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Scientific Note

Microbial Reduction of Sulfur Dioxide in Immobilized, Mixed Cultures of Sulfate-Reducing Bacteria with Sewage Digest as Carbon and Energy Source**

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Index Entries: Sulfur dioxide; flue gas desulfurization; sulfate-reducing bacteria; anaerobic digestion; immobilization.

INTRODUCTION

Sulfur dioxide (SO_2) gas is one of the most abundant air pollutants emitted in the US. The quantity emitted in the US in 1992 was estimated to be 22.73 million tons *(1)*. About 70% of the total emissions are from fossil fuel combustion. In the atmosphere, SO_2 reacts photochemically or catalytically with other constituents to form sulfuric acid or acid rain. In this article, we demonstrate a microbial process for the reduction of sulfur dioixde utilizing mixed cultures of sulfate-reducing bacteria (SRB). The cost of feed stock and the productivity of bioreactor are identified as key factors for the economic viability of the microbial process. In these regards, we have developed a continuous process of producing sewage digest, an inexpensive carbon and energy source, and have investigated the immobilization of mixed cultures of SRB in order to increase the productivity of the process.

A microbial process for reducing the SO_2 resulting from regenerable processes for flue gas desulfurization (FGD) into hydrogen sulfide (H_2S) was demonstrated by Dasu and Sublette *(2)*. They utilized a mixed culture of bacteria containing *Desulfovibrio desulfuricans* and non-SRB heterotrophs with glucose as the carbon and energy source. They proposed that the resulting H_2S may then be oxidized to elemental sulfur using the Claus process or additional microbial action. An eco-

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nomic analysis of this microbial process performed by Sublette and Gwozdz *(3)* compared the microbial process with conventional catalytic hydrogenation, and demonstrated that annual operating costs for the microbial process were much higher than those for conventional process. This was mainly owing to the cost of raw materials, namely corn hydrolysate, which served as the carbon and energy source for the SO_2-reducing culture. Municipal sewage sludge was then recommended as an alternative electron donor.

Municipal sewage is readily available at negative or near-zero cost. However, the sewage sludge must be chemically or biologically processed in order for the SO_2-reducing cultures to be able to utilize the organic materials present in this feed. A bench-scale continuous reactor utilizing a mixed septic culture of the SRB *D. desulfuricans* immobilized by coculture with floc-forming anaerobes with SO_2 as the terminal electron acceptor was operated by Selvaraj and Sublette *(4)*. Anaerobically digested municipal sewage sludge (AD-MSS) medium with inhibition of methanogenesis by 250 ppm of chloroform was used as sole carbon and energy source for the SO_2-reducing process culture. A gravity settler was used to recycle the biomass. An economic analysis of this process, with AD-MSS medium as the electron donor and carbon source rather than the expensive glucose, revealed that the SO_2-reducing microbial process would be competitive if the SRB concentration is above 30% of the total biomass *(5)*. A further requirement for economic viability is that the concentration of fermentable substrates in the AD-MSS medium be above 2500 mg/L of chemical oxygen demand (COD) *(5)*.

It is thus seen that the concentrations of biocatalyst in the reactor and of fermentable substrates in the AD-MSS medium are key impact parameters affecting the economic viability of a microbial SO_2-reduction process compared with conventional hydrogenation. In this article, our work was initially focused on developing mixed cultures of SRB to obtain improved utilization of AD-MSS medium. With the low-cost AD-MSS medium as the carbon source, work focused on the immobilization of biocatalyst by entrapment into a gel bead to increase the cell concentration in the bioreactor, thus achieving higher volumetric productivity.

In the anaerobic digestion process, organic matter in the municipal sewage solids is decomposed into methane by three main reactions: hydrolysis, acidogenesis, and methanogenesis. The first two bioreactions break the complex organic compounds in the sewage into simpler organic acids. Subsequently, methanogens convert those acids into methane. For the purposes of obtaining organic acids as carbon and energy sources for the SO_2-reducing SRB cultures, methanogenesis is therefore an undesired reaction. The methanogenic reaction can be inhibited by digesting the sewage solids at high temperatures (35–40°C) and short retention time of solids in the digester, and/or by adding an inhibiting agent, for instance, chlorinated methyl compounds *(6)*. Ghosh demonstrated a pilot-scale, two-phase anaerobic digestion process with both acid-phase and methane digesters to obtain maximum conversion of sewage into methane *(7)*. In his study, optimum acidogenic fermentation produced 9500 mg/L of organic acids when using a feed concentration of 5–7% dry wt of sewage solids and a hydraulic retention time of 3 d at 37°C. In the present study, a continuous process of producing AD-MSS media using a lower concentration of sewage solids was investigated in order to reduce the production cost of AD-MSS medium. We also studied the effects of a methane inhibitor such as chloroform and hydraulic retention time of the feed in order to maximize the organic acid yield.

The immobilization of process culture with alginate and carrageenan beads has been studied to investigate the durability of the beads under operating conditions and to assess the effects of immobilization on biocatalytic performance. The bioreduction of SO_2 to H_2S using the immobilized-cell beads in columnar and well-mixed reactors has also been investigated to maximize SO_2 throughput and minimize reactor volume and, hence, required capital investment.

MATERIALS AND METHODS

AD-MSS Medium Preparation—Continuous Process

For the continuous production of AD-MSS medium, municipal sewage solids were obtained from diffused air flotation (DAF) units of municipal sewage treatment plants at both Oak Ridge and Knoxville, TN. A 15-L glass vessel was operated as a well-mixed continuous reactor at 37°C. It received feed containing 15% wet wt of DAF solids in distilled water, initially at a flow rate of 90 mL/h. This corresponded to the hydraulic retention time of the sewage solids for 6.9 d. Note that, unlike the method described by Selvaraj and Sublette *(4)*, this uses no additional mineral salts to support the growth of SRB. The flow rate of the DAF solids feed was altered several times during the period of operation to vary the hydraulic/solids retention time in the reactor. Chloroform was added at a concentration of 100 and 50 ppm to the DAF feed solution during the course of the operation as an inhibiting agent for methanogenesis. The supernatant of the effluent was used as AD-MSS medium for the SO_2-reducing bioreactor. Effluent samples were analyzed for soluble COD and organic acids as described below.

Organisms and Culture

SRB were isolated from sewage solids obtained from the DAF unit of a municipal sewage treatment plant at Oak Ridge, TN. A serum bottle containing 100 mL of AD-MSS medium and 0.15 g of sodium sulfate was incubated at 30°C. The indigenous SRB in the sewage medium utilized the added sulfate as terminal electron acceptor and reduced it to H_2S. The stock culture of SRB was maintained by transferring this positive culture into the previously described AD-MSS medium (containing sodium sulfate) weekly.

Columnar and Well-Mixed Reactors with Immobilized Cells for SO_2 Reduction

A 1-L omni-culture fermenter (Virtis Co., Gardiner, NY) with temperature and agitation control was used to grow SRB cells. A pH controller (Chemcadet, Cole-Parmer, Niles, IL) with 6*N* phosphoric acid and 6*N* sodium hydroxide as acid and base delivery arrangements was used to monitor and maintain a constant pH of 7.0. Prior to growth of the culture on SO_2, chemostat operation was initiated with 1 L of AD-MSS medium with sulfate added (1.5 g/L of Na_2SO_4) as the electron acceptor. A 5-mL sample of the mixed SRB stock culture developed as described previously was used to inoculate this reactor, which was operated with an agitation rate at 200 rpm and temperature at 30°C. Nitrogen was purged at a flow rate of 300 mL/min in order to scrub the produced H_2S from the system. When the H_2S concentration reached about 1000 ppm, the biomass was harvested by centrifugation and resuspended in fresh AD-MSS medium (with no sulfate added). At this time, 1%

SO_2, 5% CO_2, balance N_2 gas mixture (Matheson Gas Products, Marrow, GA) was fed to the reactor at a rate of 7.6 mL/min. This corresponded to a molar SO_2 feed rate of 0.19 mmol/h. This culture was then operated in a continuous mode with the AD-MSS medium at a feed rate of 0.4 mL/min. The reactor effluent was collected under a nitrogen blanket and used as a biomass source for additional experiments as described later.

Alginate and Carrageenan Beads

A 1% solution of alginic acid (Sigma Chemical Co., St. Louis, MO) in distilled water was used to make alginate beads. The bead preparation has been described elsewhere *(8)*. In our study, the cells were collected by centrifuging 1 L of effluent of the 1-L chemostat operated at an SO_2 feed rate of 0.19 mmol/h. The resulting cell paste was then added to 300 mL of 1% alginate solution and pumped through a syringe needle (gage 22) as droplets. The droplets were fixed as beads in 0.2*M* $CaCl_2$ solution. The immobilized alginate-cell beads (0.5–1 mm) were kept in the $CaCl_2$ solution and mixed for another 2 h for complete bonding between the alginate and the calcium ion. The resulting crosslinking yielded 135 mL of cell beads, which were then transferred to a columnar reactor. A fully jacketed glass columnar reactor of internal dimensions 2.5 × 30 cm was used as a fixed-bed reactor with immobilized-cell beads. The total volume of the column was 265 mL. A continuous preparation of SO_2 solution was obtained by purging 1% SO_2, 5% CO_2, balance N_2 gas mixture into 500 mL distilled water at a flow rate of 1.6 mL/min (0.043 mmol/h of SO_2); the solution was then fed into the columnar reactor by a peristaltic pump at a rate of 0.2 mL/min. The distilled water was added continuously to the SO_2 premixing bottle to maintain the constant volume and concentration of the sulfite solution. At equilibrium, the concentration of sulfite in the premixing bottle was about 280 mg/L. The AD-MSS medium was delivered into the columnar reactor by a similar pump at a rate of 0.2 mL/min. Therefore, the final concentration of sulfite entering the column was about 140 mg/L. The retention time of the feed mixture with cell beads in the reactor was calculated to be 5.4 h, which was $^1/_8$th of the retention time in the 1-L chemostat. Owing to the limitation of the minimum volumetric flow rate of the pump (0.2 mL/min), we were not able to achieve the same retention time in both reactors. However, the SO_2 flow rate into the columnar reactor was calculated to be 0.17 mmol/(h·L) compared with 0.19 mmol/(h·L) of SO_2 into the chemostat. The reactor temperature was maintained at 30°C. The measured pH of the feed mixture (sulfite and AD-MSS medium) was between 6.75 and 6.80. No pH adjustments were made in this experiment. The effluent from the reactor was collected in a glass bottle, where nitrogen was sparged into the solution to strip off H_2S. The pH of the effluent was about 6.45. The feed and effluent samples were analyzed for sulfite and organic acids as later described.

In experiments using carrageenan beads, immobilized biocatalyst was prepared using 4% κ-carrageenan (Type NJAL-798 from FMC Corp., Chicago, IL) with 0.5% polyethylenimine (50% PEI solution from Sigma Chemical Co., St. Louis, MO) in an aqueous solution at 40°C *(9)* and the cells collected from the chemostat as described previously. In this case, 0.3*M* potassium chloride was used as fixing solution. The beads were prepared from 300 mL κ-carrageenan-PEI solution with the cell paste obtained from 1 L of effluent of the chemostat reactor. Since the κ-carrageenan-PEI solution was more viscous than the alginate solution, the beads

(2–3 mm) were larger than the alginate beads. Unlike the alginate beads, the crosslinking of the carrageenan beads in 0.3*M* KCl solution showed no reduction in the bead volume, resulting in 300 mL of the cell beads. Therefore, only one-third of the crosslinked beads could fit into the reactor. The total amount of biomass in the reactor was therefore one-third of that in either the free-cell or the alginate reactor. The flow rates of the sulfite solution and the AD-MSS medium were same as in the experiment with alginate-cell beads. A similar experiment with carrageenan beads without any cells was conducted for 7 d as a control run to investigate any reduction in the sulfite concentration owing to any chemical reaction between carrageenan and sulfite or between AD-MSS medium and sulfite in the columnar reactor.

In order to assess the operation of immobilized bioreactor under the same operating conditions as in the free-cell reactor, the 1-L chemostat arrangement described earlier was used to operate the well-mixed reactor with immobilized cells. The agitation rate was reduced to 100 rpm, just enough to maintain the uniform mixing of the beads. The SO_2 flow rate was 0.19 mmol/h. The effluent samples were analyzed for sulfite concentrations as later described. The effect of mechanical agitation on the beads was visually observed. This reactor configuration enabled comparisons to be made with equal hydraulic retention time, the same SO_2 delivery mode, and so forth.

Analytical

Hydrogen sulfide in the off-gas of the chemostat was analyzed using a gas chromatograph (Hewlett Packard [Wilmington, DE] HP 5890 Series II) equipped with a Teflon column (3 ft × 1/8 in.) packed with SuperQ (Alltech Associates, Inc., Deerfield, IL) (80–100 mesh). Temperatures of the column, injection port, and thermal conductivity detector were 50, 125, and 125°C, respectively. Helium was used as carrier gas at a flow rate of 25 mL/min. Organic acid concentration in the AD-MSS medium was analyzed using another gas chromatograph (Varian 3700) equipped with glass column (6 ft × 1/4 in.) packed with Chromosorb 101 (80–100 mesh). Temperatures of the column, injection port, and thermal conductivity detector were 200, 250, and 250°C, respectively. Helium gas was used as carrier gas at a flow rate of 50 mL/min. Sulfite was analyzed spectrophotometrically by the reaction of fuchsin and formaldehyde in sulfuric acid *(10)*. CODs were determined using Hach Chemical Co. (Loveland, CO) premeasured reagent vials.

RESULTS AND DISCUSSION

AD-MSS Medium Preparation–Continuous Process

Initially, the 15-L reactor was operated with 15% DAF solids without any addition of chloroform for a period of 51 d. Figure 1 shows the soluble COD concentrations of the effluent samples. The corresponding analysis of organic acids present in the resulting effluent as a function of time is shown in Fig. 2. Organic acids such acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caproic, were detected. The initial feed rate of the DAF feed solids was 90 mL/h, corresponding to an HRT of 7.0 d. The COD of the effluent increased from 1500 to 3500 mg/L in 10 d. At this time, the feed rate of the DAF solids was increased to 180 mL/h, in order to reduce the retention time of the feed and to increase the concentrations of organic acids by not allowing the conversion of the acids into methane as a result of methanogenesis.

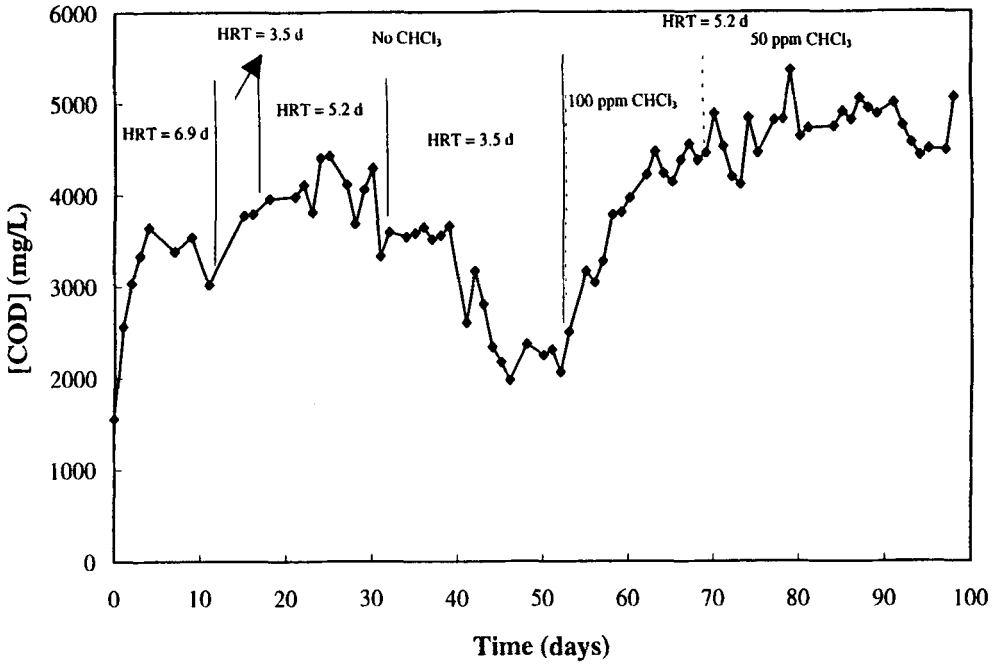


Fig. 1. Effluent soluble COD concentration during continuous anaerobic digestion of municipal sewage solids at 37°C. HRT = hydraulic retention time.

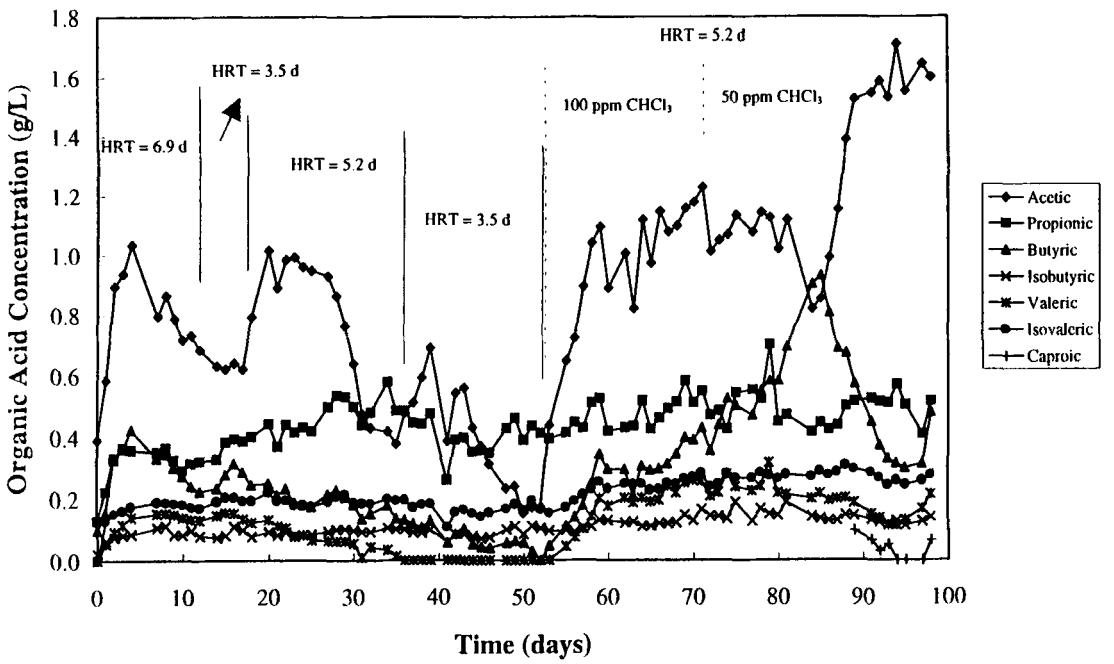


Fig. 2. Effluent organic acid concentration during continuous anaerobic digestion of municipal sewage solids at 37°C. HRT = hydraulic retention time.

However, the COD concentration as well as the organic acid concentrations decreased in 5 d, probably because of the shorter contact time of the feed solids with acid-producing organisms already existing in the reactor. Therefore, the flow rate of the feed was decreased to 120 mL/h (HRT = 5.2 d), and the reactor was operated at this condition for another 15 d. Though the COD and organic acid concentrations of the effluent increased initially, there was a sudden drop in the COD and organic acid concentrations. The COD dropped from 4300 to 3300 mg/L, possibly as a result of an increase in methanogens population in the reactor. At this time, the hydraulic retention time was reduced back to 3.5 d to wash out the methanogens in the reactor. However, the COD and the organic acid concentrations continued to drop, as shown in the Figs. 1 and 2. At this time, in order to inhibit the methanogenesis in the reactor, chloroform at a concentration of 100 mg/L was added to the feed solution. The HRT of the feed solid was increased back to 5.2 d. The reactor was operated under these conditions for 22 d. The COD concentration reached levels as high as 4500 mg/L and seemed to maintain itself at that concentration. The organic acid concentrations—specifically acetic, propionic, and butyric acid—also increased as shown in Fig. 2. At this time, the concentration of the chloroform added to the feed solution was reduced to 50 ppm in order to minimize its use. The reactor was operated for another 20 d. The COD concentration increased to about 5000 mg/L. At this time, the sum of the detected organic acid concentrations was about 3.0 g/L. The difference between the COD concentration and the total organic acid concentrations may be owing to high-mol-wt organic acids, which contribute to COD, but are not detected by our gas chromatograph technique.

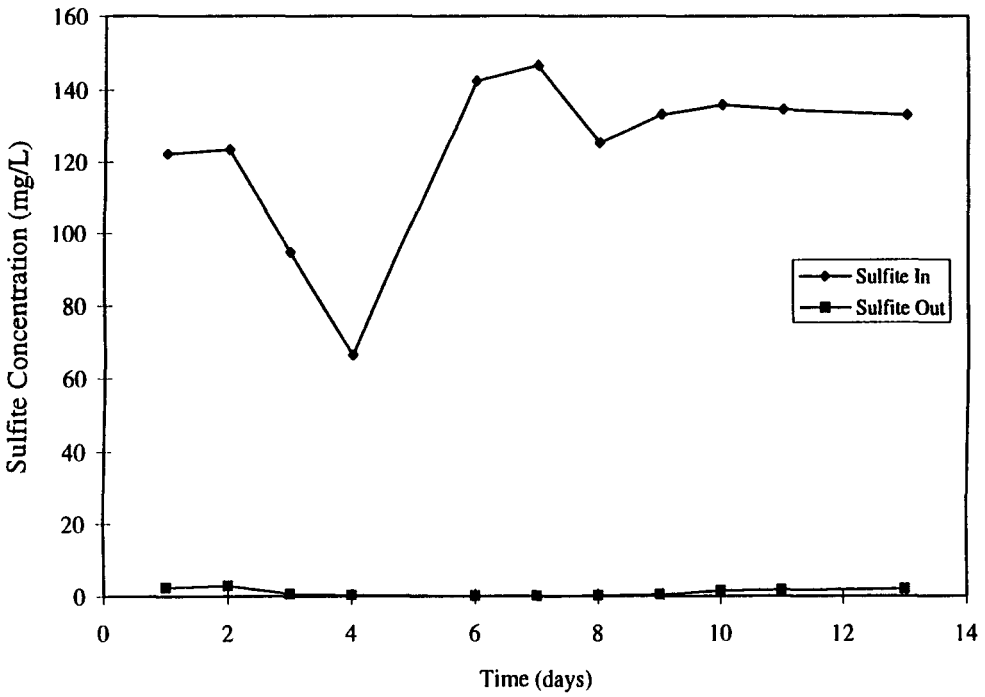
Columnar and Well-Mixed Reactors with Immobilized Cells for SO_2 Reduction

Alginate-Cell Beads

The feed and effluent sulfite concentrations of the columnar reactor are shown in Fig. 3. Complete removal of sulfite was observed in the effluent at the inlet sulfite concentration of about 140 mg/L. The SO_2 conversion rate in this immobilized-cell reactor was 0.022 mmol/h compared with 0.19 mmol/h in the free-cell reactor. Although this at first appears to indicate a decrease in biocatalyst activity, several factors must be considered. The immobilized-cell reactor, because of its decreased volume, had a retention time 1/8 that of the 1-L reactor. When compared on an equal retention time basis, the productivities seem to be comparable (0.17 mmol/h vs 0.19 mmol/h for the immobilized-cell and free-cell reactors, respectively). This is equivalent to reporting reactor productivity on a mmol/(h·L) basis. Since the immobilized-cell bioreactor has the potential to accommodate higher loading of biocatalysts, further increases in productivity are achievable. However, after 5 d, a decrease in bead height was observed as the beads started to disintegrate. This might be owing to the presence of sulfite or H_2S in the reactor or the gradual dissolution of the bead material in the AD-MSS medium. Although the immobilization process did not seem to decrease the biocatalytic performance, the alginate matrix did not appear robust enough for use in this application.

κ-Carrageenan-PEI-Cell Beads

The columnar reactor with carrageenan-cell beads was operated for more than 30 d. Initially, the sulfite concentration of the feed had great variability because of



Fig, 3. Sulfite concentration of the feed and the effluent in the columnar reactor with alginate immobilized cells as a function of time.

mechanical failure in the SO_2 feed system. As shown in Fig. 4, a small amount of sulfite was initially present in the effluent, probably because of decreased biomass in the column compared with that in the alginate bead reactor. However, after 20 d of operation, the carrageenan-cell beads in the reactor were able to convert about 250 mg/L of sulfite, which was more than that of the alginate-cell reactor. This might be owing to the growth of additional biomass on the beads during the period of operation. In addition, the bead height in this column was stable for the whole period of operation. This improved stability as compared with that of the alginate might be attributed to the concentration of κ-carrageenan-PEI solution (4% compared with 1% alginate solution) and/or resistance to chemical reactions with sulfite, H_2S, or AD-MSS medium. The control experiment with raw carrageenan beads (without cells) showed no reduction in the sulfite concentrations, indicating that the sulfite reduction in the carrageenan-cell beads was owing only to the biocatalyst in the beads. Thus, the carrageenan-cell beads were found to be active and stable under the operating conditions.

Well-Mixed SO_2-Reducing Reactor with Immobilized Cells

Because of their inadequate mechanical stability, alginate-cell beads in the well-mixed reactor disintegrated completely within 2 h of the operation at an SO_2 feed rate of 0.19 mmol/h and an agitation rate of 100 rpm. However, a reactor with the same volume of carrageenan-cell beads showed no damage to the beads at the same SO_2 feed rate and agitation rate for 7 d, and very little sulfite (about 5 mg/L) was present in the effluent. It proved once again that the carrageenan beads showed better mechanical and chemical stability than the alginate beads and that immobi-

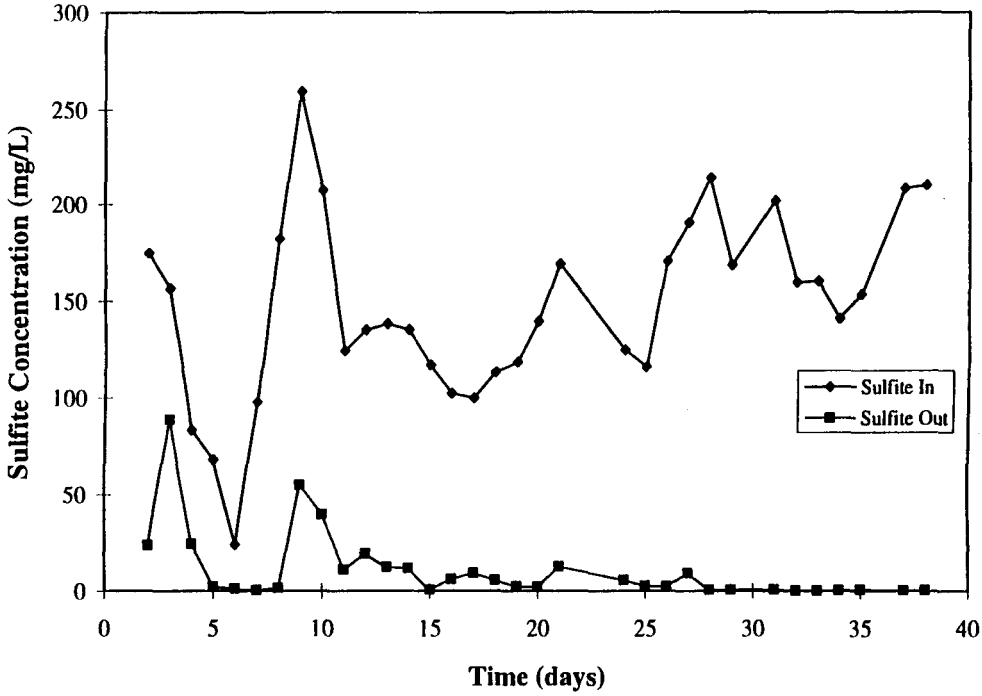


Fig. 4. Sulfite concentration of the feed and effluent in the columnar reactor with κ-carrageenan-PEI immobilized cells as a function of time.

lization does not affect biocatalyst performance. In the future, with the harvesting of larger amounts of SRB, it should be possible to increase the concentration of biocatalyst within the beads and further improve productivity.

CONCLUSIONS

A considerable increase in soluble COD and organic acid concentrations was achieved in a continuous process for AD-MSS medium preparation. This reactor utilized less DAF solids and chloroform, and used no additional salts compared with previous investigations. It resulted in a COD of 5000 mg/L, double that of previous studies. This increase in available carbon source for SO_2-reducing bioreactors, together with the corresponding decrease in required hydraulic loading to the bioreactors, serves to decrease the capital and operating cost for the proposed microbial SO_2-reduction process. Our initial investigation of the immobilization of mixed SRB cultures with alginate or κ-carrageenan gel demonstrated that biocatalyst immobilization did not impair biocatalyst performance. Sulfur dioixde throughputs of 0.19 and 0.17 mmol/(h·L) were achieved when the same amount of biomass was used in free-and immobilized-cell forms, respectively. The κ-carrageenan-cell beads were found to be more stable than the alginate-cell beads under the operating conditions. Summarizing the results of the experiments, it can be concluded that the immobilized-cell bioreactors gave equal conversion rates of SO_2 as free cells on a per-biomass basis and have the potential to increase greatly the volumetric productivity of the reactor. Owing to the limited reactor volumes available, maximal biomass loading in the immobilized-cell reactors was not realized in the

present study. Future work will increase the biomass concentration in packed and fluidized beds of immobilized biocatalyst in order to increase reactor throughput and further improve the economic viability of the proposed microbial SO_2-reduction process.

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