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Sulfate Decomposition by Bacterial Leaching

NURAN DEVECI* AND CÜNEYT GÖKTUG DELALOĞLU

Chemical Engineering Department,
Istanbul Technical University, 80626 Maslak, Istanbul, Turkey

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ABSTRACT

Sulfate disposal is the main problem of many industrial effluents, such as excess sulfuric acid, gypsum, coal desulfurization byproducts, acid-mine waters, and general metallurgical effluents. It has been established that sulfate present in wastes can be converted to elemental sulfur by bacterial mutualism. This study presents the results of an investigation of the industrial feasibility of utilizing a biological system capable of converting hydrous calcium sulfate (gypsum) to elemental sulfur. Gypsum, which was used in this study, is a byproduct of the fertilizer industry. The biological system is referred to as a bacterial mutualism, and involves *Desulfovibrio desulfuricans* for sulfate conversion and *Chlorobium thiosulfatophilum* for hydrogen sulfide conversion. Bacterial mutualism and utilization of sulfate were investigated by means of a two-stage anaerobic system. In the first stage, a gas purge system was used for sulfate conversion to sulfide, and it was found that maximum conversion is 34%. In the second stage, a static culture system was used for sulfide conversion to sulfur with a conversion of 92%.

Index Entries: Gypsum; sulfate bioconversion; *Desulfovibrio desulfuricans;* gas purge system; sulfide bioconversion; *Chlorobium thiosulfatophilum;* static culture.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Nowadays, the environmental pollution caused by industrial wastes is one of the most important problems in the world. The problem of sulfate disposal exists with many major industrial effluents, including excess sulfuric acid, gypsum, coal desulfurization byproducts, acid-mine waters, and general metallurgical effluents. In the fertilizer industry, during the production of phosphoric acid from the phosphate rock by the reaction:

$$Ca_3(PO_4)_2 + 3H_2SO_4 + 6H_2O \longrightarrow 3H_3PO_4 + 3CaSO_4 \cdot 2H_2O \quad (1)$$
(Gypsum)

a large amount of gypsum is produced as waste (*1*). We propose herein a solution to this problem that utilizes the natural biological activities of *Desulfovibrio desulfuricans, Chlorobium thiosulfatophilum,* and/or *Chromatium vinosum,* obligate anaerobes of the aquatic sulfur cycle (*2–5*). *Desulfovibrio* reduces sulfate to sulfide, and the green and purple sulfur bacteria *Chlorobium* and *Chromatium* photosynthetically oxidize sulfide to elemental sulfur according to reaction shown below (*6–8*).

$$SO_4 = \xrightarrow{\textit{Desulfovibrio}} H_2S \xrightarrow[\text{and/or } \textit{Chlorobium}]{\textit{Chromatium}} S^\circ \quad (2)$$

C. thiosulfatophilum excretes the colloidal sulfur, whereas *C. vinosum* stores sulfur intracellularly (*9,10*). It is the purpose of the studies described herein to demonstrate that a mutualism may be established between *Desulfovibrio* and either *Chromatium* or *Chlorobium,* so as to quantitatively convert sulfate to elemental sulfur. Further bacterial mutualism and utilization of sulfate were investigated by means of a two-stage anaerobic system. In the first stage, a gas purge system was used for sulfate conversion to sulfide. The gas purge rate was varied, and the amount of sulfide was determined. In the second stage, a static culture system was used for sulfide conversion to sulfur, and conversion of sulfide to elemental sulfur was determined.

MATERIALS AND METHODS

Organisms

Experiments were carried out with *D. desulfuricans* (DSM 642) and *C. thiosulfatophilum* (DSM 245), which were obtained from Deutch Sammlung Von Microorganismen Und Zellkulturen GmbH (DSM).

Medium and Static Growth Conditions

Desulfovibrio was grown in a DSM medium described in the DSM catalogue (*11*).

Solution I:

K_2HPO_4	0.5	g
NH_4Cl	1.0	g
Na_2SO_4	1.0	g
$CaCl_2 \cdot 2H_2O$	0.1	g
$MgSO_4 \cdot 7H_2O$	2.0	g
DL·sodium lactate (60%)	2.0	g
Yeast extract	1.0	g
Resazurin	1.0	g
Distilled water to	980	mL

Solution II:

$FeSO_4 \cdot 7H_2O$	0.5	g
Distilled water to	10	mL

Solution III:

Sodium thioglycolate	0.1	g
Ascorbic acid	0.1	g
Distilled water to	10	mL

Solutions I, II, and III were autoclaved separately and mixed. The pH was adjusted to 7.8 with NaOH.

Chlorobium was grown in Larsen medium (*9*).

NH_4Cl	1.0	g
KH_2PO_4	1.0	g
$MgCl_2$	0.5	g
NaCl	1.0	g
$NaHCO_3$	2.0	g
$Na_2S_2O_3$ or $Na_2S \cdot 9H_2O$	1.0	g
$Na_2S \cdot 9H_2O$ (if $Na_2S_2O_3$ is used above)	0.1	g
$CaCl_2$	0.1	g
Fe (as $FeCl_3 \cdot 6H_2O$)	500	μg
B (as H_3BO_3)	100	μg
Zn (as $ZnSO_4 \cdot 7H_2O$)	100	μg
Co (as $Co[NO_3]_2 \cdot 6H_2O$)	50	μg
Cu (as $CuSO_4 \cdot 5H_2O$)	5	μg
Mn (as $MnCl_2 \cdot 4H_2O$)	5	μg
Distilled water to	1000	mL

The pH was adjusted to 7.0–7.5 with H_3PO_4.

Sulfate Source

Gypsum (hydrous calcium sulfate), which was used in this study, is a byproduct of the fertilizer industry. The main structure of gypsum determined by X-ray analysis is $CaSO_4 \cdot 2H_2O$. There also exists $CaSO_4 \cdot 0.15H_2O$, $CaCO_3$ and $Ca(H_2PO_2)_2$. X-ray phase study of gypsum showed that the sample contained 55% gypsum ($CaSO_4 \cdot 2H_2O$).

Table 1
Sulfate-Reducing Salt Solution

Constituent	Solution, g/L
NH_4Cl	1.0
$MgSO_4 \cdot 7H_2O$	2.0
K_2HPO_4	0.5
$CaCl_2 \cdot 2H_2O$	0.1
NaCl	10.0
$CaCO_3$	1.0
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	Trace

Sulfate Reduction by *D. desulfuricans*

Bacterial mutualism and utilization of sulfate were investigated by means of a two-stage anaerobic system. The first stage is sulfate conversion to sulfide by *D. desulfuricans*. The purge system was used for sulfate conversion to sulfide. The reactor was charged with the salt solution given in Table 1, gypsum, and sodium lactate as carbon source. After sterilization, it was inoculated with *D. desulfuricans*.

This system was operated at 30–35°C. Stirring was accomplished with a magnetic stirring bar and was just fast enough to keep the gypsum suspended. Nitrogen gas was used as the carrier gas for hydrogen sulfide (*12*). The gas purge rate was varied, and the amount of sulfide was determined according to US Stell Chemists' Method (iodimetric method) (*13,14*).

Sulfide Reduction by *C. thiosulfatophilum*

The second stage in the bacterial mutualism and utilization of sulfate is sulfide conversion to sulfur. In this stage, a static culture system was used. In the anaerobic jar, photosynthetic bacterium *C. thiosulfatophilum* reduced hydrogen sulfide to elemental sulfur. The Larsen medium (*9*) was used in this stage. Experiments were carried out at 30–35°C. The amount of sulfur was determined according to the Grote Krekeler method (ASTM D-1551-65 T).

RESULTS AND DISCUSSION

In this study, bacterial mutualism and utilization of gypsum were investigated by means of a two-stage anaerobic system. In the first stage, a gas purge system was used. *D. desulfuricans* reduced sulfate to sulfide. The optimum pH and temperature ranges for hydrogen sulfide production were 7.2–7.8 and 30–35°C, respectively. Sodium lactate was used as carbon source. The gas purge rate was varied, and the amount of sulfide was determined. The changes in sulfide content in experiments are given in Tables 2, 3, and 4. The use of a purge gas to remove hydrogen sulfide

Table 2
The Changes in Hydrogen Sulfide Content
During Gypsum Bioconversion

Time, h	Hydrogen sulfide, mg/L
4	0.72
8	1.77
12	2.98
16	4.19
20	4.83
24	5.23
Reactor content	
Salt solution	1.95 L
Gypsum	17.5 g/L
Sodium lactate	21 g/L
Inoculum	50 mL
Operation conditions	
Stirring rate	180 rpm
pH	7.4
Temperature	30°C
The gas purge rate	80 mL/min

Table 3
The Changes in Hydrogen Sulfide Content
During Gypsum Bioconversion

Time, h	Hydrogen sulfide, mg/L
4	3.54
8	7.72
12	12.87
16	16.09
20	18.99
24	21.24
48	20.01
Reactor content	
Salt solution	1.95 L
Gypsum	17.5 g/L
Sodium lactate	21 g/L
Inoculum	50 mL
Operation conditions	
Stirring rate	180 rpm
pH	7.4
Temperature	30°C
The gas purge rate	20 mL/min

Table 4
The Changes in Hydrogen Sulfide Content During Gypsum Bioconversion

Time, h	Hydrogen sulfide, mg/L
4	0.5
8	1.2
12	2.0
16	4.0
20	6.4
24	9.5
48	28.8
72	27.6
Reactor content	
Salt solution	1.95 L
Gypsum	17.5 g/L
Sodium lactate	40 g/L
Inoculum	50 mL
Operation conditions	
Stirring rate	180 rpm
pH	7.4
Temperature	30°C
The gas purge rate	10 mL/min

Table 5
Sulfur Content During Hydrogen Sulfide Bioconversion

Time, h	Sulfur, mg
6	0.040
12	0.072
24	0.122
48	0.206
72	0.282
96	0.325
120	0.366
148	0.360
Operation conditions	
pH	7.0–7.5
Temperature	30°C
Amount of hydrogen sulfide used	0.421 mg

from the system was found to increase hydrogen sulfide. It was found that maximum conversion of sulfate to sulfide is 34% in 24 h.

In the second stage, a static culture system with photosynthetic bacterium *C. thiosulfatophilum* reduced hydrogen sulfide to elemental sulfur at 30–35°C and pH 7.0–7.5. The amount of sulfur was determined. Sulfur

contents are given in Table 5. Conversion of sulfide to elemental sulfur was reached 92% in 5 d.

The system vessels used in the study play an important role in obtaining maximum hydrogen sulfide and sulfur production. In addition to the variables, such as time, temperature, pH, stirring, nutrient addition, and medium exchange, ease of operation and maintenance of anaerobic conditions will also affect the production of hydrogen sulfide and elemental sulfur. The results reported here show that the production of elemental sulfur from gypsum with sulfate-reducing bacteria is technically feasible.

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