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Conversion of Hydrogen Sulfide to Elemental Sulfur by *Chlorobium thiosulfatophilum* in a CSTR with a Sulfur-Settling Separator†

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ABSTRACT

Chlorobium thiosulfatophilum may be used for the bioconversion of hydrogen sulfide to elemental sulfur or sulfate. Sulfur is the preferred product because of problems in the disposal of sulfate. A CSTR with a sulfur-settling separator has been used to preferentially produce and recover elemental sulfur. The simple nutritional requirements of the bacterium and differences in densities and average cell and sulfur particle sizes make a CSTR with a sulfur-settling separator attractive. A bench-scale study has been carried out to determine the optimum process conditions to maximize H_2S conversion, cell growth, elemental sulfur production, and to minimize sulfate production. The liquid effluent typically contained about 425–550 mg/L elemental sulfur. The sulfate concentration was maintained at levels below 100 mg/L. It was possible to remove up to 57 μmol min^{-1} L^{-1} of H_2S from the gas stream. An experiment over a period of 392 h showed stable performance.

Index Entries: Hydrogen sulfide; *Chlorobium thiosulfatophilum*; desulfurization; photobioreactor; photosynthetic bacterium.

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INTRODUCTION

Current process gas desulfurization technology employs ammonia, alkanolamines, or alkaline salt solutions as absorbents (*1*). Hydrogen sulfide, after absorption and desorption, goes through the well-known Claus process for elemental sulfur recovery. The tail gas is further treated to decrease the hydrogen sulfide concentration in a Tail Gas Clean Up (TGCU) process. Sulfur dioxide is the final sulfur product, since released gases from TGCU need to be incinerated before venting. The primary process is focused on the economics of sulfur recovery from hydrogen sulfide, whereas the secondary process must reduce the hydrogen sulfide concentration to 5–30 ppm or less for discharge (*2*).

The Claus process operates most effectively on H_2S and CO_2 feed gas streams containing at least 15 mol% H_2S (*3*). If the feed gas contains lower levels of H_2S, the Claus catalysis becomes less efficient. Commercial clean-up processes are designed to incinerate 200–500 ppm hydrogen sulfide, concentrations that are difficult to recycle to the Claus process because of limited efficiency.

Cork (*3*) suggested that biological desulfurization by *C. thiosulfatophilum* could be an alternative to the traditional Claus process. In the presence of light, *C. thiosulfatophilum* accepts electrons by biooxidation of hydrogen sulfide to produce elemental sulfur.

$$2H_2S + CO_2 + h\nu = 2S^\circ + (CH_2O) + H_2O \quad (1)$$

If more light is available than necessary for Eq. (1), elemental sulfur is oxidized further to sulfate.

$$H_2S + 2CO_2 + 2H_2O + h\nu = 2(CH_2O) + H_2SO_4 \quad (2)$$

Realizing that elemental sulfur is not the only sulfur product obtained from hydrogen sulfide, Cork and Ma (*4*) investigated the control of oxidative sulfur metabolism by the bacterium in order to maximize elemental sulfur production in a semibatch reactor. Experiments showed that reducing the hydrogen sulfide feed rate resulted in the formation of more sulfate and thiosulfate, but less molecular sulfur. An exponentially decreasing sulfate yield, and thus increased sulfur yield, was found with increasing hydrogen sulfide feed rate.

Maka and Cork (*5*) studied the effect of light intensity, surface area of the illuminated bioreactor, H_2S flow rate, and various wavelength regions of light on oxidative sulfur metabolism by *C. thiosulfatophilum* in a fed-batch reactor. Kim et al. (*6*) and Kim and Chang (*7*) studied the removal of hydrogen sulfide by *C. thiosulfatophilum* in immobilized-cell and sulfur-settling free-cell recycle reactors. In these fed-batch studies, the immobilized-cell reactor required 30% less light energy for the same conversion in comparison to the sulfur-settling free-cell recycle reactor. However, the performance of the immobilized-cell reactor dropped significantly after 40

h of operation because of sulfur deposition inside the beads. Kim and Chang also reported that 80% of the sulfur excreted by the free cells could be removed in a settler.

Kim et al. (2) studied the growth kinetics of *C. thiosulfatophilum* in a fed-batch reactor. They observed sulfide inhibition at sulfide concentrations of 5.7 m*M*, and light inhibition at average light intensities of 40,000 lx. They developed a mathematical model to describe the cell growth by considering the light attenuation factor owing to scattering and absorption and the crowding effect of the cells.

Most data available in the literature are obtained by fed-batch operation of the reactors. In this mode of operation, the gas phase flows continuously through the reactor, whereas there is no flow of the liquid phase. Reactor performance drops sharply within a few days because of sulfur deposition on the reactor surface causing light attenuation and also because of toxic metabolite accumulation. A very limited amount of data has been reported for the semisteady-state removal of hydrogen sulfide using a membrane recycle reactor with a sulfur settler (*7*). The maximum H_2S removal reported in this study was 38.5 μmol min^{-1} L^{-1}.

In this study, *C. thiosulfatophilum* has been used for the bioconversion of hydrogen sulfide to elemental sulfur or sulfate. A CSTR with a sulfur-settling separator has been used to produce and recover elemental sulfur preferentially. A bench-scale study has been carried out to determine the optimum process conditions to maximize H_2S conversion, cell growth, and elemental sulfur production in the system.

MATERIALS AND METHODS

Microorganism and Medium

Chlorobium thiosulfatophilum (ATCC 17092) was obtained from the American Type Culture Collection (Rockville, MD). It was grown on a basal medium containing (per 1 L): yeast extract (Difco), 5.0 g; Pfennig's minerals solution (*8*), 50 mL; Pfennig's trace metals solution (*9*), 1 mL; and sodium bicarbonate, 4.0 g.

Equipment and Procedures

The continuous stirred-tank reactor used was a New Brunswick Scientific (Edison, NJ) BioFlo C30 fermenter. The fermenter was modified to operate under strict anaerobic conditions with both continuous gas and liquid flow. The liquid working volume was 1250 mL, and the overhead gas volume was 750 mL. Illumination necessary for growth was supplied by two tungsten lights (120° apart) directed toward the glass fermentation vessel from a distance of approx 20 cm. Two 60-W bulbs were used in all of the experiments, except for the light-variation studies and long-term

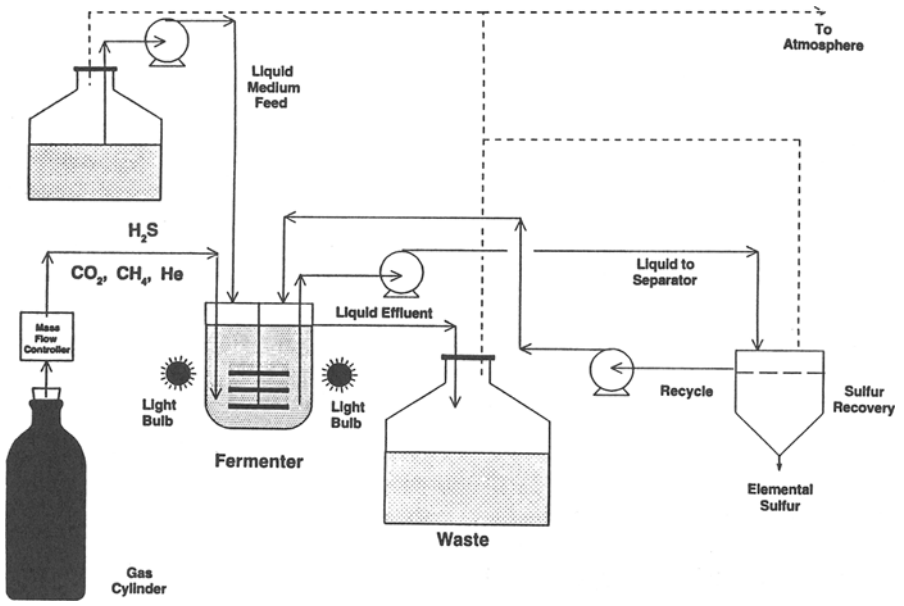

Fig. 1. Schematic diagram of the experimental setup.

behavior study. Experiments were carried out at 30°C and pH 7. The pH remained near 7, and control was not necessary. The feed gas used was a mixture of H_2S, CO_2, CH_4, and He (2.5/10.0/15.0/72.5 %v/v) and was continuously fed to the reactor at atmospheric pressure. A schematic of the equipment setup is shown in Fig. 1. A 475-mL separatory flask with a side arm was used for the rapid separation of solid sulfur from the recycle liquid stream (recycle rate: 70 mL/min). The separator recycle loop was removed for those experiments performed without sulfur recovery.

Typically experiments were started with a constant agitation rate and gas flow rate. The gas flow rate (standard mL/min, 0°C, 760 mm Hg) was measured with a mass flow controller (Edwards High Vacuum International, Wilmington, MA). The liquid flow rate was measured with a test tube and a stop watch, and by measuring the weight of the collected liquid. The conversion of H_2S, cell density, and concentrations of sulfide, sulfate, and elemental sulfur were monitored. Experiments were considered complete when the gas conversion and cell density leveled off, and steady state was reached. It typically took 24–48 h to reach steady state. The liquid flow rate was then increased for the liquid flow variation studies, or the gas flow rate was increased for the gas flow variation studies.

Analytical Techniques

Liquid and gas samples were withdrawn anaerobically from the reactors during cultivation, and analyzed for liquid- and gas-phase concentrations. Gas samples were withdrawn with a gas tight syringe. The syringes were flushed with helium before any gas or liquid samples were with-

drawn. The dry cell weight concentration was obtained by centrifuging 1 mL of liquid sample for 2 min at 15,000 rpm, discarding the top phase, and resuspending the cells in 1 mL of methanol to extract chlorophyl. After an additional centrifugation cycle, the green-colored methanol extract was combined with 1.5 mL of fresh methanol. The absorbance was measured at 670 nm on a Spectronic 21 spectrophotometer (Milton Roy Co., Rochester, NY) and converted to dry weight cell concentration using a calibration curve.

Sulfide in the liquid phase was measured in sulfide antioxidant buffer (SAOB) using a Corning Silver/Sulfide electrode (Corning Glass Works, Medfield, MA) and a Corning double-junction reference electrode in conjunction with an Orion specific ion meter, model 407A (Orion Res. Inc., Cambridge, MA). The samples in the SAOB buffer were frozen to stop microbial activity until sulfide analyses were performed. Sulfur in the liquid sample was settled by centrifugation and then mixed with acetone. The acetone was then evaporated, and the dried sulfur was dissolved in chloroform. The concentration of elemental sulfur was determined spectrophotometrically at 290 nm in combination with a standard curve (*7*). Sulfate in the liquid sample was measured turbidimetrically at 420 nm using a standard curve prepared with sodium sulfate solution (*10*). Conditioning reagent (0.25 mL) was first added to a 5.0-mL aliquot of the centrifuged sample. After stirring the solution, 1.0 mL of 0.24 g/mL $BaCl_2$ solution was added to initiate the reaction to $BaSO_4$.

Gas analyses were performed on a gas chromatograph (Hewlett-Packard 5890 Series II gas chromatograph and HP 3396A integrator, Avondale, CA) using a 3 mm × 1.8 m PTFE column packed with Chromosorb 107, 80/100 mesh (Alltech, Deerfield, IL). The oven temperature was maintained at 80°C, whereas the injector and thermal conductivity detector temperatures were 175°C. Helium at 30 mL/min was used as the carrier gas. Light intensities were measured with a LX-101 digital lux meter (Cole-Parmer, Chicago, IL).

RESULTS AND DISCUSSION

Experiments were first performed without sulfur recovery to determine an optimum liquid dilution rate for cell growth. Once the optimum liquid dilution rate was determined, further experiments were performed to determine the maximum H_2S removal rate without sulfur recovery. The sulfur settler was then added to the recycle loop to separate sulfur from the liquid effluent at different gas flow rates. A light intensity variation study was then performed to maximize the performance of the reactor. Once the optimum liquid dilution rate, gas flow rate, and light intensity were determined, further experiments were carried out for a long period of time to demonstrate the stability of the process.

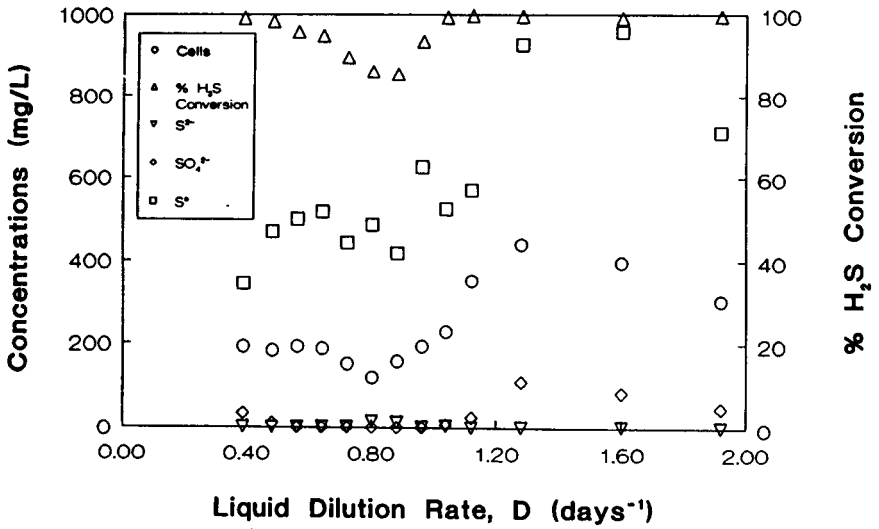

Fig. 2. Performance of CSTR without sulfur recovery using *C. thiosulfatophilum* at various liquid dilution rates (gas retention time: 62.5 min).

The reactor was first operated without sulfur recovery at a constant gas flow rate of 20 standard mL/min (62.5 min gas retention time), while varying the dilution rate (based on reactor volume), D, from 0.38 to 1.92 d^{-1}. The use of two 60-W bulbs to supply the necessary light energy had been shown in preliminary studies to give a high yield of elemental sulfur while minimizing sulfate formation (data not shown). Figure 2 shows the results of the liquid dilution rate study with cell concentration, H_2S conversion, and liquid-phase sulfur species (S^{2-}, SO_4^{2-}, and S^o) concentrations plotted as a function of dilution rate. As is noted in the figure, the H_2S conversion was nearly 100% for all of the dilution rates studied. The exception to this result is the dip in conversion found while operating at dilution rates of 0.7–1.0 d^{-1}. This was because of gradual deposition on sulfur on the reactor wall surface and subsequent light attenuation. This problem was alleviated by reducing the gas flow rate. In the absence of sufficient H_2S, the bacteria consumes the sulfur particles on the wall, and the reactor surface gradually becomes transparent. Normal reactor operation was then resumed. The cell concentration showed typical Monod behavior, increasing from 192 mg/L at a 0.4 d^{-1} dilution rate to 440 mg/L at a 1.3 d^{-1} dilution rate, before falling gradually as the dilution rate was further increased. The dissolved S^{2-} concentration, corresponding to the accumulated H_2S dissolved in the liquid phase, remained near zero for all dilution rates. Almost all of the sulfur product was present as elemental sulfur (400–1000 mg/L), whereas the sulfate concentration remained at 0–110 mg/L. In fact, the sulfate concentration was 0 at dilution rates below 1.20 d^{-1}.

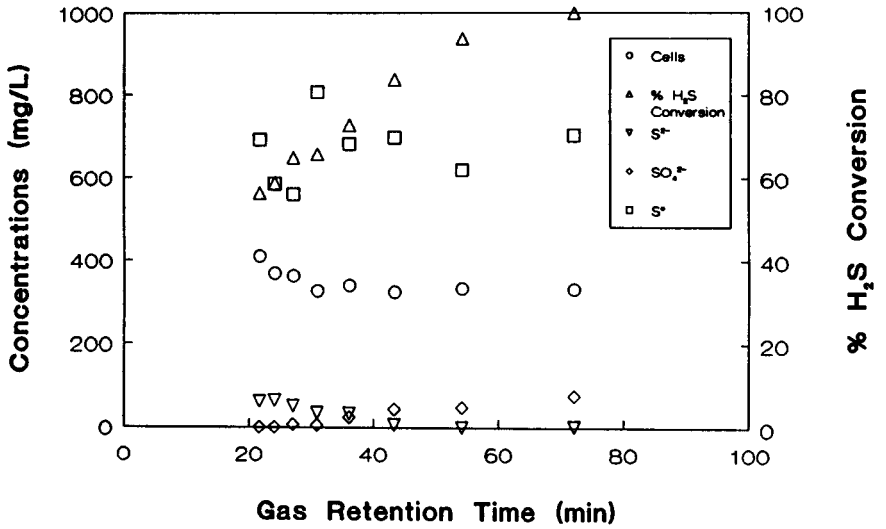

Fig. 3. Performance of the CSTR without sulfur recovery using *C. thiosulfatophilum* at various gas retention times (liquid dilution rate: 1.25 d^{-1}).

A second study was performed in the CSTR without sulfur recovery in which the gas retention time was varied at a constant liquid dilution rate of 1.25 d^{-1}. Figure 3 shows the results of this study, where cell concentration, H_2S conversion, and sulfur species concentrations are again plotted as a function of gas retention time. As is noted in Fig. 3, the H_2S conversion increased almost linearly with gas retention time, ranging from just under 60% at a 22-min gas retention time to 99.99% at a 72-min gas retention time. As expected, the cell concentration was not affected by gas retention time, maintaining approximately a constant concentration of 330 mg/L. Similarly, the liquid-phase S^{2-} concentration stayed very low at 0–65 mg/L, the SO_4^{2-} concentration remained low at 0–76 mg/L and the elemental sulfur concentration ranged from 560–800 mg/L.

A third study was performed in the CSTR, but this time with a sulfur recovery device. As is shown in Fig. 4, cell concentration, H_2S conversion, and sulfur species concentrations are plotted as a function of gas retention time for a liquid dilution rate of 1.25 d^{-1}. In the study, the H_2S conversion ranged from 59–99.99% reaching a 99.99% conversion at a gas retention time of 45 min. Thus, conversions were higher at a given retention time with sulfur recovery. The cell concentration was practically constant at 346 mg/L, a similar level as in the previous study. The S^{2-} and SO_4^{2-} concentrations were again quite low, and the sulfur concentration in the liquid effluent (not the total sulfur produced) ranged from 185–325 mg/L. Thus, sulfur recovery significantly improved H_2S conversion and thereby sulfur production.

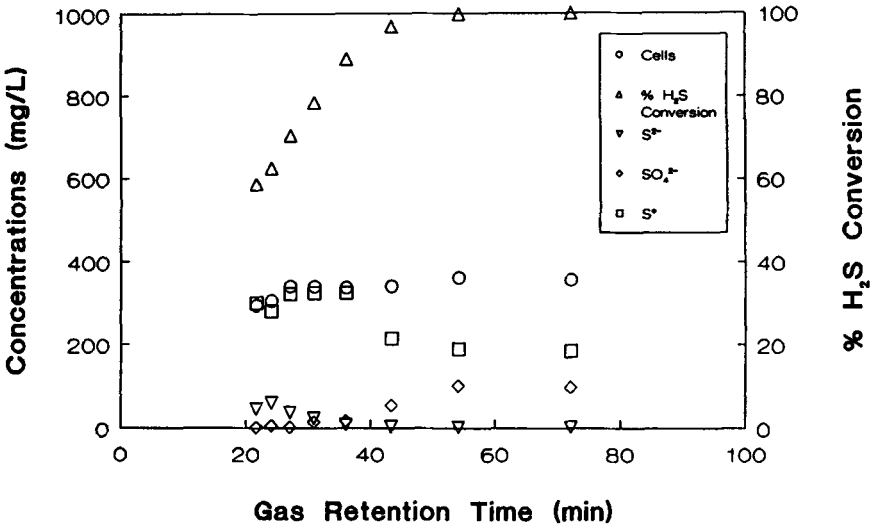

Fig. 4. Performance of the CSTR with sulfur recovery using *C. thiosulfatophilum* at various gas retention times (liquid dilution rate: 1.25 d^{-1}).

More experiments were carried out at varying light intensities and gas flow rates to observe maximum H_2S removal. These results are summarized in Table 1. As is noted, the gas retention time for complete H_2S removal was minimized at 20 min when using two 200-W light sources. Also, it is observed that the S^{2-} concentration remained essentially at zero. It was also possible to maintain the SO_4^{2-} concentration at levels below 100 mg/L. The sulfur concentration in the liquid at this gas retention time remained between 500 and 600 mg/L. Higher light intensities are expected to permit higher H_2S removal rates. However, because of physical limitations in providing sufficient reactor cooling, experiments were not carried out at higher light intensities.

After determining the optimum liquid dilution rate, gas flow rate, and light intensity to maximize H_2S removal in the system, an experiment was performed for an extended time period at these optimum conditions (1.25 d^{-1} liquid dilution rate, 19.6-min gas retention time, and two 200-W light sources) to establish the stability of the process. The results of this experiment over a period of 392 h are shown in Fig. 5. It can be seen from this figure that the reactor performance was quite stable during this period. However, the settler was not efficient in removing all of the elemental sulfur particles present in the reactor effluent. The liquid effluent contained about 425–550 mg/L elemental sulfur. The H_2S removal rate during this operation was 57 μmol min^{-1} L^{-1}, which is about 1.5 times the maximum value reported by Kim and Chang (*7*).

Table 1
Performance of the CSTR with Sulfur Recovery Using *C. thiosulfatophilum* at Different Light Intensities (Liquid Dilution Rate: 1.25 d^{-1})

Light	Gas retention time, min	Cell conc., mg/L	% H_2S conversion	S^{2-} mg/L	SO_4^{2-} mg/L	S^o mg/L
2 × 60 W	43.3	339	99.2	0.3	1	292
2 × 75 W	43.3	409	99.9	1.2	182	327
2 × 75 W	36.0	432	99.8	2.1	239	327
2 × 75 W	30.9	460	98.0	0.1	182	402
2 × 100 W	30.9	463	99.5	2.1	214	449
2 × 100 W	27.0	441	95.5	2.1	135	516
2 × 150 W	27.0	391	99.5	2.1	117	496
2 × 150 W	24.0	408	98.5	2.1	89	572
2 × 150 W	21.6	412	96.5	2.1	62	501
2 × 200 W	21.6	422	99.9	2.1	100	539
2 × 200 W	19.7	453	99.9	2.1	114	440

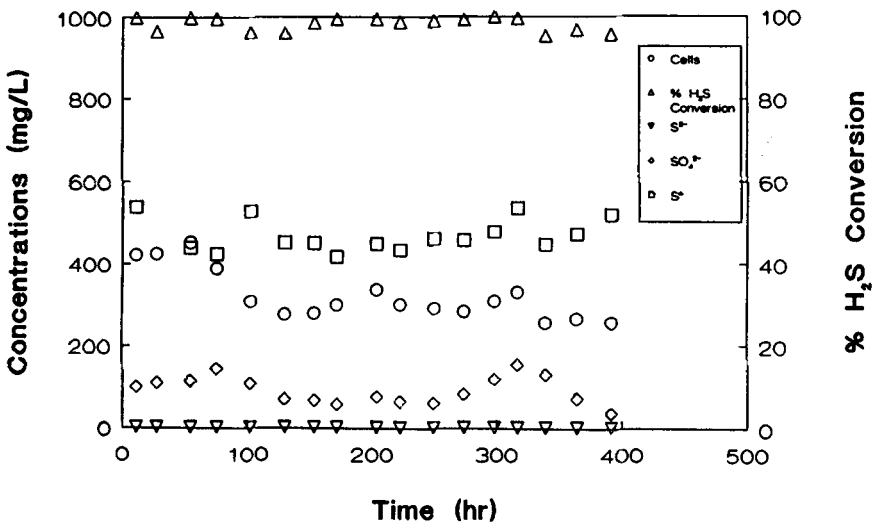

Fig. 5. Long-term behavior of the CSTR with sulfur recovery using *C. thiosulfatophilum* (liquid dilution rate: 1.25 d^{-1}; gas retention time: 19.6 min).

CONCLUSIONS

A continuous stirred-tank reactor with and without sulfur recovery has been operated using *C. thiosulfatophilum* for the conversion of H_2S to elemental sulfur. In operating the reactor system with sulfur recovery, essentially no SO_4^{2-}, an undesirable product, was produced. It was possible to remove completely up to 57 μmol min^{-1} L^{-1} of H_2S from the gas stream, which is 1.5 times the maximum value previously reported in the literature. Increasing the light intensity was shown to improve the H_2S removal capacity of the reactor, at least to the physical limits of the system.

The use of a sulfur settler in the reactor recycle loop significantly improved reactor performance. However, the settler was not efficient in removing all of the elemental sulfur particles present in the reactor effluent. A filtration device will probably be a better choice. The maximum cell density observed in the reactor system was about 450 mg/L. Therefore, the use of a cell recycle device is also expected to improve the performance of the reactor by increasing the quantity of biocatalyst present in the reactor. Significant reductions in the gas retention time are expected by employing cell recycle after sulfur recovery.

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