Bacterial Removal of Pyrite from Concentrated Coal Slurries

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Accepted for publication November 9, 1987

To be economically feasible, bacterial coal desulfurization must be done at a high rate in concentrated coal slurries. The rate may be restricted by gas transfer limitations (O_2 or CO_2), inhibition of the Thiobacilli, or cell death by mechanical abrasion. Experiments designed to differentiate between these limitations show that CO_2 transport is limiting in shake flasks for slurries of more than 20% (wt/wt) of a coal containing 1% pyritic sulfur. Air sparging removed this limitation in slurries of up to 50%, leaving inhibition as the main limitation. Heterotrophic bacteria that establish themselves in a recycled cell culture increase process rates not only by removing organic inhibitors but also by increasing the O_2–CO_2 ratio required by the biomass to the value that is supplied naturally from air.

INTRODUCTION

Several genera of bacteria, including *Thiobacillus, Sulfobus,* and *Leptospirillum,* are capable of oxidizing the pyrite in finely ground coal. In 7–10 days 90% removal of pyritic sulfur has been reported by several researchers.[1–5] This work could be the basis for the bacterial desulfurization of coal on a commercial scale in order to alleviate the problem of acid rain.

The economic feasibility of a commercial process has been studied by Detz and Barvinchak[2] and more recently by Dugan.[6] It depends critically on the details of the environmental regulations and on the costs of competing technologies, mainly stack-gas scrubbing. It is most attractive when combined with coal transportation in slurry pipelines because once the coal has been ground and suspended in water, it is in the ideal form for the microbial process. Also, the costs associated with drying the coal (or of burning it as a concentrated slurry) become chargeable to the transportation step rather than the desulfurization process. The only change required is that coal must be ground slightly finer than is usual in slurry pipelines in order to expose the pyritic inclusions to attack by the bacteria (approximately < 60 mesh vs. < 14 mesh).[7]

It has been suggested[1,8] that the desulfurization could actually be carried out in the pipeline, but there are several difficulties with this approach. Given the large variation of pressure in a pipeline, it would be difficult to keep the dissolved oxygen concentration in the range (1–10 mg/L) required by the bacteria. Temperature and pH control would also present difficulties. The combination of abrasion by the slurry and the dissolved oxygen and low pH needed by the bacteria would require special linings if the pipeline is not to corrode. The cell yields for the process are low, which means that a large inoculum of cells would need to be grown and fed into the pipeline. Finally, given the speed at which a slurry must be pumped in order to prevent settling (~5 mph), only the very longest slurry pipelines would give sufficient residence time to complete the pyrite decomposition. Pipelines built to transport western coal to the eastern United States may be long enough, but western coals contain so little pyrite (they are low in total sulfur, and the sulfur is mainly organic) that a pyrite removal process is not usually justified. Pipelines under consideration for transporting high-pyrite eastern coals are mostly much shorter.

It seems preferable to carry out the desulfurization in an aeration tank at the power plant site. Low-grade heat for temperature control and CO_2 for cell growth are then available from stack gases. Some cell recycle could be provided to inoculate the incoming coal. The required residence time is available in principle since utilities typically keep a 90-day stock of coal at the plant site to guard against interruptions in supply.

The main objection to this idea is that the aeration tank would be extremely large and expensive. Most studies of coal desulfurization have been done with 20% (wt/wt) slurries, and with this slurry a 10-day residence time would require a tank volume of approximately 500 m^3 per electrical megawatt (based on 33% power generation efficiency and a coal heating value of 10^4 BTU/lb). One way to reduce this volume is to increase the rate of the process, and several studies are proceeding in this direction. Another possibility is to increase the slurry concentration in the tank toward the 50% value typically used in pipelines. Unfortunately, it has been found[3,4,9] that slurry concentrations above 20% decrease the desulfurization rate. The objective of this study is to determine the causes of this decrease in order to allow bacterial desulfurization of more concentrated slurries.

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Biotechnology and Bioengineering, Vol. 32, Pp. 813–820 (1988)

CCC 0006-3592/88/060813–08$04.00

THEORY

Stoichiometry of Pyrite Decomposition and Cell Growth

Direct measurement of cell yields for growth on coal pyrite is complicated by the difficulty of measuring the numbers of cells adsorbed on the solid. They can, however, be estimated from the stoichiometry of pyrite decomposition and cell growth, which can be written

$$2FeS_2 + [7.5 - \frac{b}{2}(4 + m - 3n - 2p)]O_2 + bCO_2 + bnNH_3 + [1 + b(m - 3n)]H_2O \rightarrow bCH_mN_nO_p + H_2SO_4 + Fe_2(SO_4)_3 \quad (1)$$

Given the composition of the cells and one measured yield value (cell yield on pyrite, O_2–CO_2 ratio, etc.) the constant b and all the other yields can be calculated. No direct experimental measurements are available for the elemental composition of the Thiobacilli, but fortunately the elemental composition of bacteria is fairly consistent. Taking the average composition given in ref. 10 (p. 120) and various measured yields from the literature gives the results shown in Table I. The scatter in these values reflects the uncertainties in this procedure, including the exact composition of the cells, the differences between coal pyrite and pure crystalline pyrite, and the presence of maintenance metabolism and heterotrophic bacteria both of which would increase the ratio of oxygen consumption to carbon dioxide consumption. Despite these difficulties, the average values in Table I are useful estimates of process parameters.

A further source of uncertainty in the preceding calculation is the possibility that pyrite decomposition does not proceed stoichiometrically but that sulfur oxidation is favored by the cells because it yields more free energy.[14] The cell yield given by Chang and Myerson[15]—0.16 g protein/g Fe leached, which corresponds to Y_{XS} = 0.15 g cells/g FeS_2 if the cells are 50% protein—is much higher than the values in Table I. This may be due in part to the fact that the grams of iron leached is not proportional to the grams of pyrite decomposed.

Rate of Sulfur Oxidation

If pyrite degradation is not stoichiometric, it must be defined in terms either of iron removal or of sulfur removal. The latter is the obvious choice since this is the objective of the process. It is assumed that sulfate sulfur in the coal is negligible, that there is no oxidation of organic sulfur, that the rate of purely chemical oxidation of the pyritic sulfur is negligible compared to the bacterial rate, and finally that pyritic sulfur oxidation can only be done by cells adsorbed on the pyrite surface. The conservation equation for a batch process can then be written by equating the metabolic rate of the adsorbed bacteria to the rate of disappearance of pyritic sulfur from the coal and the rate of accumulation of sulfate in the liquid:

$$qX\varepsilon A(x) = -\varepsilon\frac{dS}{dt} = \frac{32}{\rho}(1 - \varepsilon)\frac{d[SO_4^{=}]}{dt} \quad (2)$$

Here, x is the fractional removal of the pyrite ($=1 - S/S_i$) and $A(x)$ is the exposed surface area of pyritic inclusions per unit volume of coal. This area is inversely proportional to the coal particle size and must decrease as the pyrite is consumed. The exact form of the function $A(x)$ depends on the details of the geometry of the coal particles and pyritic inclusions. It has been derived mathematically only for the simple case of parallel-sided inclusions in a "flat-plate" coal particle.[7]

Dividing equation (2) by εS_i and integrating the second equality shows how x can be calculated from measurements of liquid phase sulfate concentration (assuming all the ferric hydroxysulfate precipitates have first been dissolved):

$$x = \frac{32(1 - \varepsilon)}{S_i\varepsilon}[[SO_4^{2-}] - [SO_4^{2-}]_i] \quad (3)$$

The first equality becomes

$$\frac{qXA(x)}{S_i} = \frac{dx}{dt} \quad (4)$$

One important feature of this equation is that it does not contain the coal slurry concentration ε. So a plot of experimental data as x vs. t will be independent of ε unless the high solids concentration affects cell viability or the specific sulfur oxidation rate q, which depends only on the concentrations of nutrients and inhibitors to which the cells are exposed.

In equation (4) X is the mass of viable sulfur-oxidizing cells adsorbed per unit area of pyrite. Studies of the adsorption of *Thiobacillus* on coal[16] show that the cells adsorb preferentially on the pyrite and that they do so irreversibly within a few minutes of contact with the coal. If few cells are present, then the offspring of adsorbed

Table I. Yield values for pyrite decomposition.[a]

Reference	Measured value	b	Y_{XS} (g cells/g pyrite)	Y_{oc} (g O_2/g CO_2)	Y_{os} (g O_2/g pyrite)
11	NH_3–FeS_2 = $\frac{2}{67}$ mol/mol	0.26	0.027	19.4	0.92
12	O_2–CO_2 = 13 mol/mol	0.49	0.051	9.5	0.86
13	CO_2–FeS_2 = $\frac{1}{5}$ mol/mol	0.40	0.042	12.0	0.88
Average			0.040	14	0.89

[a] Assumed cell composition, $CH_{1.8}N_{0.23}O_{0.38}$ + 8% ash.

cells can remain adsorbed, and $X = X_i + S_i Y_{XS} x/A$. Thus X depends on the fractional pyrite decomposition x but is still independent of the slurry concentration as long as the inoculum concentration, X_i grams of cells per unit area of exposed pyrite, is kept constant between experiments. If many more cells are added, then the surface becomes saturated with cells and offspring cannot find room to adsorb and fall off into the liquid, X remaining constant at its maximum value. In order to maximize the desulfurization rate, the cell inoculum should be large enough to achieve this saturation condition. Myerson and Kline[17] measured the saturation-adsorbed cell concentration as approximately 800 μg protein/g coal (<250 mesh). Since the coal used contained 2% pyrite and a cell contains $O(10^{-13})$ g of protein, this corresponds to $O(10^{11})$ cells per gram of pyrite, which is precisely the inoculum size found to give the maximum rate of desulfurization.[2,18] (Adding more cells seems to reduce the rate, possibly due to autoinhibition.[1])

The figures given in the preceding illustrate the difficulty of generating the cell inoculum for a commercial process. If cells are 50% protein by weight, the average cell yield from Table I corresponds to $0.04 \times 0.5 \times 10^{13} = 2 \times 10^{11}$ cells/g pyrite. So generating the cells required to inoculate 1 ton of coal at the required level of 10^{11} cells/g pyrite requires all the cells that grow on a half ton assuming they could somehow all be desorbed from the coal. Cell recycle is therefore probably essential, although there is the possibility that a physical coal separation process could generate a high-pyrite coal fraction on which sufficient cells could be grown.

HYPOTHESES

Experiments were designed to discriminate between four possible limitations by which high slurry concentrations could reduce the metabolic rate of the bacteria.

Inhibition by Organic Compounds

It is a common observation[1,2,3,5] that mixed cultures of bacteria isolated from acid–mine drainage or developed in experiments with nonsterilized coal work better than pure cultures of the autotrophic, pyrite-oxidizing bacteria. This is usually explained by the presence of heterotrophic bacteria, which consume organic compounds that can inhibit the autotrophs. These inhibitors may leach from the coal or they may be autoinhibitory, low-molecular-weight organic acids generated by the autotrophs.[19,20] In either case, their concentration will be higher in concentrated slurries.

Cell Death by Mechanical Abrasion

Nienow and Conti[21] have shown that at slurry concentrations above 20%, the abrasion rate between particles in a stirred vessel is proportional to the square of the slurry concentration. Ebner[22] has reported seeing damaged cells under the microscope in samples taken from concentrated slurries. It is therefore reasonable to suppose that the cells are being trapped between the particles in concentrated slurries and killed by mechanical abrasion.

Oxygen Limitation

The possibility that bacterial metabolism is limited by the transfer rate of oxygen into the slurry can be assessed by comparing the supply and demand for oxygen in the process. The demand for oxygen is low principally because the process is slow. For example, if a coal has density 1.25 g/cm^3 and contains 2% pyrite and the objective is complete removal in a week, then the oxygen requirement (using the average Y_{oS} value from Table I) is Q_o = 26.5 mg O_2/L h for a 20% (vol/vol) slurry and 53 mg O_2/L h for a 40% slurry. This is much less than the maximum oxygen transfer rate ($k_L ac^*$) expected for water in 1-L shake flasks, which is typically 500 mg/L h. However, transfer into concentrated slurries is slower than into water. The data of Joosten et al.[23] for a sparged agitated tank suggest that $k_L a$ for a 20% slurry is similar to that for water, but for a 40% slurry it is an order of magnitude lower. If this holds true for shake flasks, then oxygen will become limiting in slurries of over 40%. However, this should never be a problem in sparged tanks, where $k_L a$ values are typically an order of magnitude larger than in shake flasks. The same general conclusion, that oxygen transfer limitation can be expected in concentrated slurries in shake flasks but that it can be alleviated by air sparging, would hold if this approximate calculation were repeated for any practical coal density, pyrite content, shaker speed, etc.

Carbon Dioxide Limitation

Cell growth metabolism, but not maintenance metabolism, may also be affected by a low concentration of dissolved carbon dioxide. The question is whether oxygen or carbon dioxide is more likely to become limiting.

Oxygen limitation is expected if the ratio of the oxygen requirement Q_o (mg/L h) to the maximum rate that oxygen can be supplied $(k_L ac^*)_o$ is greater than unity (see previous section). However, oxygen limitation will only be more serious than carbon dioxide limitation if this ratio for oxygen is larger than the corresponding ratio for carbon dioxide. We therefore examine the quantity

$$\frac{Q_o}{(k_L ac^*)_o} \frac{(k_L ac^*)_c}{Q_c} = Y_{oc} \left(\frac{D_c}{D_o}\right)^{2/3} \frac{P_c}{P_o} \cdot \frac{H_o}{H_c} \qquad (5)$$

It has been assumed that the mass transfer coefficient k_L is proportional to the diffusivity of the gas in water, D, raised to the two-thirds power, a common result from mass transfer theory.

Taking the values $p_c = 3 \times 10^{-4}$ atm, $p_o = 0.21$ atm for air, $Y_{oc} = 14$ (Table I), $D_c = 1.96 \times 10^{-5}$ cm^2/s, $D_o = 2.5 \times 10^{-5}$ cm^2/s, $H_o = 25$ atm L/g, and $H_c = 0.69$ atm L/g gives this quantity as 0.62. The fact that it is less than 1 suggests that carbon dioxide would become limiting first, although the value is not small enough to

make this conclusion certain. (It would depend on the relative values of the Monod saturation constants for the two gases, which have effectively been taken as zero in this calculation.) Previous experiments to resolve this question by sparging slurries with air enriched with carbon dioxide have also produced inconclusive results.[2,3,24] This is partly because many of the experiments were done with low slurry concentrations, conditions under which no limitation by either gas is expected.

Other Hypotheses

Other possible reasons exist for the poor performance of the bacteria in concentrated slurries. The other hypotheses considered are listed in this section with the reasons for rejecting them.

Coal particles may agglomerate in concentrated slurries, leaving some pyrite surfaces not "wet" and not accessible by the bacteria. However, the irregular nature of the coal particle surface and the very powerful surfactants produced by the bacteria will combine to minimize this problem.

All coals contain a range of heavy metals that may leach out and reach inhibitory levels in the liquid phase in concentrated slurries. Although this may be a problem with certain coals, the Thiobacilli in general are very resistant to inhibition by heavy metals.

There is some evidence for inhibition of *Thiobacillus* by the ferric ion. However, the solubility of Fe^{3+} is extremely pH dependent, so as long as the pH is controlled at a fairly high value, there is no reason to believe that the Fe^{3+} concentration in a dilute slurry will be higher than in a concentrated one.

EXPERIMENT

All experiments were carried out in 1-L Erlenmeyer flasks shaken at 125 rpm on a rotary shaker at room temperature. Slurries were made up by weight to 250 g, so the actual slurry volume varied from 240 mL for a 20% (wt/wt) slurry to 220 mL for a 50% slurry. The liquid contained the mineral salts from Silverman's[25] 9K media plus $6 \times 10^{-5}M$ $Fe_2(SO_4)_3$ to saturate it with ferric ions. The pH was kept in the range 2.3–2.5 by adding 6*N* NaOH every 2 days if required.

The coal was a Pittsburgh seam bituminous coal from the Arkwright mine in Monongalia, WV. It was sieved to 80×140 mesh and washed with acidified tap water (to pH 1 with H_2SO_4) to remove fines and ash constituents that could affect the pH during an experiment. It was then rinsed with distilled water and dried before use. This size fraction contained 1.0% pyritic sulfur.[1]

Thiobacillus ferroxidans (ATCC 19859) was grown on 9K media with 45 g/L $FeSO_4 \cdot 7H_2O$ as the energy source. *Thiobacillus thiooxidans* (ATCC 19377) was grown on 9K media with 5 g/L $Na_2S_2O_3$. Cells were grown in 1-L shake flasks containing 400 mL liquid at 125 rpm and room temperature. Early stationary phase cells were harvested by centrifugation at 12000*g* for 20 min. After mixing the two cultures a cell count was made in a Petroff Hauser counting chamber and sufficient cell suspension added to each slurry to give a total cell concentration in the range 10^{10}–10^{11} cells/g pyrite. This procedure gives approximately equal numbers of the two bacterial species and has been found to give a much shorter lag phase than using iron-grown *T. ferroxidans* alone.

Every 2 days 4-mL samples were taken and 1 mL 6*N* HCl was added to stop bacterial activity and dissolve iron hydroxide and hydroxysulfate precipitates. After mixing and warming, the sample was filtered and the sulfate concentration in the precipitate measured by the barium chloride turbidometric method.

Obtaining consistent representative samples of the slurry was found to be quite difficult. It is important, first, because some of the iron and sulfur liberated from the pyrite forms precipitates that stick to the coal and, second, because a systematic sampling error could alter the slurry concentration as an experiment progressed. The procedure adopted was to use a 5-mm-i.d. glass rod as a pipette while holding the flask as close to the horizontal as possible and swirling it to keep the coal in suspension. The accuracy of this procedure was confirmed by checking the slurry concentration at the end of each experiment. If a significant deviation was found, the calculation of the percentage of sulfur removal from the liquid phase sulfate concentration was based on the values of slurry concentration interpolated between the known initial and final values.[26] Changes in slurry concentration due to evaporation, checked by weight loss in flasks from which no samples were taken, were found to be small. The water loss (~0.1 g/day) would be balanced during the experiments by the addition of a base for pH control.

No attempt was made to sterilize the coal before these experiments, so the results may be affected by the activity of the coal's natural microflora. There were two reasons for this procedure. First, previous experience with this coal had showed that autoclaving had some effect on the pyrite that accelerated its decomposition.[1] Second, results obtained in this way are more applicable to the commercial scale, where pure culture operation would be impractical. The slow pyrite-decomposing activity observed in control flasks to which no cells were added was apparently due primarily to the natural bacteria on the coal. There was virtually no pyrite decomposition in uninoculated control flasks that contained 1 g/L $HgCl_2$ to stop this bacterial activity.

Several experiments were run with coal that had not been washed to remove fines, but the results were inconsistent. This suggests that fines could affect gas transfer or, due to their large surface area, cell adsorption. Investigation of this question is continuing.

RESULTS AND DISCUSSION

Addition of Activated Carbon

Experiments were run on slurries consisting of different mixtures of coal and 18×45 mesh activated carbon. Re-

sults are plotted in Figure 1 as percentage of sulfur removal vs. time. The data from the 20% coal slurry and the 40% coal slurry (all concentrations are wt/wt) demonstrate the problem we are attempting to resolve. The analysis of eq. (4) showed that these plots would be independent of slurry concentration if there was no gas transfer restriction, cell abrasion, or inhibition. Since the observed desulfurization in the 40% slurry is actually much slower than in the 20% slurry, the question is which of these limitations is responsible.

To answer this question, compare the 20% coal slurry with the 20% coal, 20% activated carbon slurry. Adding the carbon would increase gas limitation (by reducing k_La) and mechanical abrasion but should reduce inhibition of the Thiobacilli by organics since activated carbon will adsorb a wide spectrum of organic molecules from aqueous solution. So the fact that addition of activated carbon is observed to reduce the desulfurization rate (Fig. 1) tends to eliminate the inhibition hypothesis.

It could be argued that the lower rate in the mixed 20:20 slurry was due to nonselective adsorption of the Thiobacilli on any available surface. The cell inoculum size was proportional to the pyrite content of the slurry, so if cell adsorption is nonselective, the cell concentration on the pyrite [X in eq. (4)] in the 20:20 mixture would be approximately one-half that in the 20% coal slurry. (Only the external surface area of the activated carbon is available to the cells; they are too large to enter the internal pore structure.) To show that this is not the case, compare the results from the three slurries that have a total slurry concentration of 40%. Since cells were added in proportion to pyrite content, the X values would increase in the order 20:20 mixture, 30:10 mixture, and 40% coal if cell adsorption was nonselective. The fact that the desulfurization rate (dx/dt) increases in exactly the opposite order (Fig. 1) shows that cells do not adsorb nonselectively but have a high affinity for pyrite surfaces. This agrees both with previous studies[16] and with the theory of evolution; natural selection would deal harshly with any strain of organisms that relied on a solid substrate but were unable to recognize it and attach themselves to it.

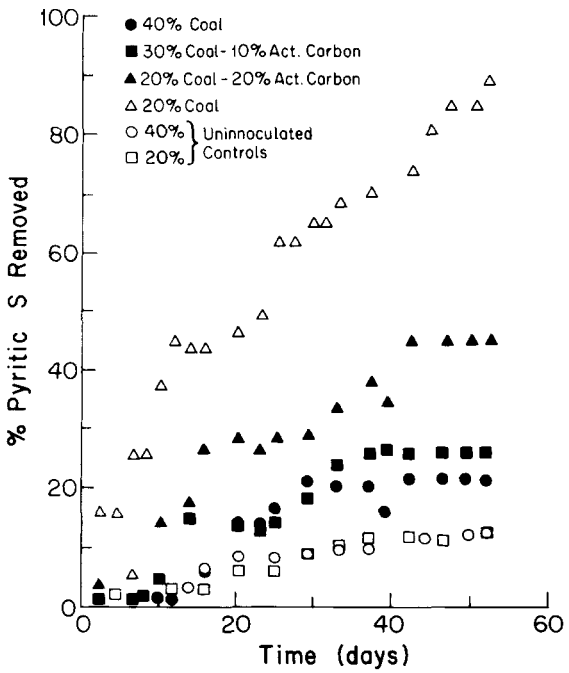

Figure 1. Effect of activated carbon on desulfurization rate.

Further analysis of the data from the three slurries with a total concentration of 40% allows us to discriminate between the two remaining hypotheses of gas transfer limitation and cell abrasion. Abrasion would be similar in all three cases, but gas limitation would become more severe in the order 20:20 mixture, 30:10 mixture, and 40% coal. This is because the maximum gas transfer rate (k_Lac^*) would be similar in all three slurries, but the requirement for the gas would be highest in the 40% coal slurry since it contains more pyrite and biomass. The observation that the desulfurization rate decreases in this order (Fig. 1) therefore supports the gas transfer hypothesis. This is reinforced by the fact that the rate (dx/dt) in the 20:20 mixture is approximately twice that in the 40% coal slurry, which means that the actual rate of bacterial metabolism (in terms of grams of pyrite decomposed per day) is the same in both flasks. This is as expected if metabolism is limited by gas transfer, and k_Lac^* is the same in both cases.

Gas Sparging

In this series of experiments different gases saturated with water vapor were sparged through the slurries while they were being shaken. Estimates made earlier showed that this would remove any gas limitation present.

Sparging a 20% coal slurry with air produced no measurable improvement in the desulfurization rate (Fig. 2), indicating, as expected, that there is no gas transfer limitation at this slurry concentration. However, the data for 35% slurries shown in Figure 3 shows a completely different pattern. Without sparging the desulfurization rate is very low, but sparging with air dramatically increases the rate, showing again that the gas transfer hypothesis is correct. To determine if the limiting gas is oxygen or carbon dioxide, slurries were also sparged with air containing 2% CO_2. This produced a drop in rate (Figs. 2 and 3) compared with the air-sparged case, which suggests that carbon dioxide at these elevated levels (60 times the concentration in air) is inhibitory to the Thiobacilli.

Data from air-sparged slurries of different concentrations are plotted together in Figure 4. It can be seen that removing the gas transfer limitation makes the concentrated (35 and 50%) slurries perform nearly, but not quite, as well as dilute (20%) slurries. The difference in ultimate pyrite removal (60 vs. 75%) is particularly serious. This residual effect could be due to inhibition or mechanical abrasion, so another set of experiments was carried out to distinguish between them.

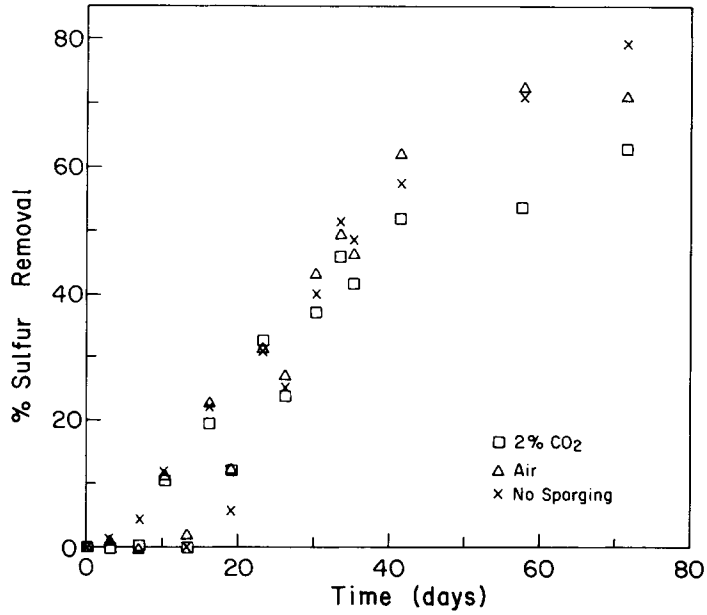

Figure 2. Effect of gas sparging on 20% slurries.

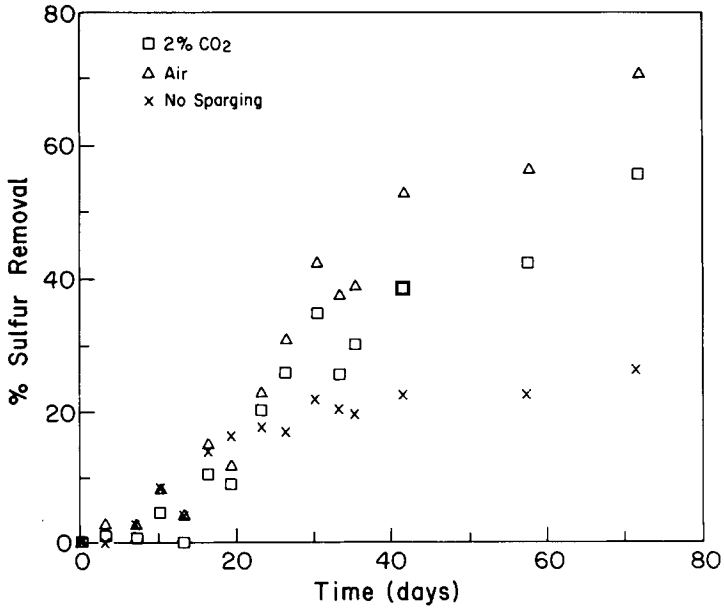

Figure 3. Effect of gas sparging on 35% slurries.

Sequential Batch Culture

The data in Figures 1–5 often show a tendency to "diauxic" behavior with a "lag" separating two regions of rapid desulfurization (clearest in the 20% coal slurry in Fig. 1). This has been observed previously[1] and explained by a combination of autoinhibition of the Thiobacilli by organics and the growth of heterotrophs living naturally on the coal. As desulfurization proceeds, the organic compounds accumulate, eventually completely inhibiting the Thiobacilli, until sufficient heterotrophic bacteria can grow to consume the organics and allow desulfurization to continue. If this is correct, the mixed culture present at the end of an experiment should contain a balanced population of heterotrophs and be much less prone to inhibition by organics. To test this, half of the slurry was removed from the flasks at the end of some of the experiments described, the coal was drained on a sieve and then rinsed with a small amount of mineral salt solution to remove as many bacteria as possible. Fresh coal was then added to the liquid to make up the original slurry concentration, and this fresh slurry was put back in the flask, which was returned to the shaker. The coal on the sieve was rinsed with distilled water, dried, and weighed, so that the new slurry in the flask could subsequently be corrected to its exact original concentration by addition of small amounts of water or coal.

A typical time course for the subsequent process is shown in Figure 5. There is an immediate improvement in rate when compared with Figure 4, desulfurization proceeds to over 90% even in the concentrated slurries, and

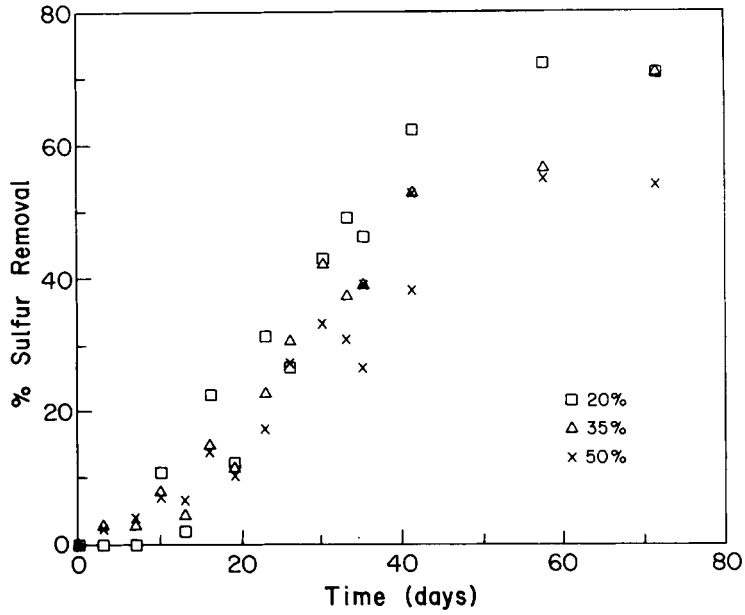

Figure 4. Pyritic sulfur removal in air-sparged slurries.

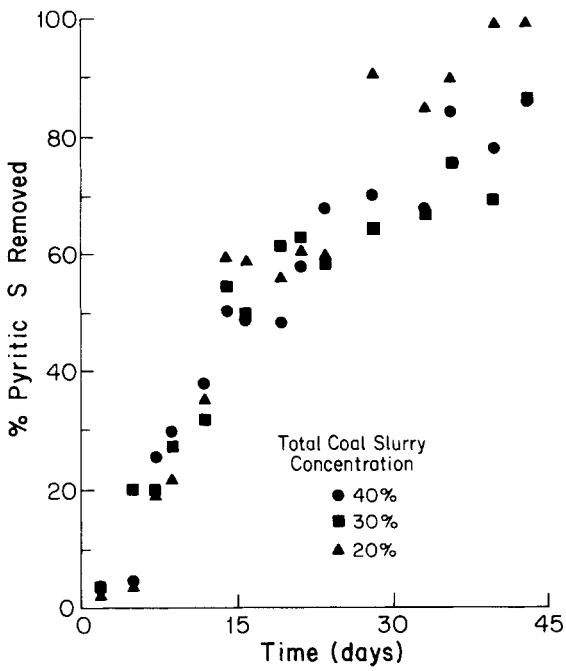

Figure 5. Performance of recycled cell culture.

there is no evidence of diauxic behavior. The improvement in rate is similar to that observed previously[1,2,3,5] using recycled cultures or mixed cultures isolated from acid mine drainage. All of this is as expected if the limiting factor in Figure 4 is inhibition of the Thiobacilli by organics, and the preceding discussion about the importance of heterotrophic activity is correct. But it is impossible to reconcile these observations with the cell abrasion hypothesis.

It should be noted that the cell concentration in the recycled culture is unknown, so some of the improvement between Figures 4 and 5 could be due to an increased cell concentration. However, this effect cannot be large because all experiments were done with a cell inoculum large enough to saturate the available pyrite surface. Thus the desulfurization rate is independent of the exact cell concentration.

Figure 5 also shows that the desulfurization rate obtained with the recycled culture is independent of slurry concentration and is close to that observed with an autotrophic culture in a 20% coal slurry (Fig. 1). This is surprising. The 40% slurry data in Figure 5 should be comparable with the 20:20 mixed slurry data in Figure 1; both experiments were done in unsparged flasks, both slurries contained 20% coal and 20% inert solids (previously desulfurized coal in one case and activated carbon in the other), and neither experiment should be affected by inhibition. Yet the recycled culture gives a much higher rate. How can these results be reconciled with the belief that the desulfurization rate in concentrated slurries in unsparged shake flasks is limited by gas transfer restrictions?

Estimates made earlier showed that for transfer from air, carbon dioxide is slightly more limiting than oxygen. However, this was based on an average of published values for the ratio of O_2 to CO_2 (Y_{oc}) required by the cells. Heterotrophic bacteria consume O_2 and produce CO_2, so their presence must increase Y_{oc}. Indeed, the wide variation in the Y_{oc} values in Table I probably reflects the varying amounts of heterotrophic activity in the experiments on which they are based. Taking the highest value, the one with the most heterotrophic activity, would have produced the conclusion that oxygen and carbon dioxide were equally limiting. (One can speculate that evolution would inevitably produce a bacterial ecosystem for which air is a "balanced diet"). It is therefore possible that the purely autotrophic bacteria in the 20:20 slurry in Figure 1 were restricted by the availability of carbon dioxide and that the heterotrophic bacteria in the corresponding slurry in Figure 5 produced enough carbon dioxide to eliminate this restriction. The heterotrophs would, of course, increase the demand

for oxygen in the slurry, but this need not give rise to an oxygen-limited situation; a small percentage increase in oxygen transfer could produce a large percentage increase in carbon dioxide availability.

CONCLUSIONS

During the bacterial decomposition of coal pyrite by the Thiobacilli in shake flasks, carbon dioxide availability reduces the rate in slurries containing more than 20% coal. Inhibition by organic compounds, either produced by the Thiobacilli or leached from the coal, plays a lesser role. Recycled mixed cell cultures, which must be used in any practical coal desulfurization scheme, develop a population of heterotrophs. These accelerate the process not only by removing the inhibitors but also by generating carbon dioxide, which can be used by the Thiobacilli. For these recycled cultures transfer from air provides nearly the correct balance of oxygen and carbon dioxide, so using a gas stream enriched in carbon dioxide is unnecessary. Sparging air through the slurry should provide adequate amounts of both gases, even in slurries of 50% coal.

NOMENCLATURE

A	exposed pyrite surface area per unit mass of coal (function of x)
a	gas–liquid interfacial area per unit volume of slurry
c^*	saturation concentration of dissolved gas
D	diffusivity of gas in water
H	Henry's law constant
k_L	mass transfer coefficient for gas
p	partial pressure of gas in gas phase
Q	mass of gas required by cells per liter of slurry per hour
q	specific sulfur oxidation rate
S	mass of pyritic sulfur per unit mass of coal
$[SO_4^{2-}]$	concentration of sulfate in liquid phase (mol/L)
t	time
x	fractional removal of pyritic sulfur
X	cells adsorbed on unit area of pyrite
Y_{XS}	cell yield on pyrite
Y_{oc}	mass ratio of O_2 to CO_2 required by cells
Y_{os}	mass of O_2 required by bacterial oxidation of 1 g pyrite

Subscripts

c	carbon dioxide
O	oxygen
i	initial condition

Greek letters

ε	mass fraction of coal in slurry
ρ	density of liquid phase (gm/L)

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