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Production of Sulfur from Gypsum as an Industrial Byproduct

SERGE HILIGSMANN,*,[1] SOPHIE DESWAEF,[2] XAVIER TAILLIEU,[1] MICHEL CRINE,[2] NICOLAS MILANDE,[3] AND PHILIPPE THONART[1]

[1] Wallon Center for Industrial Biology, Chemical Engineering Laboratory (BG), University of Liege, B-4000 Sart-Tilman, [2] Genetic Chemistry Laboratory, University of Belgium, Liege, Belgium; and [3] Bertin Society, Bayonne Center, Industrial Zone, Tarnos, France

ABSTRACT

Biological sulfate reduction was investigated at the bench and pilot scales in order to determine optimum culture conditions. Efficient strains of sulfate-reducing bacteria (SRB) were selected by classical microbiological methods and by mutagenesis. Improvement factors, including stripping, scale-up, sulfate, and organic substrate concentrations, have been studied in batch bioreactors.

Two types of pilot-scale bioreactors have been adopted, the first being completely mixed with free cells and the second having two stages with immobilized cells on a fixed bed. An overall bioconversion capacity of 11 kg/m^3·d of gypsum and 1.2 kg/m^3·d of dissolved organic carbon has been achieved in the two-stage bioreactor.

Index Entries: Gypsum; biodegradation; sulfate-reducing bacteria; sulfide; lactic acid; acetic acid; whey.

INTRODUCTION

Gypsum ($CaSO_4 \cdot 2H_2O$) is produced by increasingly more industrial processes, for instance, when calcium carbonate or lime is used to neutralize effluents containing sulfuric acid or when apatite ($Ca_3(PO_4)_2 \cdot CaF_2 \cdot CaCO_3$), the raw material used in phosphoric acid manufacture, is attacked by sulfuric acid.

The precipitated toxic metals that are often contained in this gypsum (mercury, lead, arsenic, zinc, cadmium, and so forth) and the large amounts of gypsum produced (hundreds of millions of tons produced yearly throughout the world *[1]*) imply that there is, so far, no convenient method for its disposal without causing damage to the environment. Apart from some local opportunities, such as its inclusion in plasters or in cements, gypsum (as industrial byproduct) is usually disposed of in controlled sites or discharged in the seas, threatening to pollute soils and waters.

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

The biotechnological process reported here takes advantage of the so-called dissimilatory sulfate-reducing bacteria (SRB), which can produce sulfide from sulfate and oxidize an organic substrate into carbonate *(2,3)* according to:

$$SO_4^{2-} + \text{organic substrate} \rightarrow S^{2-} + CO_3^{2-} \quad (1)$$

The solid effluent, containing a majority of carbonate and impurities precipitated as sulfides, could be recycled repeatedly in neutralization in order to concentrate the toxic metals. Alternatively, the sulfide could be recycled in the manufacture of sulfuric acid, after chemical transformation into sulfur (derivatives of the Claus process using catalysts *[4]*).

Most of the available literature describes the reduction of highly soluble sodium or ammonium sulfate *(5,6)* or the reduction of relatively low concentrations of $CaSO_4 \cdot 2H_2O$ (slightly above the solubility threshold *[7–10]*). However, preliminary experiments have shown a more efficient gypsum reduction at concentrations higher than the solubility threshold. Experiments would then have to cope with a multiple-phase culture medium. However, these sulfate reduction conditions have not been investigated in detail.

The purpose of this study (carried out by four research groups within the European Community *[11]*) was to investigate and improve a biotechnological process that could economically cope with the disposal of industrial gypsum and its relatively expensive chemical reduction, which requires a great deal of energy (reaction of gypsum with coal at 700–1200°C to form CaO, CO_2, and SO_2 *[4]*).

To achieve this objective, only cheap residual organic substrates from food or other industries were considered. Therefore, incompletely lactate-oxidizing sulfate-reducing bacteria (LSRB), which produce acetate, were chosen for study because of their fast and minimally restricted metabolism compared to other metabolic processes oxidizing organic substrates *(3,12–14)*. Nevertheless, they were combined with acidogenic bacteria in order to convert the raw residual organic substrate into the suitable electron donor for SRB *(15)*.

Only complete dissolved organic carbon (DOC) degradation–sulfate reduction processes were promoted. Therefore another type of SRB was studied, the completely acetate-oxidizing sulfate-reducing bacteria (ASRB), which is able to degrade acetate only to carbonate, with further reduction of gypsum *(12,13)*.

Efficient pure strains of SRB were selected by classical microbiological methods and by mutagenesis (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine [NTG]). Some improvement factors have been investigated in 2.5- and 20-L bench-scale batch bioreactors: stripping, scale-up, sulfate, and organic substrate concentrations.

Two types of continuous pilot-scale bioreactors (completely mixed with free cells and two-stage with immobilized cells on a fixed bed) have been adopted to study feasibility and bioreactor engineering aspects of gypsum reduction. Particular attention was paid to the nature of the organic substrate, association with acidogenic bacteria, immobilization support, retention time, and organic inputs.

METHODS

Media

Bench-scale experiments used slightly modified culture media B, E, and C described by Postgate *(3)*. Medium B (in g/L of demineralized water): KH_2PO_4, 1; $MgSO_4 \cdot 7H_2O$, 2; $FeSO_4 \cdot 7H_2O$, 0.5; $CaSO_4 \cdot 2H_2O$, 1; NH_4Cl, 1; yeast extract, 1; ascorbic

acid, 0.2; sodium thioglycolate, 0.1; sodium lactate, 3.5 (for LSRB); sodium acetate trihydrate, 4 (for ASRB). Medium C: KH_2PO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.06; $FeSO_4 \cdot 7H_2O$, 0.004; $CaSO_4 \cdot 2H_2O$, 3.5; $CaCl_2 \cdot 2H_2O$, 0.02; NH_4Cl, 1; yeast extract, 1; ascorbic acid, 0.2; sodium thioglycolate, 0.1; sodium lactate, 6 (for LSRB); sodium acetate trihydrate, 4 (for ASRB). Medium E: KH_2PO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.2; $(SO_4)_2Fe(NH_4)_2$, 0.2; Na_2SO_4, 1; $CaCl_2 \cdot 2H_2O$, 0.5; NH_4Cl, 1; yeast extract, 1; ascorbic acid, 0.2; sodium thioglycolate, 0.1; sodium lactate, 3.5 (for LSRB); sodium acetate trihydrate, 4 (for ASRB); resazurin (redox indicator), 0.001; agar, 15. Hydrochloric acid and sodium hydroxide were used to adjust pH. All chemicals were of analytical or extra-pure quality and supplied by Merck (Darmstadt, Germany), Union Chimique Belge (Leuven, Belgium), or Sigma (St. Louis, MO).

Pilot-scale experiments used industrial gypsum from a fertilizer manufacture (analysis after drying at ambient temperature, in %w/v: H_2O, 26–28; CaO, 32–33; SO_3, 44–46; P_2O_5, 0.4–0.6; SiO_2, 1.2–1.4; K_2O, 0.01–0.02; Al_2O_3, 0.028; Fe_2O_3, 0.017; MgO, 0.008; Na_2O, 0.05; and impurities in mg/kg: As, 1–7; Cd, 4–13; Co, <1; Cr, 8; Cu, 9; Mn, 3.8; Hg, <0.01; Mo, 3.2; Ni, 11; Pb, 0.8; Se, 2; Ti, 400–700; V, <0.5; Zn, 15–40; Fe, 120. pH: 2.8–3.5).

The cheese whey used as organic substrate contained (%w/v): H_2O, 2–4; lactose, 70; proteins, 11–12; fats, 1.5; minerals (Cl^-, NO_3^-, Na^+, K^+, Ca^{2+}, NH_4^+), 9. pH of a 10% solution in water: 6.0.

Strain Isolation Method

The Postgate *(3)* general method for strain isolation was applied to samples of natural (poorly salted or unsalted) environments, such as manures (pig, bovine, and rabbit), or an estuary that had been hugely contaminated by gypsum, soil, landfill, and water from a pond *(16,17)*. The screening for ASRB and LSRB was carried out on the same raw samples.

The many facultative anaerobic contaminants present in samples did not permit pure strains to be obtained after three or four consecutive repetitions of the isolation procedure. Progressively more selective media had to be adopted: medium E containing 1 g/L of zinc sulfate (zinc toxicity should be reduced for SRB by ZnS precipitation), medium B containing 6000 United States Pharmacopeial (USP) U/L of polymyxin B sulfate (this antibiotic is usually efficient against enterobacteria), medium E containing 0.8 g/L of $Na_2S \cdot 9H_2O$, but no iron (contaminants are usually sensitive to high concentrations of sulfide). In this case, all colonies should be cultivated in medium B to verify sulfate reduction.

Mutagenesis

Mutations were induced by the chemical mutagen NTG. Mutants more efficient at producing sulfide would be able to grow on medium containing a high concentration of selenate, a powerful and specific competitive inhibitor of sulfate reduction owing to its structure being analogous to that of the sulfate ion. Indeed, the enzyme system has some 40-fold greater affinity for the inhibitor, selenate, than for its natural substrate, sulfate. Consequently, selenate would act against the accumulation of sulfate in the organism, rather than on its actual reduction *(3)*.

Cells of the pure SRB strain (further called wild-type strain) in exponential growth phase were suspended in dilution medium (2 g/L Tween-80, 1 g/L bactopeptone, 5 g/L NaCl of demineralized water). One drop of a saturated NTG solution

was added to each 10-mL aliquot of cell suspension. The suspension was distributed in sterile centrifugation vessels and incubated at 30°C for different periods of time (ranging from 15 min to a few hours). At the end of the incubation time, the suspensions were centrifuged at 11,000*g* for 15 min.

The supernatant was replaced by sterile dilution medium in which the centrifuged cells were once more suspended and washed twice. Eventually, the centrifuged cells were mixed in a minimum of dilution medium, diluted, and inoculated (according to the strain isolation method described above), in two series of medium E test tubes, one with 1 g/L of Na_2SO_4 and the other with a supplement of 6 g/L Na_2SeO_4. Colonies of mutant bacteria grew in the solid medium containing selenate. Less than 1% of cell survival has been observed on selenate-free medium after the action of NTG.

Analytical Methods

Sulfate was determined by the turbidimetric method: sulfate ions are precipitated by barium and the solution turbidity was analyzed by a spectrophotometer at 450 nm *(18)*. Sulfide was determined photometrically using the methylene blue method applied to 1-mL liquid samples that had been collected in 5 mL of a 2% (w/v in water) zinc acetate solution *(19,20)*. However, when sulfide stripping was performed in bench-scale experiments, zinc sulfide was, once or twice a day, gravimetrically determined after 15 min of stripping with 70% N_2–30% CO_2 and precipitation in 4% (w/v in water) zinc acetate solution.

In bench-scale experiments, lactate and acetate were determined with a Waters (Milford, MA) 590 HPLC equipped with a SHODEX P1011 precolumn and a SHODEX 1011 ion-exchange column at 90°C. For eluant, 1.5 m*M* H_3PO_4 in water was used at a flow rate of 0.7 mL/min. Samples were filtered on 0.22-μm membranes after substitution of calcium for sodium on DOWEX 50 W resin. A Waters 410 refractive index detector and a Waters Module 745 integrator were used. In pilot-scale experiments, carbohydrates (sugars, polysaccharides, and oligosaccharides) were photometrically determined at 485 nm after reaction with phenol in presence of sulfuric acid *(21)*.

DOC was photometrically determined: the sample was acidified and then stripped by oxygen to remove inorganic carbon; hydroxyl free radicals were formed from potassium persulfate by UV irradiation, and were reacted with the sample organic carbon to form CO_2 and water. The CO_2 diffused through a membrane to a phenolphthalein solution, the absorbance of which was measured at 550 nm. Hydroxylamine was used to prevent interference owing to the chlorinated compounds contained in the samples *(22)*.

Volatile fatty acids (VFA) were determined with a Merck-Hitachi HPLC (Pump: L-6000, UV-visible detector at 210 nm: L-4200, column thermostat: T-6300) equipped with a Supelcogel (Bellefonte, PA) GL-C610h-SP column operating at 20°C. For eluant, 10 m*M* H_3PO_4 in water was used at a flow rate of 0.5 mL/min.

Bench-Scale Bioreactors

A 2.5-L glass vessel (2-L culture vessel, Bibby Steriun Ltd., Stone, UK) with a stainless-steel lid equipped with septum, shaft with two Rushton turbines (four blades, height 10 mm, diameter 45 mm), 0.45-μm gas filters, and tubings for sampling, gas inlet, gas outlet, and temperature regulation was used as the bench-scale

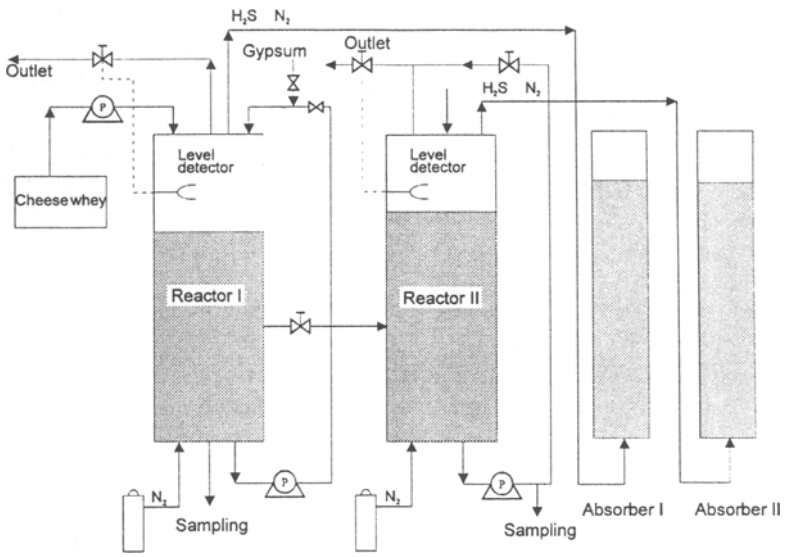

Fig. 1. Flow sheet of the fixed-bed pilot-scale setup.

bioreactor (constructed at University of Liege). Medium C (2 L) was autoclaved at 120°C for 20 min, cooled under nitrogen, and inoculated with 250 mL of cell culture in medium B.

A 20-L full stainless-steel fermenter (LSL Biolafitte SA, St. Germain en Lave, France) equipped with pH regulation, shaft with three Rushton turbines (four blades, height 20 mm, diameter 100 mm), gas filters, septum, and tubings as in the small bioreactor was used to study the scale-up improvement factor. A volume of 12 L of medium C was sterilized (by steam at 120°C) in the fermenter, cooled under nitrogen, and inoculated with 2 L of cell culture in medium B.

Needles placed through the septum were used to maintain pH (Ingold combined probe, Mettler-Toledo, Urdorf, Switzerland) in the range 7.15–7.35 (the probe deviation owing to sulfide was taken into account) by means of sterile HCl (3*N*) or NaOH (3*N*) addition. Redox-poising agents like thioglycolate or ascorbic acid were added during inoculation to prevent their degradation by oxygen during cooling. The stirring speed and temperature were kept constant at about 100 rpm and 30°C, respectively. The redox potential of samples was determined with an Ingold Platinum combined probe. During culture, the bioreactors were overpressurized by about 0.1 bar.

Pilot-Scale Bioreactors

The fixed-bed pilot-scale setup is shown in Fig. 1. In both reactors (constructed with stainless steel), the biologically active volume was 30 L. The reactors contained a solid support matrix (Plasdek type, PVC material, 97% internal porosity, 50 kg/m^3 density, 157 m^2/m^3 specific area). Gypsum and cheese whey inputs have been progressively increased (in the first stage) from 6.5 kg/m^3·d of industrial gypsum and 3 kg/m^3·d of whey to 18 and 8.5 kg/m^3·d, respectively. The medium (gypsum and whey in water) was continuously pumped from the bottom to the top of both

columns. Sulfide was continuously stripped by nitrogen gas (at a 40 L/h flow rate) and absorbed in a sodium hydroxide (7.5*N* in water) solution.

The first stage was inoculated with sediments obtained from the Seine estuary and worked discontinuously (no whey nor gypsum input or output) for a few weeks in order to improve immobilization of the bacteria. After this period, it was continuously operated at a hydraulic retention time of 6 d. The second stage was inoculated with the wild-type ASRB strain, which had been investigated in bench-scale experiments. The culture medium was directly transferred from the first stage. However, in the second stage, the retention time was 12 d (twice that of the first stage).

The mixed pilot-scale setup was similar to the former, but featured only one stage (constructed with stainless steel). The biologically active volume was 30 L, the stirring speed was 120 rpm, and the hydraulic retention time was 10 d. Industrial gypsum and cheese whey inputs have been progressively increased from 0.85 and 3.5 kg/m^3·d to 13 and 43.5 kg/m^3·d, respectively. The medium was also continuously pumped (at a 2000 L/h flow rate) from the bottom to the top of the bioreactor, and sulfide was continuously stripped by nitrogen gas (at a 40 L/h flow rate). The inoculum was also made of sediments obtained from the Seine estuary. In both pilot-scale setups, temperature was maintained at 35°C, pH between 6.0 and 8.0, and redox potential between –140 and –240 mV.

RESULTS

Selection of SRB Strains

The selection of SRB strains from natural samples provided one ASRB strain purified from rabbit manure and four LSRB strains purified from bovine manure (BM), soil (S), Seine estuary (E) (France), and pond water (W). All five strains were studied in 2.5-L bioreactors (conditions described in Methods).

At the end of bioconversion (i.e., after about 150 h), the ASRB strain induced a sulfide concentration of 66.6 mg S^{2-}/L; and the LSRB induced 218 mg S^{2-}/L for the BM strain; 153 mg S^{2-}/L for the E strain; 87 mg S^{2-}/L for the S strain, and too weak a value, to be correctly measured, for the W strain. Moreover, the BM and E strains had regularly consumed the 5.5 g/L of sodium lactate during the ± 150 h of culture.

As illustrated in Fig. 2, plots for wild-type strain and mutant displayed different patterns depending on the organic substrate and the selection type. Therefore, results should also mention maximum sulfate reduction yield, calculated in industrial gypsum equivalent ($CaSO_4 \cdot 2H_2O$ + 30% free water), as in pilot-scale results. Therefore, BM strain daily reduced, at its maximum exponential phase, 470 g of raw industrial gypsum Eq/m^3 of culture medium; E, 217; S, 238, and the ASRB strain, 127 g/m^3·d.

Improvement of Sulfide Production in Bench-Scale Bioreactors

The most productive of both organic substrate-oxidizing strains, ASRB and BM, were investigated to determine which improvement factors could influence sulfide production and sulfate reduction yield. For ASRB, the factors studied were: temperature (from 30 to 38°C), the concentration of gypsum and acetate in medium (medium C: 3.5 and 4 g/L, respectively; rich medium: 7.5 and 8 g/L), and mutagenesis. For LSRB, the improvement factors studied were: the sulfide stripping (i.e., sulfide remov-

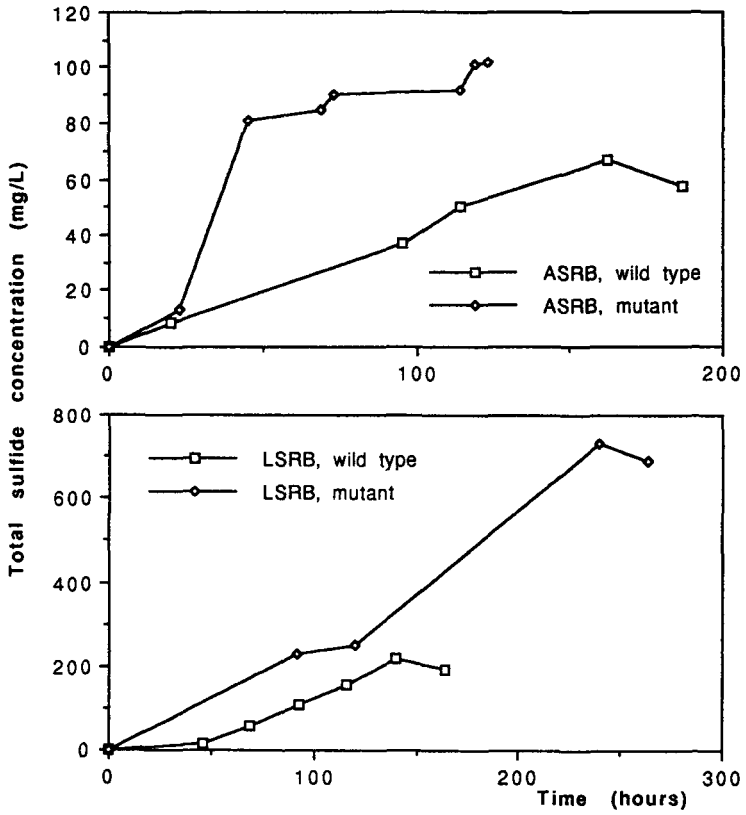

Fig. 2. Total sulfide concentration (mg S^{2-}/L) in the culture medium C during 2.5 L batch cultures with ASRB and LSRB strains, before (wild-type) and after mutagenesis.

ing from culture medium by means of a nitrogen flow as described), the scale-up (from 2.5- to 20-L bioreactor), the concentration of gypsum and lactate in medium (medium C: 3.5 and 6 g/L, respectively; rich medium: 10 and 10 g/L), and mutagenesis.

The results are given in Table 1, which shows the maximum sulfide concentration induced in the culture medium (mg S^{2-}/L; in the case of stripping, it has no meaning) and the maximum sulfate reduction yield (kg of industrial gypsum daily reduced/m^3 of medium). Figure 3 shows the sulfate reduction yields obtained with both SRB types under various conditions of culture.

Pilot-Scale Experiments

Different raw organic substrates were used, such as sugar-refinery molasses, cheese whey, and the mixture of cheese whey and methanol in various proportions (ratio methanol:lactose in weight being 1:1, 3:1, and 9:1). Best results, especially in terms of DOC in effluent, have been obtained with cheese whey.

In the single-stage mixed bioreactor, at a 6.2 kg/m^3·d gypsum input, a yield of 5.5 kg/m^3·d of gypsum reduction was observed (i.e., 90% of the gypsum fed, related to 85% of DOC degradation). A better gypsum reduction yield was obtained at a 13 kg/m^3·d gypsum input: 7.8 kg/m^3·d of gypsum reduction (i.e., only 60% of the gypsum supplied, related to 38% of DOC degradation).

Table 1
Results of the Investigation
of Some Improvement Factors for ASRB and LSRB[a]

Strains	Maximum sulfide concentration S^{2-} mg/L	Maximum sulfate reduction yield, industrial gypsum kg/m³·d
ASRB		
Wild-type		
38°C	85.5	0.329
Rich medium	95	0.400
Mutant		
Medium C	101	0.450
Rich medium	90	1.56
LSRB		
Wild-type		
Stripping	NM[b]	1.2
Scale-up	450	4.07
Mutant		
Medium C	731	0.743
Rich medium	871	1.47
Stripping	NM[b]	1.93

[a] Maximum sulfide concentrations reached in the culture medium and maximum sulfate reduction yield are given.

[b] No meaning because the stripping removes the hydrogen sulfide from the culture medium.

With regard to the fixed-bed bioreactor, at a 18 kg/m³·d gypsum input, the gypsum reduction yield reached 11 kg/m³·d (i.e., 60% of the gypsum fed). At this yield, 1.2 kg/m³·d of DOC was oxidized, which means 45% of the DOC fed. However, during biomass growth and immobilization, a higher DOC degradation yield of 55% was observed. It must be emphasized that a DOC conversion inferior or equal to 40% was attributed to lactic acid oxidation.

The important DOC concentration in the first-stage output is constituted primarily (16 g/L) of acetic acid, which was poorly oxidized. These results show the benefit of working with two stages, reducing organic carbon concentration in effluent, and also increasing gypsum reduction yield. It should be mentioned that, at such high inputs, pH is not easily stabilized; higher inputs lead to bioconversion breakdown. Thus, it seems that there is an input limit of organic substrate that cannot be exceeded without risk of serious acidification and growth inhibition.

Part of the effluent of the first stage, which contained primarily acetic acid, was transferred to the second reactor. Thus, the concentration of gypsum and organic substrate available for ASRB was progressively increased in the second stage. At the highest gypsum input (3.3 kg/m³·d), 3 kg/m³·d of industrial gypsum were reduced (i.e., 90% of the gypsum supplied in the second stage), and DOC conversion reached 0.25 kg/m³·d (35% of DOC fed in the second stage). Therefore, an overall degradation of 65% of the DOC main input was achieved.

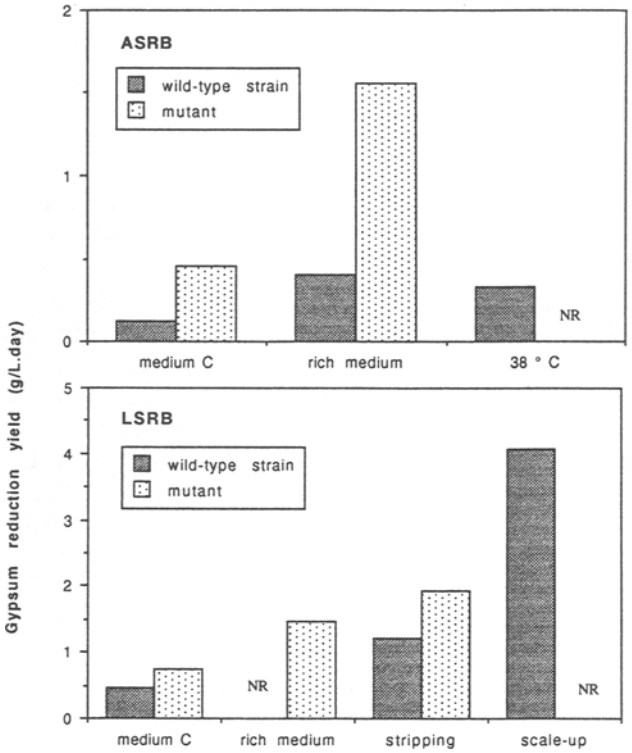

Fig. 3. Maximum sulfate reduction yields obtained in various batch culture conditions with ASRB and LSRB strains, before (wild-type strain) and after mutagenesis. NR, not realized.

DISCUSSION

Bench-Scale Experiments

Bench-scale experiments realized with pure strains of bacteria have revealed some improvement factors that could increase the gypsum bioconversion yield. The results of Fig. 3 emphasize the important improvement carried out by the mutation of both SRB types. Indeed, for ASRB, the improvement factors (i.e., the ratios of the maximum sulfate reduction yield carried out by the mutant to that carried out by the wild-type strain) on medium C and on the rich medium are 3.54 and 3.9, respectively. For LSRB, the improvement factors on medium C, without stripping and with stripping of hydrogen sulfide, are 1.58 and 1.61, respectively.

Although improvement carried out by the mutation is greater for ASRB, it should be noted that, with the wild-type strain, sulfide concentration inside the bioreactor follows a linear, rather than exponential, course indicating a lower yield (the patterns obtained at 38°C and in rich medium are similar to that shown in Fig. 2).

Hauser and Holder *(23)* and Postgate *(3)* suggested that nonexponential growth of SRB cultures may be attributed to the loss of readily available iron (essential for SRB) from solution, particularly when it is precipitated by sulfide. Further investigations are required to explain the beneficial influence of the bacterial mutations. One possible explanation could be an improved production of iron chelating agent(s), a higher rate of incorporation of iron into the cell, or a lower requirement for iron.

The production of iron chelating agents has been postulated by Hauser and Holder *(23)*. Under conditions where the dissolved iron concentration is very low, SRB would produce highly specific chelating agents (more powerful than citrate contained in medium C), which could be released into solution or located on the cell surface. This hypothesis is suggested by tactophily (tendency to adhere to surfaces often observed in freshly isolated culture of SRB *[3]*).

The rate of incorporation of iron and the requirement of metabolism for iron depend, among other things, on electron carriers. The most common electron carriers in SRB are cytochromes, ferredoxins, rubredoxins, and flavodoxins *(3,13)*. These proteins do not have an identical iron amount or well-known roles in each of the SRB strains. Furthermore, some carriers, such as flavodoxin and ferredoxin, when they are both present in the cell, may replace each other *(13)*. These are some factors on which mutations might have to operate to improve ASRB bioconversion.

In bioconversions with LSRB, the steady state was obtained following the complete exhaustion of lactate. The maximum sulfide concentration was reached at approx 870 mg/L S^{2-}. The relative distribution of H_2S and HS^- at pH 7.25 (mean of the pH regulation range; pK_a of H_2S is 7.0 at 25°C) is about 1:1.78. Therefore, the steady-state concentration of free H_2S did not exceed 330 mg/L. The mean concentration of H_2S is not far below its toxic level (i.e., 550 mg/L, *[6,24]*). This means that further improvement factors should be investigated with gas stripping at each stage to avoid limitations caused by H_2S toxicity.

Summarizing the results of the laboratory experiments, it can be concluded that a gypsum reduction higher than 10 kg/m^3·d is easily achievable if all the improvement factors are combined with each other (sulfide stripping, 38°C instead of 30°C, a more concentrated medium of gypsum and organic substrate, mutagenesis, and scale-up). A gypsum reduction yield above 10 kg/m^3·d was eventually reached in pilot-scale bioreactor without mutant biomass. However, bioconversion with ASRB is still not satisfactory. Mutations have induced a lower reliance on iron, but the steady state is rapidly attained, at a time when all organic substrate has not been consumed. Further experiments will be performed to explain the reasons for this inhibition. Possible causes include H_2S inhibition or lack of vitamins. We will also try to obtain other ASRB strains.

Pilot-Scale Experiments

Experiments realized in a mixed bioreactor have shown that gypsum reduction yield increases as a function of sulfate concentration, reaching 7.8 kg/m^3·d at an industrial gypsum concentration of about 50 g/L (i.e., when 40% of the 13 kg/m^3·d of gypsum input is not converted). However, it is important to mention that gypsum is only soluble in water at about 1 g/L. Therefore, a great deal of solid matter is present in the culture medium. Thus, the presence of solid is probably one of the reasons contributing to observed phenomenon. It would act as an immobilization support.

Nevertheless, the gypsum input should not exceed 6.5 kg/m^3·d because the converted fraction of gypsum begins to decrease rapidly below 90%, and acetate tends to accumulate, creating effluent of high DOC (another fundamental problem that is to be solved). We have partly resolved this issue in the fixed-bed setup: a second stage is joined to the main bioreactor in order to enhance acetate oxidation. The conditions (mainly the hydraulic retention time) are modified to allow better development of ASRB.

On the whole, the first stage works at a high sulfate reduction yield (11 kg/m^3·d of industrial gypsum), whereas output effluent still contains about 40% of the gypsum fed. This gypsum will finally be reduced in the second stage by ASRB, which, in addition, will oxidize the acetate produced by the LSRB in the first stage. Analyzing the results obtained in the second stage, it must be noted that gypsum concentration should be raised again, since near-total gypsum reduction is obtained, but acetate remains (35% of the DOC supplied, that is, 5.5 g/L of COD). Our results also clearly demonstrate that the association of acidogenic and SRB is relatively efficient in a sole reactor, as already mentioned by Salmon et al. *(15)*.

Further experiments at the pilot scale will determine the most convenient type of bioreactor to carry out gypsum bioconversion at the industrial scale. In these experiments, a more sophisticated setup will be assessed, including an evaluation of the number of stages, the use of mutants at each step, and the influence of carbonate production on cell immobilization and on support.

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