0.4 to 0.2 ml/min eight days prior to the start of Experiment 4. The permeate purge rate was maintained at 0.2 ml/min. These medium and culture purge rate changes had a much greater effect on culture activity.

A comparison of Experiments 3 and 4 shows a 65.5 percent increase in cell concentration due to the decrease in culture purge from 0.4 to 0.2 ml/min. However, the culture make rate only slightly fell from 0.44 to 0.40g/day. If the culture activity remained exactly the same, the decrease in culture purge rate from 0.4 to 0.2 ml/min. should have doubled the cell concentration and resulted in the same cell make rate. This result indicates that increasing the cell retention time actually slows down individual cell activity. The specific H_2S and CO_2 uptakes were reduced from 0.049 and 0.068 mmol/min., respectively, to 0.038 and 0.059 mmol/min., respectively. However there were still some improvement in the average H_2S and CO_2 uptakes, increases of 13.5 percent and 28.4 percent, respectively.

The settler collected 99.1 percent of the measured sulfur. The settler collected 0.934 mg/min of sulfur and 0.0076 mg/min of sulfur was lost to the culture purge. However, as was seen in Experiment 3, the sulfur balance was poor. The average H_2S uptake rate was 0.0617 mmole/min., or 2.14 mg/min. sulfur consumed. The accountable sulfur removed was 0.934 mg/min. in the settler and 0.0076, 0.0144, and 0.0024 mg/min sulfur from permeate and culture purge streams as elemental sulfur, sulfate, and sulfide, respectively. The total accountable sulfur removed was 0.9584 mg/min, 44.7 percent of sulfur consumed. Compared to the 71 to 81 percent sulfur balance seen in Experiments 1 and 2 with no cell recycle, the 44.7 percent sulfur balance indicates that there is still a significant amount of sulfur trapped in the hollow fiber even with hollow fiber position changed. Compared with the 52.2 percent sulfur balance found in Experiment 3, the balance with the new hollow fiber position was even poorer. However, considering the difficulty in sulfur purification from the settler and the sometimes erratic sulfur analysis in general, the difference is not high enough to judge which position is better. Maybe a better alternative system design should consider a

separate cell recycle loop operated with just enough culture flow to take out the desired amount of permeate. Since the permeate purge rate is only 0.2 ml/min., a culture circulation rate of 5 ml/min. will give enough circulation to meet the required culture circulation rate to permeate flow rate ratio of 25. As the system was operated in Experiments 3 and 4, a 20 minute settler retention time requires a 50 ml/min. circulation rate for a 1L settler. Culture flow through hollow fiber was about ten times more than needed for the required permeate purge rate. Therefore, if the cell recycle system is separated from the sulfur settler system, the sulfur trapped in the hollow fiber can be significantly reduced.

TASK 4. ADVANCED BIOREACTOR CONCEPTS

Maximizing CO_2 Utilization with *C. thiosulfatophilum*. The objective of these experiments was to maximize the utilization of CO_2 by *C. thiosulfatophilum*. To obtain the maximum CO_2 utilization, sulfide added in the form of H_2S needs to be further oxidized from elemental sulfur to sulfate. This further oxidation will increase CO_2 consumption from 0.5 to 2.0 moles per mole of H_2S uptake. Thus, the fermenter operating principle was to enhance cell concentration, keep the sulfide concentration low and supply sufficient light for growth. The fermenter was kept at pH 7 using 10 percent NaOH for pH control. The temperature was maintained at 29 to 30°C, and the culture volume was held at approximately 1600 ml. Agitation was kept at 400-530rpm. The feed gas was a blend of pure CO_2 and a 2.5%H_2S and 97.5% N_2 mixture. The light source was one 100-W tungsten bulb initially, but was later increased to two 100-W tungsten bulbs near the end of the reporting period in Experiment 5. The medium feed was the Green Sulfur Bacteria medium, a mixture of salts, trace metals, and vitamin B_{12}. The initial medium feed rate was 580 ml/day, but this rate was increased to 860 ml/day during Experiment 2 and was maintained at this rate for the remainder of the reporting period. The fermenter was initially operated as a straight through CSTR. Later, a hollow fiber/cell recycle system with permeate purge was added in Experiment 3 and was kept in the reactor system for the remainder of the reporting period. The system was also initially operated without a sulfur settler. Later a sulfur settler was added during Experiment 4, and the settler was kept in operation for the remainder of the reporting period.

Medium Flow Rate Experiments. Experiments 1 and 2 were designed to study the effect of medium feed rate in a CSTR without cell recycle. An increase in the medium feed rate provides more nutrients to the culture and therefore enhances culture growth and gas uptake. However, a higher medium rate also results in a higher culture purge rate. Therefore, an optimized medium feed rate does exist.

Experiment 1 was designed to observe the culture conditions when the medium flow rate in the reactor was set at 0.40 ml/minute. The reactor system was set up as a straight through CSTR with no sulfur settler. All other culture conditions were as listed above. Experiment 2 was designed to observe the change in culture conditions when the medium flow rate in the reactor was increased from 0.4 ml/min (Experiment 1) to

0.60mL/min. All other reactor parameters were kept the same. Results from Experiments 1 and 2 are shown in Table 35.

When the medium feed rate was increased by 50 percent from 0.4 to 0.6 ml/min.; the average H_2S uptake increased by 50 percent and the average CO_2 uptake increased by 80 percent. The H_2S and CO_2 feed rates were also increased 39.3 percent and 58.2 percent, respectively. The cell make rate also increased about 56.9 percent. However, the average cell concentration increased only 10.2 percent. This low cell concentration increase indicates that the 0.6 ml/min medium rate is approaching the optimum liquid retention time. Higher medium feed rates will exceed the cell make rate and wash out more cells, making it difficult to increase the cell concentration. However, the average cell generation rate increase and average H_2S uptake rate increase were very close to medium feed rate increase. This indicates there is a nutrient limitation problem. An increase in medium component concentrations should improve the culture growth capacity. However, the current medium makeup is already difficult to dissolve completely. The medium must be kept stirred to keep the precipitates suspended for transport into the reactor. For long term study, individual nutrients should be studied to identify the limiting compound in the medium. If the limited compound is difficult to dissolve at higher concentrations, methods to enhance solubility should be studied.

Table 35. Comparison of Different Medium Flow Rates and Cell Retention Times in a CSTR without Cell Recycle, without Sulfur Settling

Experiment	1	2
Reactor Run Time (hr)	367	528
Cell Concentration (g/L)	0.491	0.541
H_2S Conversion (%)	86	86
CO_2 Conversion (%)	17[a]	21[a]
Sulfur in Reactor (ppm)	73	115
Sulfate (ppm)	177	113
Sulfide (ppm)	12	8
Liquid Retention Time, LRT (hr)	66.5	46.8
Gas Retention Time, GRT(min)	50.1	34.7
Cell Retention Time, XRT (hr)	66.5	46.8
Cell Make Rate (g/day)	0.288	0.452
H_2S Feed Rate (mmol/min)	0.028	0.039
CO_2 Feed Rate (mmol/min)	0.215	0.340
Culture Temperature (ºC)	30.2	30.4
Culture pH	7.05	7.10
Specific CO_2 Uptake (mmol/g•min)	0.025	0.046
Specific H_2S Uptake (mmol/g•min)	0.024	0.032
Average CO_2 Uptake (mmol/min)	0.020	0.036
Average H_2S Uptake (mmol/min)	0.018	0.027
CO_2/H_2S Ratio	1.17	1.418
Predicted CO_2/H_2 Ratio	1.44	1.45

[a]Negative numbers were not included in the calculations

The sulfur balances in Experiments 1 and 2 were very poor. The sulfur consumption rates, or input into the reactor system, based on average H_2S uptakes, were 0.576 and 0.864 mg/min., for Experiments 1 and 2, respectively. The measured sulfur removal in Experiment 1 was 0.0292, 0.0236, and 0.0048 mg/min. as elemental sulfur, sulfate, and sulfide. The combined total outlet sulfur was only 0.0576 mg/min., or 10 percent of the total sulfur uptake. Experiment 2 was only slightly better. The measured sulfur out was 0.069, 0.0226, and 0.0048 mg/min as elemental sulfur, sulfate, and sulfide, respectively. The total outlet sulfur added up to 0.0964 mg/min., or 11.2 percent of the total sulfur uptake. In the fermenter studies without cell recycle, a 70 to 80 percent sulfur balance is achieved when a sulfur settler system was implemented. This indicates that the majority of sulfur product in this system is still trapped inside fermenter as part of the cell/debris/sulfur coating seen on the glass reactor walls, or really any surface inside the fermenter. This result also indicates the settler system used in earlier studies is quite effective in collecting sulfur.

A comparison of the sulfur products (elemental sulfur, sulfate and sulfide) between Experiments 1 and 2 indicates that the increase in H_2S uptake from Experiment 1 to Experiment 2 all went to elemental sulfur production. The sulfate and sulfide rates in Experiment 2 (0.0226 and 0.0048 mg/min., respectively) were very close to those in Experiment 1 (0.0236 and 0.0048 mg/min., respectively). However, the elemental sulfur removal in Experiment 2 was 0.069 mg/min., more than double in Experiment 1 (0.0292 mg/min.). This indicates that there is another limitation in sulfate production, most likely the light was insufficient to convert sulfur to sulfate.

Installation of a Cell Recycle System. The purpose of this experiment was to observe changes in overall culture conditions when the cell retention time was increased by adding a cell recycle system with permeate purge. Culture was continuously circulated through a 0.2 μm hollow fiber membrane. Permeate purge was drawn out of the hollow fiber and discarded at a flow rate of 0.2 ml/min. The medium flow rate was kept at 0.60 ml/min. All other culture conditions and parameters remained the same. Results from Experiment 3 are shown in Table 36.

A comparison of Experiment 3 with Experiment 2 showed that the installation of cell recycle enhanced the cell concentration from 0.541 to 2.78 g/L, an improvement of 178 percent. However, the cell make rate only increased from 0.452 to 0.651 g/day, or only a 44 percent improvement. The cell retention time increased from 46.8 to 90.3 hr, a change of 93 percent. The significant cell concentration increase was due to the slower culture purge rate, a 50 percent drop from 0.6 to 0.3 ml/minute. The average H_2S and CO_2 uptake rates increased from 0.027 and 0.036 to 0.040 and 0.071 mmole/min, respectively, for an average H_2S uptake increase of 48 percent and an average CO_2 uptake increase of 97 percent.

Table 36. Comparing Culture Parameters with Cell Recycle and Permeate Purge, a Sulfur Settler, and the Addition of a Light Source

Experiment	3	4	5
Reactor Run Time (hr)	336	182	606
Settler Liquid Retention Time (min)	No Settler	9.5	9.5
Cell Concentration (g/L)	1.51	1.71	1.79
H_2S Conversion (%)	85	86	86
CO_2 Conversion (%)	24[b]	24[b]	20[b]
Sulfur in Reactor (ppm)	99	99	149
Sulfate (ppm)	63	67	72
Sulfur collected in settler (mg/min)	-----	0.428	0.793
Sulfur in culture purge (mg/min)	0.0297	0.0198	0.0298
Sulfide (ppm)	9	6	5
Liquid Retention Time, LRT (hr)	45.1[c]	46.0[c]	44.7[c]
Gas Retention Time, GRT(min)	23.3	24.1	18.6
Cell Retention Time, XRT (hr)	90.3[c]	96.3[c]	87.3[c]
Cell Make Rate (g/day)	0.651	0.737	0.813
H_2S Feed Rate (mmol/min)	0.062	0.061	0.080
CO_2 Feed Rate (mmol/min)	0.391	0.391	0.391
Culture Temperature (ºC)	29.7	29.7	30.0
Culture pH	7.02	7.12	7.07
Specific CO_2 Uptake (mmol/g•min)	0.034	0.039	0.028
Specific H_2S Uptake (mmol/g•min)	0.017	0.022	0.031
Average CO_2 Uptake (mmol/min)	0.071	0.074	0.077
Average H_2S Uptake (mmol/min)	0.040	0.050	0.068
CO_2/H_2S Ratio	1.79	1.61	1.16
Predicted CO_2/H_2 Ratio	1.05	0.949	0.545
Predicted CO_2/H_2S Ratio, Excluded Sulfur in Settler	1.05[a]	0.955[a]	0.966[a]

[a]Calculations did not include the sulfur collected from the settler.
[b]Negative numbers were not included in the calculations
[c] Calculations did not include the liquid volume in the sulfur settler

Unfortunately, the installation of a cell recycle system complicates the predicted CO_2/H_2S uptake ratio calculation. For reactor operation without cell recycle the product concentration ratio equals the product producing rate. However, the hollow fiber membrane and permeate purge will hold elemental sulfur in the reactor longer by dropping the culture purge rate, from 0.6 to 0.3ml/minute in this experiment. This artificially increases the elemental sulfur concentration. Therefore, the sulfate production rate equals the sulfate measured concentration (63 ppm) times the total liquid out flow rate, permeate purge plus culture purge (0.60 ml/min. or a 45.1 hour liquid retention time), giving a sulfate production rate of 63 / 45.1 = 1.39. However, the elemental sulfur production rate equals the measured sulfur concentration (99 ppm) times the culture purge rate only (0.3 ml/minute or a 90.3 hour cell retention time) giving a sulfur production rate of 99 / 90.3 = 1.10. The average sulfate to sulfur production ratio then becomes 1.39 / 1.10 = 1.27 to 1. Based on this production ratio, the corrected predicted

CO_2/H_2S uptake ratio increased from 0.949 (based on product concentration ratio) to 1.34 (based on product producing rate ratio). The corrected value is closer to the actual average gas uptake ratio of 1.79 but it is still farther off than could be expected.

Installation of a Sulfur Settler. The purpose of this experiment was to observe changes in overall culture conditions with the installation of a 125 ml sulfur settler with a LRT of 10 minutes. The sulfur settler was operated culture-filled with almost no headspace. A pump was used to transfer the culture from the reactor to the settler at an approximate flow rate of 10 ml/minute. There was no pump used to return the culture to the reactor. Instead, the culture was pushed out of the settler by the incoming culture. The sulfur settler was changed, on average, once per week. The collected sulfur was then separated from the cells to be dried and weighed to determine sulfur production in mg/minute based on the total time the settler was in place. The cell recycle system with permeate purge was kept operating under the same parameters as described in Experiment 3, *i.e.* a 0.2 µm hollow fiber membrane and a 0.3 ml/min. permeate purge rate. The culture flow to the settler was separate from the culture flow to the hollow fiber. There was one only port for culture removal from the reactor and was divided into two flows. One flow went to the hollow fiber, and the other flow went to the settler. The return culture flows from the settler and hollow fiber were merged prior to entry back into the reactor. The medium flow was kept at 0.6 ml/min. All other culture parameters and conditions were kept the same. Results from Experiment 4 are shown in Table 36.

Note that there was about 500ml of culture lost from the system, and medium was used to replace the lost volume. Overall, the loss in culture performance was small. The installation of the sulfur settler enhanced the cell concentration from 1.51 to 1.71 g/L, a 14 percent improvement. The cell make rate also increased from 0.651 to 0.737 g/day, or a 13 percent increase. The average H_2S uptake increased 25 percent, from 0.04 to 0.05 mmole/min. However, the average CO_2 uptake only increased 4.2 percent. This indicates that most of the H_2S uptake increase went to elemental sulfur production. As expected, the settler removed sulfur from the fermenter and improved sulfur production. The average measured sulfur and sulfate concentrations in the fermenter did not change significantly after the installation of the settler. However, the total accountable sulfur measured significantly increased from 0.0297 mg/min. without the sulfur settler (Experiment 3) to 0.428 mg/minute from the settler and 0.0198 mg/min. from the culture purge when the settler was in use (Experiment 4). As discussed in the earlier, until the sulfur settler was used, a significant amount of sulfur was trapped in the fermenter as part of the cell/debris/sulfur paste that clings to all surfaces and this could not be accounted for.

The sulfur balance in the system improved once the sulfur settler was installed. The average H_2S uptake rate was 0.05 mmole/min., or 1.60 mg/minute of sulfur taken up into the system. The settler collected an average of 0.428 mg/minute of sulfur. Counting the elemental sulfur coming out only in the culture purge gives a sulfur purge rate of 0.0198 mg/minute. This shows that the settler collected about 95.6 percent of the sulfur produced. This is not as good as the settler used in the other reactor setup,

which had a larger settler volume and settler retention times of 15 to 20 minutes. The sulfate and sulfide are both capable of passing through the hollow fiber in the cell recycle system. Based on both permeate and culture purge rates, the sulfate and sulfide removal rates were 0.0134 and 0.0036 mg/minute as sulfur, respectively. The total sulfur removal rate was 0.465 mg/min., or 29.1 percent of sulfur consumed.

The operation of the small settler was not smooth from beginning. The earlier amounts of collected sulfur were much lower than the latter stages of settler operation. If the calculations are based on the latter stage data, the average sulfur collected increased to 0.653 mg/min., or the total sulfur removed from the system increased to 0.690 mg/min. This is 43.1 percent of the sulfur consumed. Both sulfur balance numbers of 29.1 percent or 43.1 percent are still low, but this is much better than the 10 percent sulfur balance calculated in Experiments 1 and 2. As discussed in the earlier study, the sulfur balance could be as high as 70 to 80 percent without using a cell-recycle system, but the sulfur balanced dropped to 40 to 50 percent with the installation of a hollow fiber. The 30 to 40 percent sulfur balance found in Experiment 4 is acceptable since a cell recycle system with hollow fiber was in use.

Additional Light Source. This experiment was used to observe the change in culture conditions when a second 100-W tungsten bulb was added to the system. The bulbs were positioned 180° apart, with both bulbs directed toward the CSTR. All reactor parameters and conditions were maintained the same, including the sulfur settler and cell recycle systems. The results from Experiment 5 are shown in Table 36.

The addition of the second 100-W tungsten bulb only slightly increased the cell concentration from 1.71 to 1.79 g/L, a 4.6 percent increase. The cell make rate increased from 0.737 to 0.813 g/day, or a 10.3 percent improvement. However, the H_2S gas feed rate was able to be increased from 0.061 to 0.08 mmole/min., a 31.1 percent increase. The average H_2S uptake also increased about 36 percent, from 0.05 to 0.068 mmole/minute. As for the average CO_2 uptake, the margin of improvement was similar to the cell concentration increase. The average CO_2 uptake increase was 4.1 percent, from 0.074 to 0.077 mmole/minute. This result indicates that the increase in the H_2S feed rate all went to elemental sulfur production. The increase of light intensity from 100 to 200 W did not significantly enhance culture performance in this case, especially the CO_2 uptake. Although more light was given, the surface capable of receiving the light was still the same. With cell/debris/sulfur cake easily formed on that surface, light penetration was poor. A more effective light deliver system is required in order to observe the advantage of increasing the light intensity.

Like Experiment 4, the sulfur balance significantly improved with the sulfur settler installation. The average H_2S uptake rate was 0.068 mmole/min., or 2.18 mg/minute of sulfur delivered into the system. The settler collected average 0.793 mg/min. sulfur. With elemental sulfur coming out only in the culture purge, the sulfur from that purge rate was 0.0298 mg/minute. The settler collected about 96.4 percent sulfur produced. The sulfate and sulfide are both capable of passing through the hollow fiber in the cell recycle system. Based on both permeate and culture purge rates, the sulfate and

sulfide removal rates were 0.0144 and 0.0030 mg/minute as sulfur. The total sulfur removal rate for the system was 0.840 mg/min., or 38.6 percent of sulfur consumed. As discussed in Experiment 4, this value for a sulfur balance is quite acceptable when using a cell recycle system because of the sulfur that is inevitably trapped in the membrane.

Improvement of Sulfur/Cell Separation in the Sulfur Settler. Cells are mixed with removed sulfur in the sulfur settler, therefore an undetermined amount of cells are lost in the system with sulfur recovery. In an effort to improve sulfur and cell separation in the sulfur settler and minimize cell loss, a mild amount of turbulence was added to the sulfur settler by bubbling helium into the settler. *C. thiosulfatophilum* cells are more buoyant than the sulfur solids, which is why the sulfur settler works. By slowly bubbling helium into the settler, it was speculated that the turbulence would help to keep the cells suspended, while still allowing the sulfur solids to settle out. Helium was chosen so that the additional gas would not interfere with cellular activity or with GC analysis of the effluent gas.

Throughout the study, the sulfur settler was changed about once per week. The sulfur collected in the settler was separated from the culture broth and cells, then dried and weighed. The weight of recovered sulfur was then used to determine sulfur production in mg/minute based on the total time the settler was in place. The reactor volume was kept at 1.7 L and the medium feed rate was about 580 ml/day. A cell recycle system with a 0.2 µm hollow fiber membrane filter was also used in the system. The hollow fiber was positioned after the sulfur settler so that the culture was pumped out of the sulfur settler, and then through the hollow fiber before returning to the reactor. A permeate purge of 290ml/day was maintained. The sulfur settler liquid retention time was kept at 20 minutes. The volume of culture in the settler was maintained at 1L. The settler was not run completely full, leaving 100ml of headspace. The settler headspace was connected to the effluent, waste system to prevent air contamination. The culture temperature and pH were kept at 7.0 and 30°C, respectively. A solution of 10%NaOH was used for pH control. The light source was one 100 W tungsten bulb aimed directly at the fermenter. The agitation rate was 550 rpm. The feed gas was a blend of pure CO_2 and a 2.5%H_2S and 97.5% N_2 mixture. The medium feed was the Green Sulfur Bacteria medium, a mixture of salts, trace metals, and vitamin B_{12}. During this study, the H_2S feed rates were continuously adjusted to the maximum the culture could handle as indicated by a comfortable, apparent H_2S conversion of 80% or above. The sulfur collected in the settler throughout this experiment is summarized later in Table 38.

To determine how the helium bubbles affected the amount of cells lost in the sulfur collected from the sulfur settler, the mixture of sulfur and cells was tested for cell density before helium addition and when helium was flowing at the different flow rates. Each time the settler was changed, the broth remaining in the settler was removed, leaving the settled sulfur/cell mixture. The mixture was stirred to a homogeneous mix. Then a sample of the sulfur/cells was tested for cell density using the methanol extraction method. The results of the cell density measurements are also shown later in Table 37.

Helium Agitation. Helium was bubbled into the Sulfur Settler at a Flow Rate of 2 ml/min. An initial experiment was performed for a period of 358 hours to observe the effect of the mild agitation on the amount of cells collected with the sulfur in the sulfur settler. The helium was bubbled into the settler through a 6-inch, 18 gauge needle inserted through the stopper at the top of the settler. The end of the needle was approximately half-way down into the culture. All culture conditions and parameters were as listed above. The results from this initial experiment (Experiment 1) are shown in Table 37. For an easier comparison of helium flow vs. no helium flow in the sulfur settler, the averaged results from an experiment without helium flow are also shown in Table 37.

Following the initial experiment, the helium flow rate was increased to 4 ml/minute for an operating period of 365 hours (see also Table 37, Experiment 2). When changing the sulfur settler for the first time in Experiment 2, it was noticed that the culture flow from the reactor into the settler was plugged. It is unknown how long there was no culture flow, or only partial culture flow, to the settler. The data collected while there was no culture flow to the settler was not included in the averaged data for Experiment 2.

Experiment 1 was repeated for a period of 198 hours. It was impossible to determine when the culture flow to the settler stopped, so the data collected during Experiment 1 may or may not have been with sulfur separation. Experiment 1 was repeated in Experiment 3 by dropping the helium flow rate back to 2 ml/minute for a more reliable comparison. The results from Experiment 3 are shown in Table 37.

Results and Comparison of Experiments 1, 2 and 3. The first two weeks of helium agitation experiments showed a significant decrease in the amount of sulfur collected in the settler. The amount dropped from 0.947 mg/minute to 0.368 mg/minute during the first week, and then dropped further to 0.136 mg/minute during the second week (see Table 38). Along with the drop in collected sulfur, the cell density, H_2S uptake and CO_2 uptake also declined (see Table 34 – Experiment 1 vs. Previous Work). At the same time, the sulfur concentration in the reactor increased from 19 ppm before Experiment 1 to 23 ppm during Experiment 1. When Experiment 1 was repeated as Experiment 3, the amount of sulfur collected was 0.986 mg/min when culture flow to the settler was well established. All of the data indicate that less culture flowed to the settler some time during Experiment 1. Therefore, the data collected in Experiment 1 were not used for data analysis of experimental results.

Cell Loss in the Settler and Collected Sulfur with and Without Helium Addition. For two weeks prior to the start of Experiment 1, whenever the sulfur settler was changed, the cell/sulfur mixture was tested for cell density to determine a baseline of normal cell loss without helium flow into the settler. The average cell density for those two weeks was 6.76 g/L. The average rate of sulfur collected in the settler during the same time period was 0.978 mg/minute (see Table 38). During Experiment 2, when 4ml/min of helium was bubbled into the settler, the cell density average increased to

13.8 g/L and the sulfur collection rate increased slightly to 1.18 mg/minute. The helium flow improved the sulfur settling rate by 20.6 percent, but it also increased the cell loss rate by 104 percent.

During Experiment 3 with a 2 ml/min. helium flow rate, the cell density measured in the settled sulfur dropped significantly to 2.39 g/L while the sulfur collection rate was 0.986 mg/min. Compared to experiments without helium flow, a 2 ml/minute helium flow rate in the settler reduced the cell loss by 64.6 percent while keeping the sulfur collection rate essentially the same. Comparing 2 ml/min. to 4 ml/min. helium flow rate, the cell loss was 83.7 percent less with 2ml/min., but the sulfur collection rate was also 16.4 percent less.

These results indicate that a small amount of turbulence in the settler reduces the amount of cells lost due to settling without inhibiting the collection of sulfur. However, too much turbulence (4 ml/min) can cause a significant increase in cell loss while improving the sulfur collection rate, but the improved sulfur collection was not significant enough to justify the more than doubled rate of cell loss.

Effect of Helium Flow on Culture Performance. A comparison of the culture parameters without helium flow to the results of Experiment 2 (4ml/min.) show very little change in culture performance when helium was bubbled into the sulfur settler (see Table 36). The most significant change was a drop in cell density from 1.38 g/L to 1.02 g/L, or a drop of 25.9 percent. This drop was probably due to the doubling of the cell loss rate in the settler. Interestingly, the sulfur production rate increased with 4ml/min. agitation by the same amount from 0.9378 mg/min. to 1.183 mg/min., or a 26.1 percent increase. The sulfur production rate includes the sulfur settler collection rate and the sulfur washout rate based on reactor sulfur concentrations and the culture purge rate. The H_2S and CO_2 average uptake rates both decreased slightly, with a 6.0 percent drop for H_2S and a 7.5 percent drop for CO_2, so there were fewer cells using almost the same amount of substrate. A similar effect could be achieved by increasing the gas feed rate. More gas available per cell increases sulfur production and lowers sulfate concentrations. A 4 ml/min helium bubbling rate into the settler increased the rate of cell loss in the settler but did not seem to inhibit gas uptake. However, the turbulence in the settler improved sulfur production instead of sulfate production. If CO_2 sequestering is the goal, higher sulfate production would of course be desired.

When the helium flow rate was lowered to 2 ml/minute in Experiment 3, the results were mixed and unexpected. Although the cell density measurements from the sulfur settler showed a large decline in the cell loss rate, the average cell density in the reactor declined 20.9 percent, from 1.023 to 0.809 g/L, instead of improving. The H_2S uptake rate dropped slightly from 0.0630 to 0.0612 mmol/min., (2.8 percent), but the sulfur production rate dropped 16.4 percent from 1.183 to 0.989 mg/minute. At the same time, the CO_2 uptake increased from 0.0962 to 0.133 mmol/minute. An increase in CO_2 uptake should result in either increased cell growth or an increase in sulfate production, but both the cell make rate and sulfate production dropped. Also, the drop in sulfur production does not fit the measured parameters. The overall H_2S uptake only

dropped 2.8 percent, and the lower cell density should have resulted in a higher sulfur production rate if the H_2S uptake rate remained the same. All the results in Experiment 3 were counter-intuitive. It is possible that the one week of data collected at the 2 ml/minute helium flow rate was not sufficient for a representative average.

Table 37. Comparison of Different Helium Flows in the Sulfur Settler. Results from Experiments 1, 2 and 3

Experiment	4 – Previous Data	1	2	3
Reactor Run Time (hr)	----	358.3	365.5	197.7
Settler Liquid Retention Time (min)	20	20	20	20
Helium Bubbling Rate (ml/min)	----	2	4	2
Cell Concentration (g/L)	1.38	1.07	1.023	0.809
H_2S Conversion (%)	83	81.80	87.12	82.98
CO_2 Conversion (%)	13[c]	9.90[c]	11.78[c]	13.65[c]
Sulfur in Reactor (ppm)	19	23.3	13.5	14.1
Sulfur Collected in Settler (mg/min)	0.934	0.252	1.18	0.986
Sulfate (ppm)	108	66.4	98.8	87.7
Sulfide (ppm)	6	7.44	2.99	5.94
Liquid Retention Time, LRT (hr)	70.8[b]	70.8[b]	70.8[b]	70.8[b]
Gas Retention Time, GRT(min)	18.9	21.5	20.9	19.4
Cell Retention Time, XRT (hr)	141.7[b]	143[b]	142[b]	142[b]
Cell Make Rate (g/day)	0.399	0.616	0.589	0.466
H_2S Feed Rate (mmol/min)	0.074	0.0701	0.0730	0.0762
CO_2 Feed Rate (mmol/min)	0.383	0.383	0.383	0.383
Culture Temperature (ºC)	29.6	29.9	29.4	29.3
Culture pH	7.07	7.15	7.04	7.14
Specific CO_2 Uptake (mmol/g•min)	0.059	0.0513	0.0594	0.0918
Specific H_2S Uptake (mmol/g•min)	0.038	0.0328	0.0383	0.0494
Average CO_2 Uptake (mmol/min)	0.104	0.0873	0.0962	0.133
Average H_2S Uptake (mmol/min)	0.067	0.0566	0.0630	0.0612
CO_2/H_2S Ratio	1.57	1.60	1.53	1.86
Predicted CO_2/H_2S Ratio	0.522	0.567	0.516	0.517
Predicted CO_2/H_2S Ratio excluded Sulfur in Settler	1.55[a]	1.22[a]	1.55	1.50[a]

[a] Calculations did not include the sulfur collected from the settler.
[b] Calculations did not include the liquid volume in the sulfur settler.
[c] Negative numbers were not included in the calculations

Table 38. Measured Cell Density and Collected Sulfur in the Sulfur/Cell Mixture Taken from the Sulfur Settler with and without Helium Flow

Run Time (days)	Amount of Sulfur (mg)	Production Rate (mg/min.)	Cell Densitiy (g/L)	Helium Flowrate (ml/min.)
6.97	10091	1.005	5.37	0
7.05	9616	0.947	8.15	0
7.68	4074	0.368	7.60	2
7.30	1429	0.136	4.72	2
8.30	15826	1.320	8.51	4
6.96	10447	1.042	19.10	4
6.97	9896	0.986	2.39	2

Effects of Acetylene on Sulfate Production. A reactor was also used to test the effect of increased nutrition and the effects of acetylene on the sulfur to sulfate ratio. The reactor volume was 1.6L and medium feed rate was 860 ml/day. A cell recycle system with a 0.2 µm hollow fiber and a sulfur settler was also used. The culture flow to the settler was separate from the culture flow to the hollow fiber, so that there was only one port for culture removal from the reactor. This port was divided into two flows, one flow went to the hollow fiber, and the other flow went to the settler. The return culture flows from the settler and the hollow fiber were merged prior to entry back into the reactor. The sulfur settler maintained a liquid volume of 125 ml and was operated culture-filled with almost no headspace. A pump was used to transfer the culture from the reactor to the settler at an approximate flow rate of 10 ml/min, giving a liquid retention time of 10 minutes in the settler. There was no pump used to return the culture to the reactor; the culture was pushed out of the settler by the incoming culture. Throughout the experiment, the sulfur settler was changed (cleaned out) on an average of every 4 days. The sulfur collected in the settler was separated from the culture broth and cells, then dried and weighed. The weight of recovered sulfur was then used to determine sulfur production in mg/min based on the total time the settler was in place. A permeate purge of 430 mL/day was maintained during the reporting period. The culture temperature and pH were kept around 7.0 and 30°C, respectively. A solution of 10% NaOH was used for pH control. The light source was two 100 W tungsten bulbs aimed directly at the fermenter. The agitation rate was 480-540 rpm. The feed gas was a blend of pure CO_2 and a 2.5%H_2S – 97.5% N_2 mixture. The medium feed was the Green Sulfur Bacteria medium, a mixture of salts, trace metals, and vitamin B_{12}. The sulfur collected in the settler throughout this reporting period is summarized in Table 39, along with the experimental results.

Medium Concentration Doubled to Increase the Nutrients Delivered to the Culture. During previous operation, product ratio heavily favored sulfur over sulfate, despite the fact that the reactor conditions should have favored sulfate production. The H_2S conversion was kept high, an average of 85 percent, and the light source was doubled. High sulfide conversion, or low dissolved sulfide concentration, and excess light should favor sulfate production. Instead, as can be seen in Table 39, the predicted CO_2/H_2S ratio of 0.545 based on product concentrations heavily favored sulfur

production. It was therefore speculated that the culture may have some nutrient limitation, preventing the further oxidation of sulfur to sulfate.

At the start of the experiment, the medium component concentrations, including the B_{12} vitamin solution, were doubled while holding all reactor conditions the same. The experiment was run for 555 hours. With this increased nutrition, the average cell concentration increased by 9.5 percent from 1.79 to 1.96 g/L (see Table 39). The CO_2 uptake increased from 0.0775 to 0.104 mmol/minute, or 34.2 percent, while the H_2S uptake remained essentially the same. The culture seemed to benefit from the additional nutrients. The increase in CO_2 uptake seems to have gone entirely into cell production, because the sulfate concentration did not increase, but the sulfur production rate increased. This was unexpected because a higher cell density with the same H_2S uptake should have the same effect as lowering the H_2S uptake per cell. The products should have shifted to additional sulfate production. Instead, the sulfate concentration remained constant and the sulfur production rate increased from 0.838 mg/minute to 1.37 mg/minute. Clearly, the culture was still in sulfur production mode, despite all of the favorable sulfate conditions.

The addition of acetylene into the fermentation broth was thought to have the effect of inhibiting the production of sulfate, and therefore shifting the product ratio toward sulfur production. Therefore the fermenter needed to be in sulfate production mode. At the end of Experiment 1 the H_2S feed rate was decreased by 50 percent to stimulate sulfate production. The sulfate concentration in the fermenter increased from the Experiment 1 average of 73.6 ppm to approximately 400 ppm just before Experiment 2 was started.

Acetylene was pumped into the system at 0.5 ml/min. using a peristaltic pump and size 14 Tygon® tubing. The delivery side of the pump connected to the return culture line of the sulfur settler and hollow fiber system. A low pressure of 2 to 4 psig was maintained on the acetylene regulator to prevent air leakage into the system. All reactor conditions remained the same, including the increase in nutrients from Experiment 1. The experiment ran for 98 hours.

Acetylene contact had no effect on sulfate concentrations in the reactor. Before the acetylene was started, the sulfate concentration was 400 ppm. The average sulfate concentration during Experiment 2 was 436 ppm. The predicted CO_2/H_2S ratio, based on products, increased from 0.519 in Experiment 1 to 0.924 in Experiment 2 as would be expected with the increase in sulfate production. The cell density was lower in Experiment 2, but this is to be expected with the large drop in H_2S feed rate. The same can be said about the large drop in the sulfur collection rate and concentration in the reactor. Overall, no real effect could be attributed to acetylene addition.

Since there were no discernible changes in culture performance that could be interpreted as a direct result of acetylene addition, the acetylene flow rate was doubled to 1 mL/min for an additional 71 hours. All other culture parameters remained constant. When the acetylene flow was increased, the cell density dropped by 28.0 percent from

1.36 to 0.979 g/L (see Table 39). The sulfate concentration increased from 436 ppm to 552 ppm, and the sulfur collection rate in the settler dropped from 0.21 mg/minute to 0.042 mg/minute. Since the only change made to the reactor was the increase in acetylene, it is reasonable to say that the drop in cell density was due to acetylene contact. The H_2S uptake remained the same while the CO_2 uptake declined. This, coupled with the drop in cell density, would normally suggest that sulfur production should increase while sulfate production dropped. (Fewer cells at the same H_2S uptake rate has the same effect as increasing the H_2S feed rate when the cell density is constant.) Instead, sulfur production dropped and the sulfate concentration increased. Thus, higher acetylene contact did not inhibit the production of sulfate, and may have hurt cell growth.

Table 39. Effect of Additional Nutrients and Acetylene on the Product Ratio

Experiment	5 – Previous Results	1	2	3
Reactor Run Time (hr)	----	554.6	97.7	71.2
Settler Liquid Retention Time (min)	10	10	10	10
Cell Concentration (g/L)	1.79	1.96	1.36	0.979
H_2S Conversion (%)	85.89	88.68	99.88	99.32
CO_2 Conversion (%)	19.64[c]	35.44[c]	28.43[c]	32.77[c]
Sulfur in Reactor (ppm)	149	116	17.2	14.8
Sulfur Collected in Settler (mg/min)	0.793	1.34	0.21	0.042
Sulfate (ppm)	72.2	73.6	436	552
Sulfide (ppm)	4.59	2.96	0	0
Liquid Retention Time, LRT (hr)	44.7[b]	45.0[b]	44.2[b]	44.8[b]
Gas Retention Time, GRT(min)	18.6	20.1	34.6	35.1
Cell Retention Time, XRT (hr)	87.3 [b]	91. 2[b]	89.8[b]	90.6[b]
Cell Make Rate (g/day)	0.813	1.71	1.20	0.854
H_2S Feed Rate (mmol/min)	0.080	0.0777	0.0374	0.0371
CO_2 Feed Rate (mmol/min)	0.391	0.391	0.391	0.391
Culture Temperature (ºC)	30.0	29.7	29.6	29.7
Culture pH	7.07	7.11	7.12	7.09
Specific CO_2 Uptake (mmol/g•min)	0.0279	0.0440	0.0224	0.0273
Specific H_2S Uptake (mmol/g•min)	0.0309	0.0284	0.0225	0.0334
Average CO_2 Uptake (mmol/min)	0.0775	0.104	0.0574	0.0375
Average H_2S Uptake (mmol/min)	0.0680	0.0687	0.0402	0.0402
CO_2/H_2S Ratio	1.16	1.55	1.40	0.935
Predicted CO_2/H_2 Ratio	0.545	0.519	0.924	1.44
Predicted CO_2/H_2 Ratio excluded Sulfur in Settler	0.966[a]	0.866[a]	1.81[a]	1.89[a]

[a]Calculations did not include the sulfur collected from the settler.
[b] Calculations did not include the liquid volume in the sulfur settler.
[c]Negative numbers were not included in the calculations

TASK 6. MARKET ANALYSIS

This process generates income from the sale of by-products, protein and sulfur, as well as credits for carbon dioxide removal. The available markets for protein and sulfur are summarized in the following sections. Carbon dioxide credits are not well developed in the U.S., hence, the economics are predicated upon the required CO_2 credits to obtain beak-even economics.

***Clostridium thiosulfatophilum* Cells as a Single Cell Protein Source for Livestock or Poultry.** A secondary use of the *C. thiosulfatophilum* bacterial cells could be as a single cell protein supplement source for livestock or poultry feed. The bacteria from the culture purge stream of the fermenter could be separated, washed, dried and then fed to poultry or livestock. Table 40 shows results from analyses of protein, fat, and amino acid content performed on the bacteria. In order to determine if this bacterium would be suitable as a supplemental protein source, feeding trials will be necessary. Knowing the protein, fat and amino acid content gives a basis to for initiating feeding trials. It is not so important that the bacteria have a certain profile to be useful as a protein source, since supplements can be added to compensate for any amino acid level that is below specifications for the livestock. It is important to note the high protein composition (57%) and the wide range of amino acids present.

At the appropriate time in scale-up, feeding trials should be conducted to determine if the protein is palatable, and to see what effects, if any, the bacteria have on growth and overall health of the poultry or livestock. Feeding trials were not performed as part of this project. A trial would require a very large quantity (100 lbs) of the dried bacteria. That amount of bacteria would be impossible to obtain even from several small lab-scale fermenters. As an example, a good cell density in a lab scale fermenter is 2 g/L. With a culture purge flow of 0.4 mL/min, approximately 1.15 g of cells could be collected per day. Thus, it would take 108 years to collect 100 pounds of bacteria using a 1L lab-scale fermenter. Hence, feeding trials are deferred until the pilot plant phase of this project.

Table 40. Protein, Fat and Amino Acid Profile of *C. thiosulfatophilum* Cells

Component	% (by wt.)
Fat	4.69
Protein	56.7
Amino Acids	
Aspartic Acid	6.30
Threonine	2.60
Serine	2.01
Glutamic Acid	7.42
Glycine	3.35
Alanine	4.59
Cystine	0.57
Valine	3.74
Methionine	2.09
Isoleucine	3.18
Leucine	4.50
Tyrosine	2.04
Phenylalanine	2.71
Lysine	3.60
Histidine	1.02
Arginine	2.99

Protein Markets. Protein for animal feed supplement comes primarily from oilseed meal. Oilseeds comprise soybean, rapeseed, cottonseed, sunflower, peanut, palm kernel and copra. Soybean production far exceeds any of the above oilseeds worldwide and in the United States and this study will use soybeans as the reference protein source. Oil is first extracted from the oil-seeds for human consumption. The remaining meal is sold as protein for animal feed. Again, soybean meal is by far the largest source of animal feed protein. Soybean meal use for livestock goes primarily to poultry feed with swine and beef usage being about one-half and about one-fourth the poultry usage as shown in Table 41 below.

Table 41. U.S. Soybean Use by Livestock 2006

	Million Short Tons
Poultry	17.8
Swine	8.8
Beef	3.5
Dairy	1.6
Petfood	1.1
Other	0.8
Total	33.6

Soybean meal production in the U.S. has averaged about 40 million short tons per year over the last several years. The average price for soybean meal has between $160 and $250 per ton, as noted in Table 42 below. However, sharp price increases for soybeans have resulted from increased oil usage for biodiesel production. The current price is about $ 340 per ton.

Table 42. U.S. Soybean Meal Production

	Million short tons	Average Price (U.S. dollars)
1994	33.3	163
1995	32.5	236
1996	34.2	262
1997	38.2	185
1998	37.8	139
1999	37.6	168
2000	39.4	174
2001	40.3	168
2002	38.2	182
2003	36.3	256
2004	40.7	183
2005	41.2	174
2006	42.4	178
2007	43.1	205
2008	42.2	336

Soybean meal's higher market value per ton reflects its greater inherent value to the animal production industry. Soybean meal is the standard to which other sources of protein and amino acids must be compared (Bajjalieh, 2002). Table 43 shows nutritional values of typical soybean meal on an as-fed basis (without hulls), compared with the values for *C. thiosulfatophilum* produced from this study. As noted, *C. thiosulfatophilum* offers a superior ration of protein.

Table 43. Nutritional Composition of Soybean Meal and *C. thiosulfatophilum*.

Component	Unit	Soybean Meal	*C. thiosulfatophilum*
Crude Protein	%	47.5	56.7
Dry matter	%	90	
Crude fat	%	3	4.69
Non dietary fiber	%	8.9	
Energy			
Swine (Maine)	kgal/kg	2,020	
Poultry (Maine)	kgal/kg	2,440	
Crude Protein			
Arginine	%	3.48	2.99
Histidine	%	1.28	1.02
Isoleucine	%	2.16	3.18
Leucine	%	3.66	4.50
Lysine	%	3.02	3.60
Methionine	%	0.67	2.09
Methionine + cystine	%	1.41	.57
Phenylalanine	%	2.39	2.71
Phenylalaline +	%	4.21	
Tyrosine	%	1.85	2.04
Threonine	%	0.65	2.60
Tryptophane	%	2.27	

source (Bajjalieh, 2002 and BRI data)

The United Soybean Board has undertaken a project focused on the compositional improvement of commodity soybeans. The project is known as the Better Bean Initiative (BBI). Among other issues, the BBI plans to improve the inherent nutrient composition of soybeans relative to specified end uses. The BBI soybean meal targets comprise: increasing methionine fraction to 2.1 percent, lysine to 3.0 percent and threonine to 1.9 percent. Comparing the nutritional value of *C. thiosulfatophilum* to that of soybean meal, all three of these objectives are met with the single cell protein in this study.

Sulfur Market. The U.S. demand for sulfur has been reasonably stable for the past ten years. From 2001 (12,730 thousand long tons) through 2005 (10,650 thousand long tons) the growth was slightly negative (- 5.1 percent per year). However, projected growth is a positive 2 percent per year (ChemProfiles, 2002). Worldwide, the demand is about 50 million long tons annually, and expected to grow about 1 percent per year (Ober, 2001). The U.S. is the largest producer and consumer of sulfur.

About 96 percent of sulfur is converted into sulfuric acid, with about 50 percent of the acid used in fertilizer production. Small amounts of sulfur are used in production of

carbon disulfide, sulfur dioxide, phosphorous pentasulfide; and in pulp and paper processing and rubber vulcanizing (Ober, 2001).

Sulfur production is divided into two sectors, discretionary and nondiscretionary. Discretionary sources include the mining, primarily by the Frasch process, of sulfur or pyrites and production depends on the economics. Nondiscretionary sources include sulfur or sulfuric acid recovered as an involuntary by-product of natural gas and crude petroleum. Nondiscretionary sources represent about 95 percent of worldwide sulfur production. Nondiscretionary sulfur is primarily dependent on world demand for fuels, rather than on demand for sulfur (Ober, 2001).

Today, most sulfur is produced as elemental sulfur recovered from H_2S from natural gas or refinery acid gas streams. The U.S. producers of sulfur are listed in Table 44. Historically, the price of sulfur has varied between $25 and $100 per long ton. In the early 1990s, sulfur prices were more than $100 per long ton but have been below $80 per long ton since 1993 due to excess sulfur supplies. Significantly decreased production in the domestic phosphate fertilizer industry resulted in dramatically lower sulfur consumption and correspondingly lower prices. Sulfur prices fell to below $30 per long ton for a short period in mid-2001 due to excess supplies as phosphoric acid production was reduced. The market for sulfur might be adversely affected by large CO_2 capture, with attendant sulfur production. Hence, an average sulfur price of $50 per ton is used in the economic projections in this study.

Table 44. Sulfur Producers in the United States

Sulfur Producer	Locations	Capacity*
AtoFina Petrochemicals	2	135
BP	18	1,375
ChevronTexaco	8	1,190
CITGO Petroleum	3	370
ConocoPhillips	11	485
Delhi Gas Pipeline	4	120
Dow, Freeport, TX		150
Dynergy Midstream Services	5	285
El Paso Refining and Chemical		100
ExxonMobil	13	2,110
Jupiter Sulfur	3	145
Koch Industries	10	400
Lyondell-Citgo, Houston TX		320
Marathon Ashland Petroleum	8	390
Motiva Enterprises	4	780
Premcor	2	175
Pursue Energy, Thomasville MS		580
Republic Refining, Puckett MS		185
Shell	13	1,065
Sunoco	2	115
Tosco	4	275
Trident NGL	2	240
Valero Energy	9	440
Vintage Petroleum	2	115
Western Gas	3	160
Others**		420
Total		12, 125

*Thousands of long tons per year of elemental sulfur, excluding values produced or reclaimed in the form of sulfuric acid, hydrogen sulfide, or pyrites. Elemental sulfur is recovered from oil refinery acid gas streams, containing H_2S and SO_2, and natural gas where H_2S is recovered from the raw gas.

**Companies whose refinery or natural gas recovery capacity totals less than 100,000 long tons per year.

Source: www.the-innovations-group.com/chemprofiles/sulfur.htm (2002).

TASK 7. PROCESS ECONOMIC ANALYSIS SUMMARY

A summary of the economic evaluation of biologically removing H_2S and CO_2 from a natural gas stream is presented in the following. The details of the analysis are presented in the Appendix. The design is based upon treating 120 million SCF/day of sour gas containing 2.5 percent H_2S and 7.5 percent CO_2. Three cases are considered: biological oxidation into sulfate and cell mass; anaerobic conversion with green sulfur bacteria to elemental sulfur; and anaerobic conversion into sulfate. The latter two cases require light and two sources are considered: light supplied by LED generated with purchased electricity or generated with solar energy.

As examined in detail in the Appendix, both anaerobic systems were uneconomical. The solar lighted systems made small positive returns without capital charges, but are unprofitable when a reasonable seven percent capital charge is included.

The economics of the aerobic conversion to sulfate is given in Table 45. The total capital cost for this installation is $89 million. The operating costs total $34.7 million, including base, nutrients, utilities, labor and fixed charges. Revenue from the sale of SCP and removal of H_2S totals $44.7 million. After taxes, a cash flow of $14.9 million is generated, which provides a 16.7 percent return on investment.

It is concluded that provision of light for anaerobic photosynthetic reaction to utilize H_2S and CO_2 is not economical. A reasonable return is available for aerobic conversion of H_2S to sulfate, with the primary source of revenue from desulfurization.

Table 45. Economic Evaluation for Aerobic Sulfur Oxidizer *Thiomicrospira Crunogena*

	Aerobic Sulfur Oxidizer
Fixed Capital Investment	$89,086,000
Operating Cost	$ / year
Calcium Hydroxide (Lime Hydrate), $95/ton	9,722,000
Medium $0.055/lb cell	1,658,000
Process Water	91,000
Electricity, $0.05/kw hr	4,778,000
Steam, $5.00/m Btu	2,193,000
Cooling Water, $0.20/k gal	569,000
Waste Water Treatment	700,000
Labor, $40,000/person	1,680,999
Supervision & Over head, 50% of Labor	840,000
Maintenance, 2% FCI	1,782,000
Insurance & Taxes, 2% FCI	1,782,000
Depreciation, 10% FCI	8,909,000
Total Operating Cost	34,704,000
Revenues	
SCP, $648.00/ton	11,228,000
Credit for H_2S Removal, $0.833 k SCF	33,487,000
Total Revenue	44,715,000
Profit Before Tax	10,011,000
Taxes	4,005,000
Profit After Tax	6,006,000
Cash Flow	14,915,000
Return	16.74%
Pay out	5.97 years

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APPENDIX

PROCESS ECONOMIC ANALYSIS

The economic evaluation is based on the design to treat 120 million SCF/day gas containing 2.5 percent H_2S and 7.5 percent CO_2. There are three process designs used to evaluate the potential economics (Figures A1 – A5). The first case is to utilize a sulfur oxidizer to aerobically convert sulfide and carbonate in a sour natural gas scrubber purge into sulfate and cell mass. The second and third cases use green sulfur bacteria to directly react H_2S and CO_2 in the sour natural gas inside anaerobic fermentor, requiring external light. The difference between these two Cases is that Case 2 produces elemental sulfur and Case 3 produces sulfate. Also two light supply systems are investigated. The LED system utilizes electricity to generate the light required. The Sun Light system utilizes solar light collection and fiber optics to transport light into the fermentor. However, because sun light is limited, the fermentation is assumed to be operated one-third of the time and assumes the culture can remain dormant during dark periods. Therefore, the fermentors for the Sun-light system will be three times larger than LED system. Figures A1 through A5 show the process diagram for these Cases. The detail evaluations for these cases are listed in the Appendix

Table A1 provides the design criteria used in each case. There are 8333.28 lb-mole/day H_2S and 25,000 lb-mole/day CO_2 in the feed gas. Assuming the cell body has 50 percent carbon, cell production is projected from CO_2 fixation to be 90,000, 100,000, and 400,000 lbs/day for case 1, 2, and 3 respectively.

Table A1. Design Criteria for all Cases

	Case 1	Case 2	Case 3
Culture	Sulfur Oxidizing Bacteria	Green Sulfur Bacteria	Green Sulfur Bacteria
Fermentation	Aerobic	Anaerobic & Light	Anaerobic & Light
Sulfur Product	Sulfate	Sulfur	Sulfate
O_2 per mole of Sulfide Converted	4 moles	0 moles	0 moles
O_2 conversion	60%	0%	0%
Gas Retention Time	5 min	23 min	23 min
Fermentor Pressure	2 atm	5 atm	5 atm
CO_2 fixed per mole sulfide	0.45 moles	0.5 moles	2.0 moles
Photons Required Per molecular sulfide	None	4 photons	16 photons
CO_2 Fixed	3750 lb-mole/day	4167 lb-mole/day	16,667 lb-mole/day
Cell Production	90,000 lb/day	100,000 lb/day	400,000 lb/day

To estimate the fixed capital investment from delivered equipment cost, the Chilton method is used (Chilton, 1960). The selection and factors for this process are listed in Table A2. Since this is a speculated process, a contingency of 25 percent of total physical cost is used. The new facility also increases the factor for utilities. The factor to convert delivered equipment cost to Fixed Capital Investment is 3.836. However, this ratio is not used on all equipment. Agitator cost is one of the more expensive items in the list and it could be as high as 34 to 39 percent of all equipment cost. The installation and set up of the agitator is much less involved than other equipment items. The agitator is a module of equipment and therefore fixed capital

investment to equipment ratio is reduced to 1.5 times. The light supply system is also treated in the same manner.

Table A2. Chilton Method Used

No.	Item	Selection	Factor	Reference No.	Factor Applied to No.1
1	Delivered Equipment Cost		1.0	1	1.0
2	Installation		0.43	1	1.43
3	Piping	Fluid	0.4	2	0.572
4	Instrumentation	Some	0.1	2	0.143
5	Building & Site Development	Out Door	0.10	2	0.143
6	Auxiliaries	New Facility	0.25	2	0.3575
7	Out side Lines	None	0.0	2	0.0
8	Total Physical Cost = Sum from No. 2 to No.7		1.92	2	2.6455
9	Engineering & Construction	Simple	0.2	8	0.5291
10	Contingency	Speculative	0.25	8	0.6614
11	Size Factor	Large	0.00	8	0.0
12	Total Fixed Capital = Sum of No. 8 to No. 11		1.5	8	3.836

Based on medium formulation used in the laboratory, the largest medium cost is in the sodium chloride to maintain an ionic strength in the fermentation broth as in sea water. Sodium chloride is not used by the culture. Therefore, to reduce cost it can be assumed that the plant is built in coastal area and sea water can be pumped in with 300 ft-lbf/lb head. The next major cost is the nitrogen source. Assuming 14 percent of cell weight is nitrogen, 12,600 lb/day or 525 lb/hr nitrogen is needed, or 37.5 lb-mole/hr NH_3, using Case 1 as an example. Using an ammonia price of $500/ton, the ammonia cost is $159.38/hr. Assuming the rest of the medium is $46.87/hour. Then the medium cost is about $206.25/hour, or $0.055/lb of cells. The medium cost for Case 2 and Case 3 are also set as $0.055/lb of cell mass.

Base usage to maintain pH varies for each case. In Case 1, H_2S and CO_2 need to be scrubbed from sour natural gas in four 10 foot diameter by 30 foot high scrubbers at a pH of about 8.5, since the first pKa for H_2S is at about 6.97. The gas retention time in the scrubber is above one minute if the gas pressure is over 9 atmospheres. The scrubbing water to gas feed ratio used is 18 gallons per 1000 SCF of sour natural gas. This ratio will result in 1.5 percent sulfide solution in the scrubber purge water comprising about 2.80 percent dissolved carbonic acid, bi-carbonate and carbonate. Since the pH is around 8.5, dissolved sulfide will stay as HS^- and dissolved CO_2 as bi-carbonate. Therefore, each mole of dissolved sulfide and CO_2 require one mole of NaOH or ½ $Ca(OH)_2$ added to maintain the pH in the operating range. Sodium hydroxide usage is expected as 688 lb-mole/hr or 344 lb-mole/hr calcium hydroxide. For Case 2 and 3, reactions take place with gas scrubbing. When carbon dioxide is fixed, Na^+ or Ca^{+2} are balanced with carbonate released and available to be reused. For Case 2, each mole of elemental sulfur produced, one mole Na^+ or ½ mole Ca^{+2} will be released to be reused, reducing the caustic soda requirement. One third of the base usage in Case 1 is estimated for Case 2. As for Case 3, since sulfate is the final product of sulfide, each mole of sulfide requires 2 moles of Na^+ or 1mole Ca^{2+} to keep the broth at the high pH, even though all Na^+ or Ca^{+2} in carbonate will be released and reused. The base usage is than set as 2 X 8333.28 lbmole/day sodium hydroxide or 8333.28 lb mole/day calcium hydroxide. Current Sodium hydroxide price is about $300/ton and would significant portion of operation cost. According 2006 Minerals yearbook

(Miller, 2007), the average calcium hydroxide prices for 2006 was $89.20/ton. Assuming using $95/ton, the base cost is significantly reduced.

Revenues include single cell protein and credit for removing sulfur. It is assumed that dried culture can be sold as a replacement for soybean with the price adjusted to protein percentage. The average protein concentration in the U.S. is 35 percent (Rankin, 2007). The *Clostridium thiosulfatophilum* culture cell has a measured protein content of 56.7. A bushel of soybean weighs about 60 pounds with a 13 percent moisture content. The mid 2008 price is around $6 per bushel. So the *C. thiosulfatophilum* culture at 13 percent moisture should sell for $648/ton. Since H_2S and CO_2 come from sour natural gas, there is a credit for sweetening sour natural gas. The H_2S removal cost can be distinguished into two scale ranges (McIntush, *et. al*., 2002). For a small amount of sulfur, less than 0.2 long tons per day, an H_2S scavenger can be used. Even though operating cost may range from $10,000 to $100,000 per LT sulfur removed but capital costs are generally low. For larger scale treating, greater than 30 to 100 LT/D, Amine/Claus/tail gas treatment combinations are more economical. Operating costs are generally low, in the range of $100/LT to $200/LT, but capital costs can be high. Since 121.21 LT/D sulfur is removed, the credit should be based on the Amine/Claus/tail gas treatment combinations. After correcting with the Chemical Cost Index, the operating cost should range between $120/LT to $240/LT. Higher end, $240/LT, is used in this estimation. The capital estimation is based on Garrett (1989). The capital cost for natural gas purification is 170 millions dollars for sour gas with sulfur and liquid recovery for 120 million SCFD. The capital is $251.8 million. Assuming a 7 percent interest rate with a 20 years return (0.0944 x FCI), the capital cost is equivalent to $23.8 million per year, or $585/LT sulfur removed. The projected credit for sulfur removal credit should be $240 + $585 = $825/LT of sulfur removed, or $0.833/1000 SCF. Currently natural gas prices are over $8.00 per million Btu, (about 1000 SCF). Sweetening cost is about 10 to 15 percent of natural gas, which is a reasonable estimation. There is no credit for CO_2 removal as bi-carbonate or carbonate dissolved at high pH since during waste water treatment these compounds will become CO_2 gas again.

The economic evaluation for an aerobic sulfur oxidizer is listed in Table A3. The fixed capital investment is $89 million. The operating cost, including 10 percent depreciation is $34.7 million per year. The revenues include SCP and credit for H_2S removal from sour natural gas is $44.7 million per year. The profit before tax is $10 million per year. The profit after tax and cash flow is $6 million and $14.9 million per year respectively. The return of investment is 16.74 percent and capital pay back period is 5.97 years.

The economic evaluations for anaerobic fermentation are presented as required CO_2 credit to break even on operating and total costs, without profit. Total cost including operating cost and capital pay out charge which is estimated in 20 years with 7 percent interest, or 0.0944 times of Fixed Capital Investment per year. Table 44 lists economical evaluation for sulfur production. Anaerobic sulfate production is discussed in Table A5.

Table A3. Economic Evaluation for Aerobic Sulfur Oxidizer *Thiomicrospira Crunogena*

	Aerobic Sulfur Oxidizer
Fixed Capital Investment	$89,086,000
Operating Cost	$ / year
Calcium Hydroxide (Lime Hydrate), $95/ton	9,722,000
Medium $0.055/lb cell	1,658,000
Process Water	91,000
Electricity, $0.05/kw hr	4,778,000
Steam, $5.00/m Btu	2,193,000
Cooling Water, $0.20/k gal	569,000
Waste Water Treatment	700,000
Labor, $40,000/person	1,680,999
Supervision & Over head, 50% of Labor	840,000
Maintenance, 2% FCI	1,782,000
Insurance & Taxes, 2% FCI	1,782,000
Depreciation, 10% FCI	8,909,000
Total Operating Cost	34,704,000
Revenues	
SCP, $648.00/ton	11,228,000
Credit for H_2S Removal, $0.833 k SCF	33,487,000
Total Revenue	44,715,000
Profit Before Tax	10,011,000
Taxes	4,005,000
Profit After Tax	6,006,000
Cash Flow	14,915,000
Return	16.74%
Pay out	5.97 years

Evaluation for Case 2, anaerobic sulfur producer, is listed in Table A4 with two different light supply systems. The Fixed Capital Investment for LED and Sun-Light systems are $133.2 and $461.4 million respectively. The Sun-Light system requires three-times the fermentors and the sunlight collection system is more expensive. However, the operating cost is much less for the Sun-Light System compared to LED system, $35.9 vs. $84.9 million per year. The major difference is the electricity usage of $64 million per year for the LED vs. only $1.9 million per year for the Sun-Light system. But capital related operating charges, maintenance & insurance & taxes, are higher for the Sun-Light System. The capital repayment charges are $12.6 million per year for the LED system and $43.561 million per year for the Sun-Light System; resulting in $97.465 and $79.469 million per year in total cost. The revenue, excluding any CO_2 credits, is $48.2 million per year for both systems. The CO_2 credits that would be required to break even is $36.7 million per year for the LED system and $12.3 million for the Sun-Light system. These credits would translate into $1764.7/LT and $1120.1/LT CO_2 for LED and Sun-Light Systems.

Table A4. Economic Evaluation for Sulfur Production by Green Sulfur Bacteria

	LED	Sun-Light
Fixed Capital Investment	$ 133,196,000	$ 461,450,000
Operating Costs	$ / year	$ / year
Medium, $0.055/lb Cell	1,843,000	1,843,000
Calcium Hydroxide (Hydrate Lime), $95.00/LT	3,241,000	3,241,00
Process Water, $0.25 /K gal	133,000	133,000
Electricity, $ 0.05/kw-hr	64,043,000	1,930,000
Steam $5.00/m Btu	4,167,000	4,167,000
Cooling Water , $0.05/k gal	2,317,000	2,317,000
Waste Water treatment	795,000	795,000
Labor, $40,000/man	2,016,000	2,016,000
Supervision & Over head, 50% of Labor	1,008,000	1,008,000
Maintenance, 2% FCI	2,664,000	9,229,000
Insurance & Tax , 2% FCI	2,664,000	9,229,000
Total Operating Cost	84,891,000	35,908,000
Capital Repayment, 7% 20 yrs, 0.0944 * FCI	12,574,000	43,561,000
Total Cost	97,465,000	79,469,000
Revenues, excluding CO_2 Credit		
SCP, $648.00/ton, 114942 lb/day	12,476,000	12,476,000
Sulfur, 133.332 TPD, $50/Ton	2,233,000	2,233,000
Credit for H_2S Removal, $0.833/1000 SCF, 120 m SCFD	33,487,000	33,487,000
Total Revenues	48,196,000	48,196,000
Required Total CO_2 Credit to Breakeven on Operating Cost	36,695,000	-12,288,000
Required CO_2 Credit to Breakeven on Operating Cost	$ 1314.34/LT	0
Required Total CO_2 Credit to Breakeven on Total Cost	49,269,000	31,273,000
Required CO_2 Credit to Breakeven on Total Cost	1764.72$/LT	1120.14$/LT

Table A5 lists economic evaluations for anaerobic sulfate production. The fixed capital investment for LED is much smaller than the Sun-Light anaerobic case, $282.0 million vs. $1133.5 million. However, operating cost for Sun-Light system is only $76.089 million per year while LED system required $283.657 million per year. LED system has less capital repayment, $26.6 million per year vs. $107 million per year in Sun-Light System. Total costs are $310.3 and $183.1 million per year for the LED and Sun-Light systems. The revenue, excluding CO_2 credits, is $85.6 million per year for both anaerobic systems. The revenue for Sun-Light system is $8.477 million per year more than the operating cost. So, there is no need for CO_2 credits to break even on operating cost. However, the LED system requires $1782.87 per metric ton credit to break even with the operating cost. For break even with total cost, the Sun-Light

System requires $882.34 /LT CO_2 credit to break even and LED System requires $2,021.29/LT credit.

Conclusion

In conclusion, the aerobic sulfur oxidizer has the best economic projection, not only does it not need any CO_2 credit to break even with the total cost, but also produces a return of investment of 16.74 percent. An additional sour gas scrubber is needed for this aerobic fermentation and the least amount of CO_2 is fixed, but no light is required so both capital and operating costs are lower. The Sun-Light System in both sulfur and sulfate production do not need any CO_2 credit to break even in operating cost but require high CO_2 credit, $ 882.34/LT for sulfate and $1120.14 /LT for sulfur production, to balance total cost due to high capital. As for the LED system, the high electricity usage inhibits the economics with the other cases.

Table A5. Economic Evaluation for Sulfate Production by Anaerobic Green Sulfur Bacteria

	LED	Sun Light
Fixed Capital Investment	$ 282,036,000	$ 1,133,552,000
Operating Cost	$ / year	$ / year
Calcium Hydroxide (Lime Hydrate), $95.00/LT	9,813,000	9,813,000
Medium $0.055/lb cell	7,370,000	7,370,000
Process Water	328,000	328,000
Electricity, $ 0.05/kw hr	243,095,000	1,984,000
Steam, $5.00/m Btu	4,603,000	4,603,000
Cooling Water, $0.2/k gal	1,159,000	1,159,000
Waste Water Treatment	1,471,000	1,471,000
Labor, $40,000/man	3,024,000	3,024,000
Supervision & Over head, 50% of Labor	1,512,000	1,512,000
Maintenance, 2% FCI	5,641,000	22,671,000
Insurance & Taxes, 2% FCI	5,641,000	22,671,000
Total Operating Cost	$283,657,000	$76,089,000
Capital Repayment, 7%, 20 year return, 0.0944 x FCI	26,624,000	107,007,000
Total Cost	310,281,000	183,096,000
Revenues		
SCP, $648.00/ton,	51,079,000	51,079,000
Credit for H_2S Removal, $0.833/k SCF	33,487,000	33,487,000
Total Revenues	84,566,000	84,566,000
Required Total CO_2 Credit to Breakeven on Operating Cost	199,091,000	-8,477,000
Required CO_2 Credit to Breakeven on Operating Cost	$1782.87/LT	Not Need
Required CO_2 Credit to Breakeven on Total Cost	225,715,000	98,530,000
Required CO_2 Credit to Breakeven on Total Cost	$2021.29/LT	$882.34/LT

Figure A1. Case 1 *Thiocrospira Crunogena* CO_2 Fixation

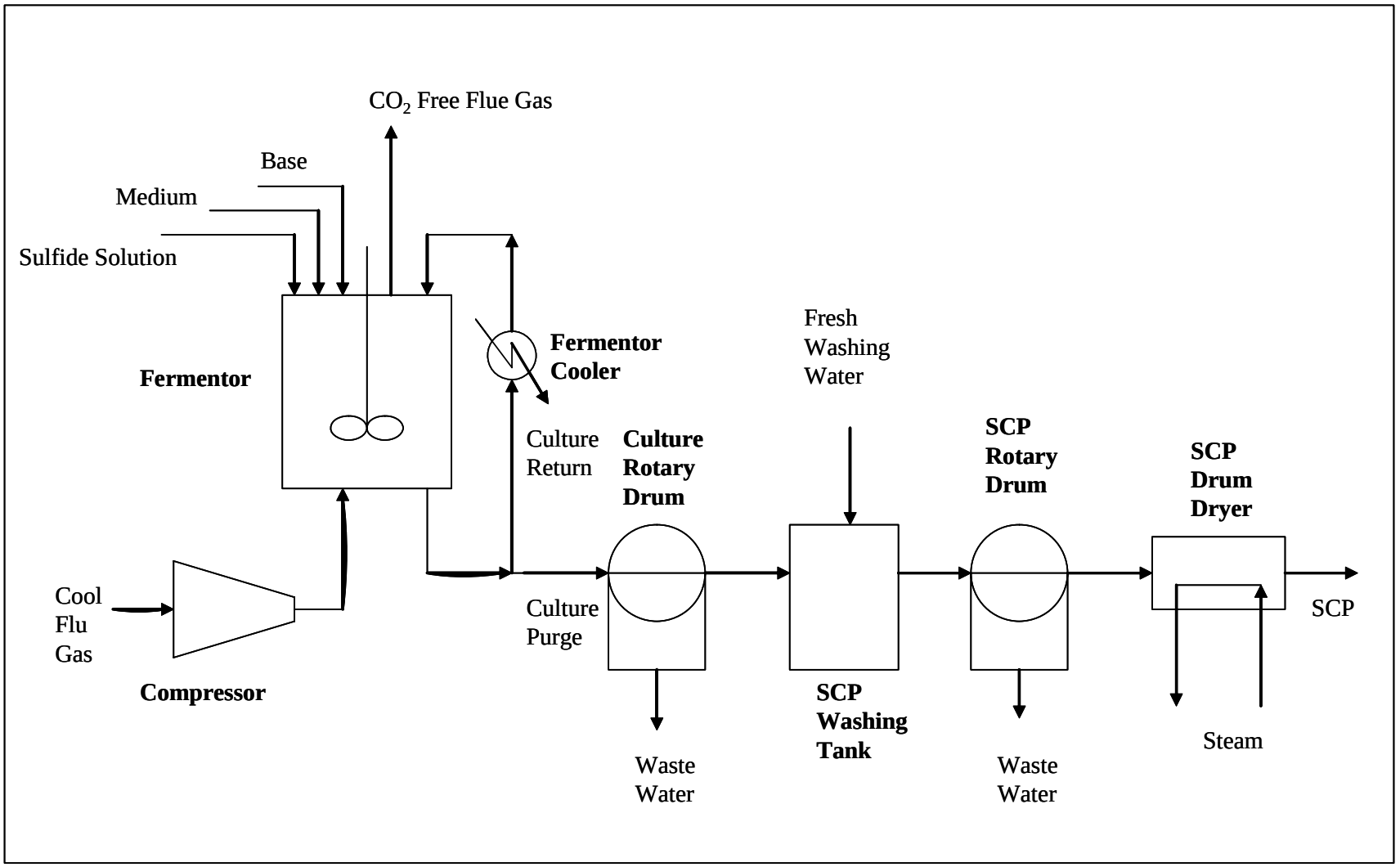

Figure A2. Case 2a Sulfur Production from Green Sulfur Bacterial Using LED Light

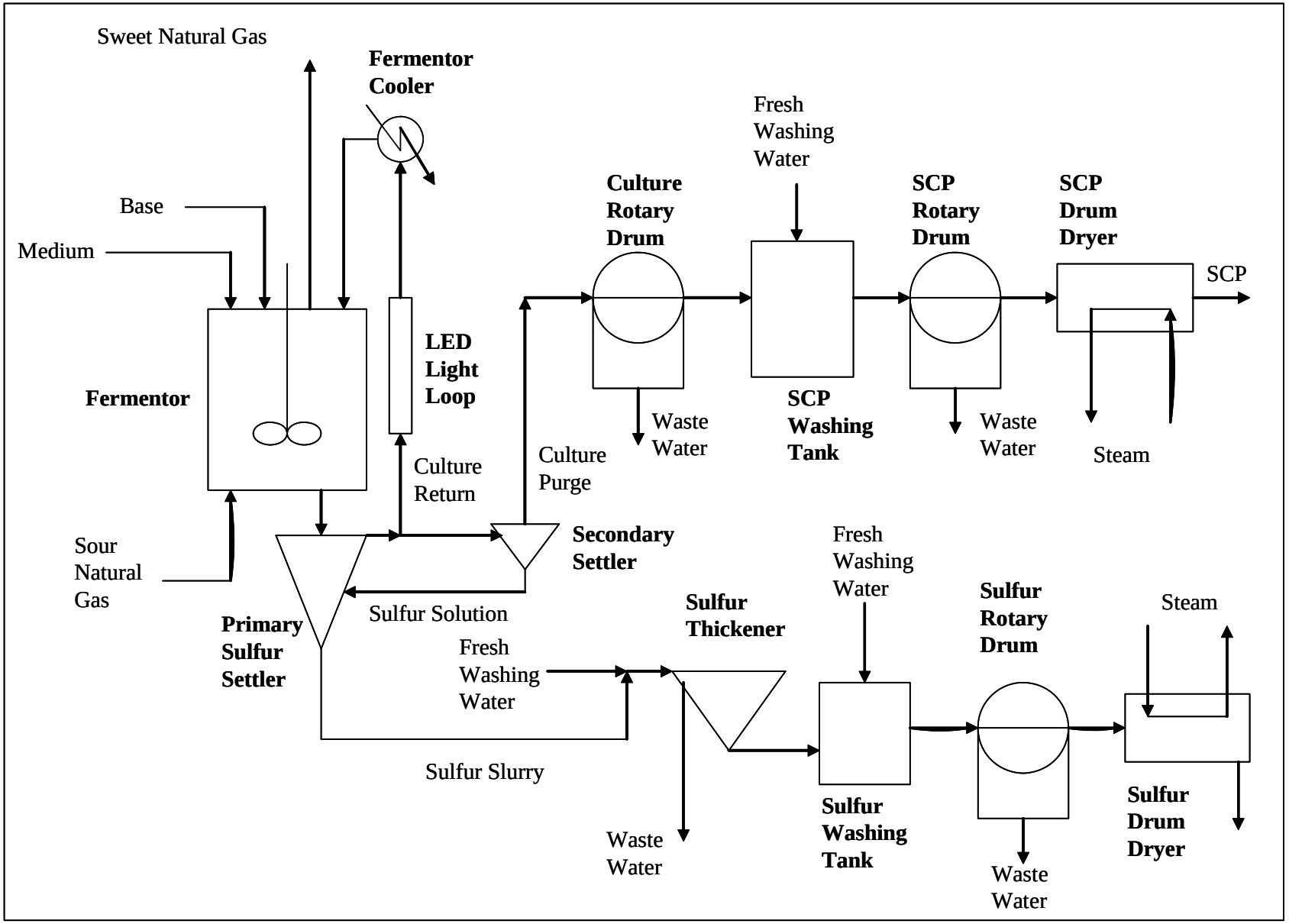

Figure A3. Case 2b Sulfur Production from Green Sulfur Bacterial Using Sun light

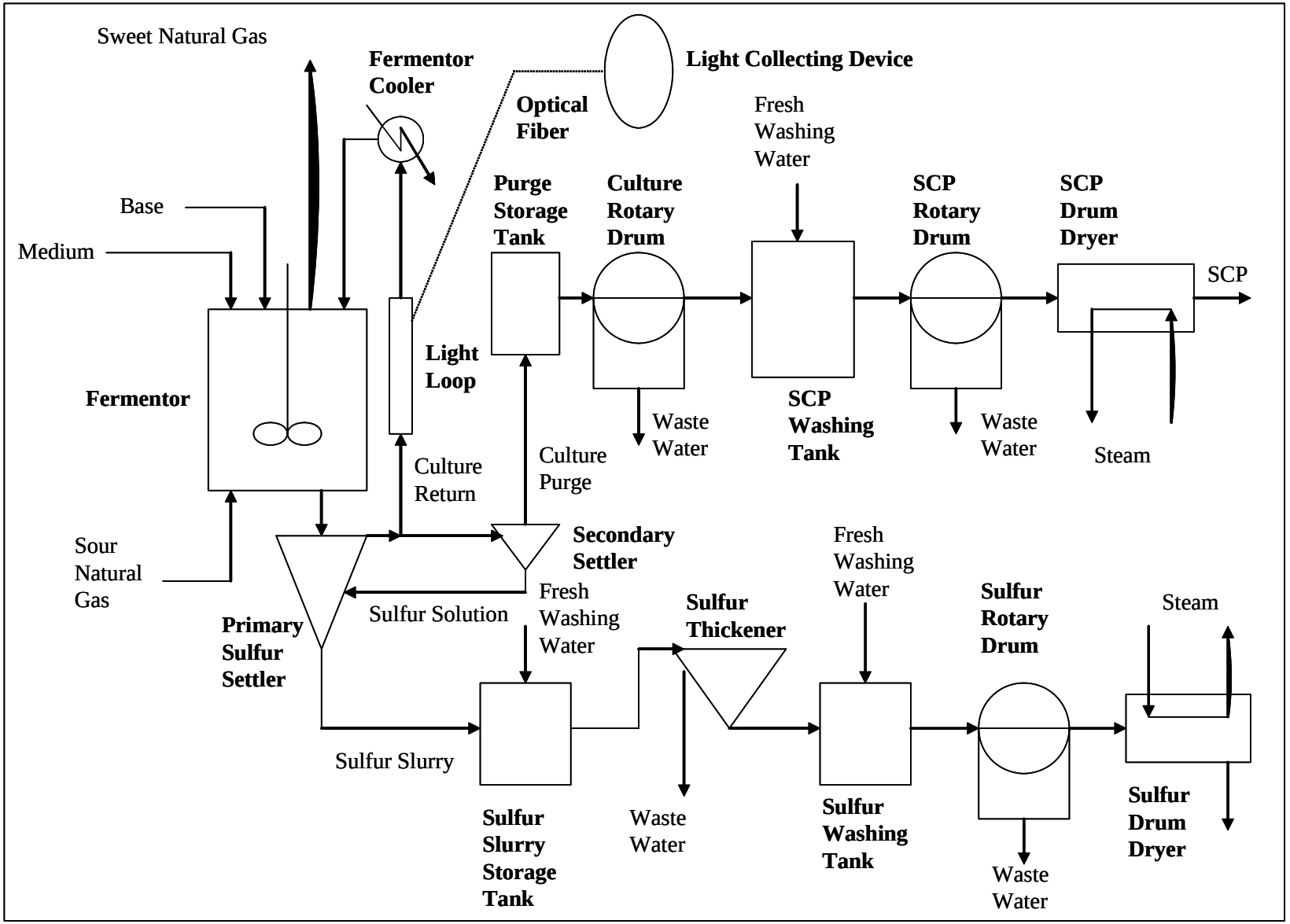

Figure A4. Case 3a Green Sulfur Bacterial to Sulfate Using LED Light

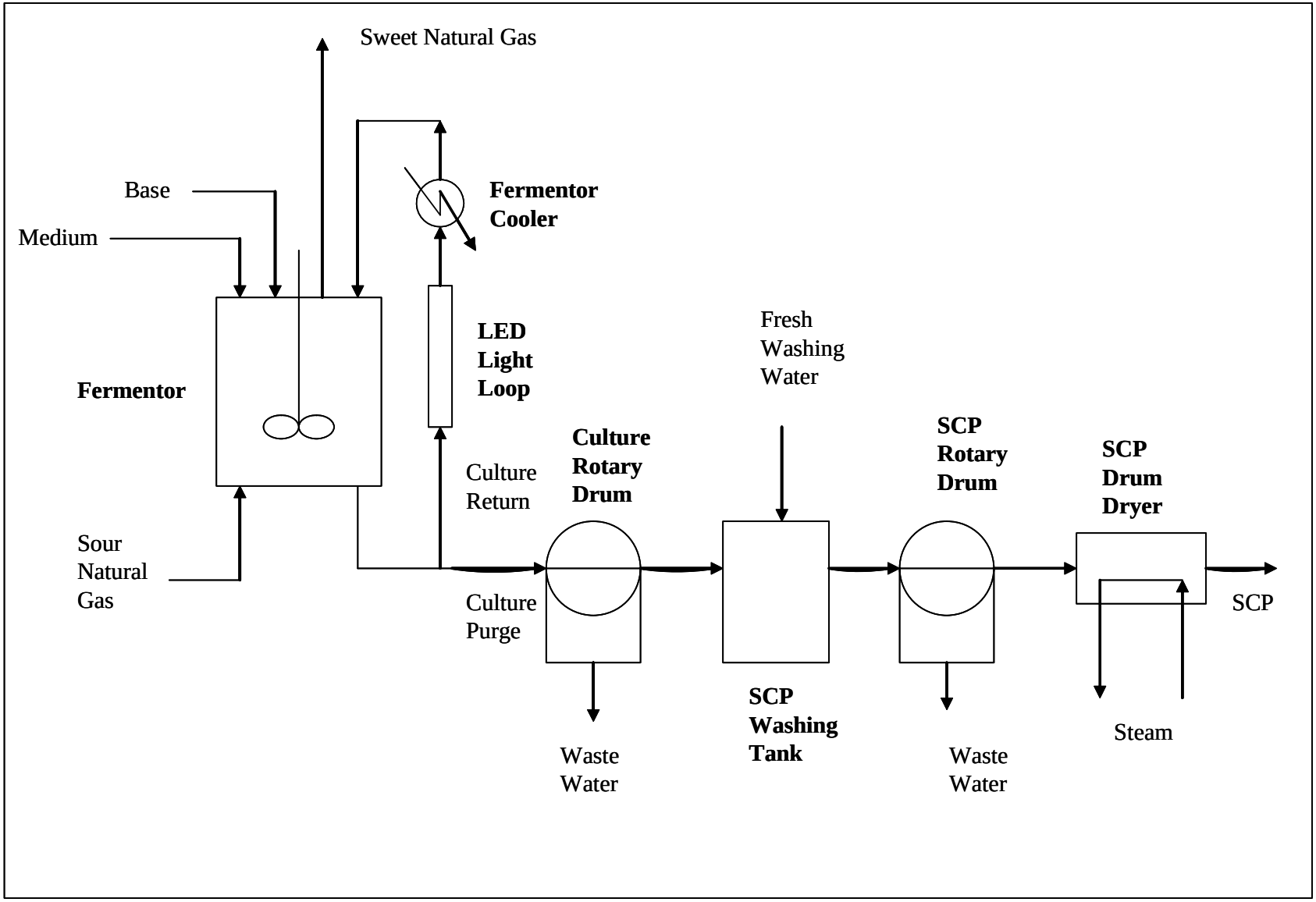

Figure A5. Case 3b Green Sulfur Bacterial to Sulfate Using Sun Light