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Continuous microbial leaching of a pyritic concentrate by *Leptospirillum*-like bacteria

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Summary. Continuous leaching of a pyritic flotation concentrate by mixed cultures of acidophilic bacteria was studied in a laboratory scale airlift reactor. Enrichment cultures adapted to the flotation concentrate contained *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. During the late stationary growth phase of these thiobacilli growth of *Leptospirillum*-like bacteria was observed, too. In discontinuous cultivation no significant influence of *Leptospirillum*-like bacteria on leaching rates could be detected. During continuous leaching at pH 1.5 *Leptospirillum*-like bacteria displaced *Thiobacillus ferrooxidans*. The iron leaching rate achieved by *Leptospirillum*-rich cultures was found to be up to 3.9 times higher than that by *Leptospirillum*-free cultures.

Introduction

In most studies of microbial oxidation of metal sulphides pure cultures of *Thiobacillus ferrooxidans* were used, although other species, e. g. *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, may play an important role, too. In order to avoid formation of elemental sulphur which may inhibit sulphide oxidation, mixed cultures of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* were applied. Leaching results varied with substrate and strain of *Thiobacillus ferrooxidans* present in the culture (Bosecker et al. 1978; Ebner 1978; Norris and Kelly 1978; Norris and Kelly 1982). Besides *Thiobacillus ferrooxidans* the iron-oxidizing *Leptospirillum ferrooxidans* and *Leptospirillum*-like bacteria were reported to oxidize pyrite when grown in mixed culture with sulphur-oxidizing thiocabilli (Balashova et la. 1974; Norris and Kelly 1978) or even in pure culture (Norris 1983).

In most cases of industrial applications continuous processes of bacterial leaching are more suitable than batchwise operation. Several continuous processes have been described so far, e. g. leaching of zinc sulphide (Gormely et al. 1975; Sanmugasunderam et al. 1985), chalcopyrite (McElroy and Bruynesteyn 1978), pyrite (Chang and Myerson 1982), removal of pyrite from coal (Myerson and Kline 1984). In spite of possible advantages of mixed cultures, in these studies only pure cultures of *Thiobacillus ferrooxidans* were used. Investigations of continuous leaching with mixed cultures are rarely found in literature. Mixed cultures of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* have been reported to enhance continuous solubilization of arsenic from sulphidic concentrates (Groudeva and Groudev 1984). Though the development of a *Leptospirillum*-containing mixed culture from a pure culture of *Thiobacillus ferrooxidans* during continuous leaching of copper sulphide has been reported (Groudev 1986), there is no information available about continuous bacterial leaching experiments with mixed cultures containing both thiobacilli and *Leptospirillum*-like bacteria.

Materials and methods

Microorganisms. Cultures used in this study were originally supplied by Preussag AG Metall, Goslar (FRG). Microorganisms relevant to leaching were enriched from the natural microflora of the Rammelsberg Mine, Goslar (FRG) on a pyritic flotation concentrate. In our laboratory the cultures were maintained at 30° C in shake flasks with 9K-Medium (Silverman and Lundgren 1959) containing 100 g/l flotation concentrate as sole energy source. After several months of subcultiva-

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tion all cultures contained predominantly *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. In the stationary growth phase *Leptospirillum*-like bacteria were observed. Pure cultures of these autotrophic organisms were able to oxidize ferrous sulphate and pyrite, but could not use elemental sulphur or sulphidic copper and zinc as energy source. Their generation time was 19 h when grown on ferrous sulphate.

Depending on culture conditions helical forms were observed as well as vibrios. These characteristics agree with former descriptions of *Leptospirillum*-like bacteria, a group of organisms resembling *Leptospirillum ferrooxidans*, but consisting of several distinct species or even genera, which have not been classified so far (Norris 1983; Harrison and Norris 1985; Harrison 1986). No heterotrophic acidophilic bacteria or anaerobic organisms were found in enrichment tests with suitable selective media.

Flotation concentrate. The pyritic concentrate used in this work was obtained from a flotation process of Preussag AG Metall, Goslar (FRG). Particle sizes of 90% (w/w) of the solids were found to be below 60 μm. Elemental analysis gave the following composition: 39% Fe, 0.2% Cu, 1.8% Zn and 44% sulphidic sulphur.

Experimental conditions. All experiments were performed in a glass airlift reactor with internal loop (Kiese et al. 1980). The reactor was equipped with a conical, stainless steel bottom (cone angle of 60° C) and a perforated plate as gas distributor. An aeration rate of 10 l/min with an operating volume of 4.5 l guaranteed homogeneous suspension even at 240 g/l pulp density. Continuous leaching experiments were carried out using the set up shown in Fig. 1. Low dilution rates necessary to avoid wash out of leaching organisms could only be achieved by intermittent feed of medium. Every 30 minutes fresh medium was fed from the reservoir into the airlift reactor. In the reservoir flotation concentrate was suspended in water containing all nutrient salts of the 9K-medium. With pH of 5.5 in the reservoir any bacterial pre-leaching was avoided. For discontinuous leaching experiments 9K-medium containing 100 g/l flotation concentrate as sole energy source was inoculated with 1% (v/v) of a mixed culture grown in shake flasks at 30° C with 100 g/l flotation concentrate. To perform experiments without *Leptospirillum*-like bacteria, inocula were taken from cultures in the logarithmic growth phase, while *Leptospirillum*-containing inocula were taken from stationary phase cultures. Prior to inoculation pH was adjusted to 2.3 using 5 mol/l sulphuric acid. Experiments were carried out at 30° C.

Continuous operation was started in the logarithmic growth phase of a culture grown with 240 g/l flotation concentrate. For pH control 5 mol/l sulphuric acid and 10% (v/v) ammonia were used. Pulp density of the feed suspension was 240 g/l. All experiments were carried out at 30° C.

Analytical procedures. Samples were analyzed for viable cells by the most probable number (MPN) method (Oberzill 1967). Serial dilutions (1:10) with five parallels were carried out with 1 ml samples in 9K-medium containing ferrous sulphate as substrate for iron-oxidizing bacteria. Activity of *Thiobacillus ferrooxidans* could be discriminated from growth of *Leptospirillum*-like bacteria by microscopic examination. For the determination of viable cell numbers of *Thiobacillus thiooxidans* a medium containing elemental sulphur as sole energy source was used. Analysis of dissolved iron, copper and zinc was carried out by atomic absorption spectrometry (AAS). Prior to

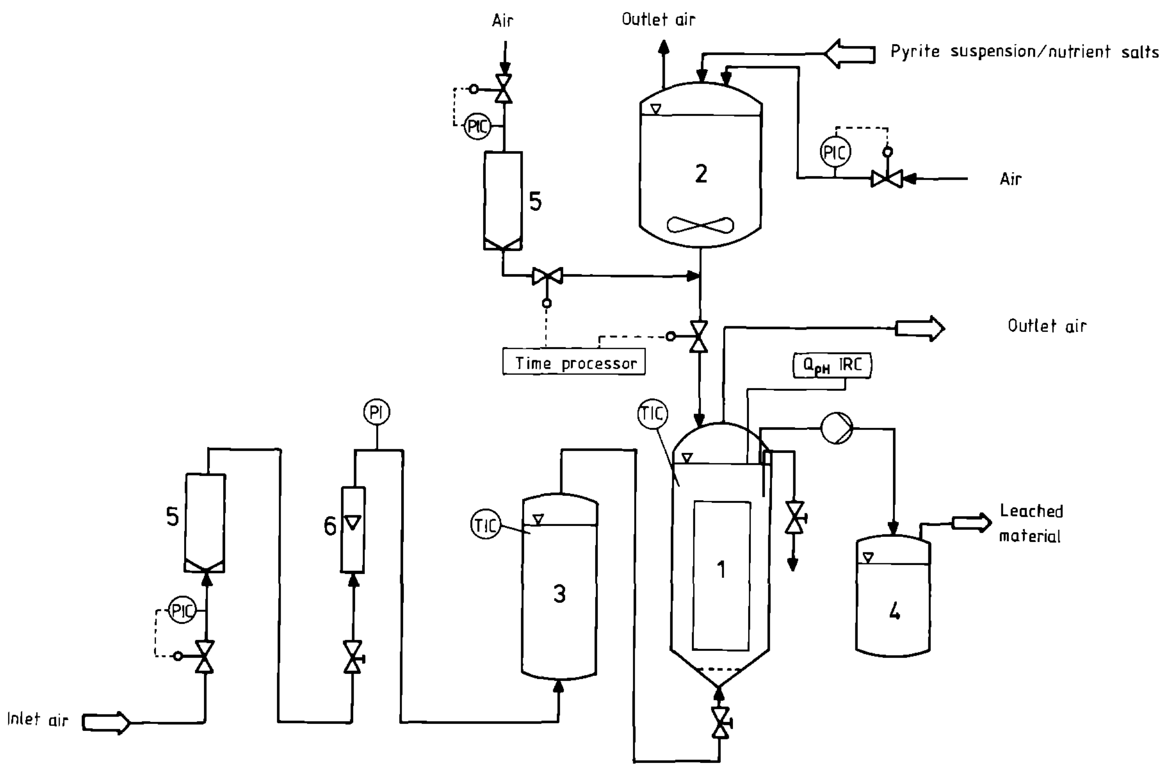

Fig. 1. Continuous leaching apparatus. *1* Airlift reactor; *2* Reservoir; *3* Air saturation; *4* Residue receiver; *5* Filter; *6* Flowmeter

analysis of the liquid phase, samples were filtered through a membrane (0.1 μm pore size) to remove solids.

Results and discussion

Discontinuous leaching

In order to compare leaching rates of cultures containing either *Thiobacillus ferrooxidans* or *Leptospirillum*-like bacteria as sole iron-oxidizing organism two different enrichment cultures were used as inocula for discontinuous leaching of flotation concentrate. According to viable cell counts these cultures contained *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* (culture I) resp. *Leptospirillum*-like bacteria and *Thiobacillus thiooxidans* (culture II). Growth of both cultures during discontinuous leaching is shown in Fig. 2.

Viable cell counts indicate that growth of *Thiobacillus thiooxidans* is coupled to growth of *Thiobacillus ferrooxidans*. This can be explained by intermediate formation of elemental sulphur during oxidation of pyrite by *Thiobacillus ferrooxidans* (Kandemir 1983). This elemental sulphur serves as energy source for *Thiobacillus thiooxidans*. Rapid growth of *Thiobacillus thiooxidans* prior to sulphur production during growth of *Leptospirillum*-like bacteria could be observed (culture II). Subsequent decrease of the viable cell number of sulphur-oxidizing bacteria was caused by the exhaustion of their energy source (sulphur). When generation of elemental sulphur started at the beginning of the growth of *Leptospirillum*-like bacteria, growth of *Thiobacillus thiooxidans* could proceed, too. This was confirmed by results of elemental sulphur analysis.

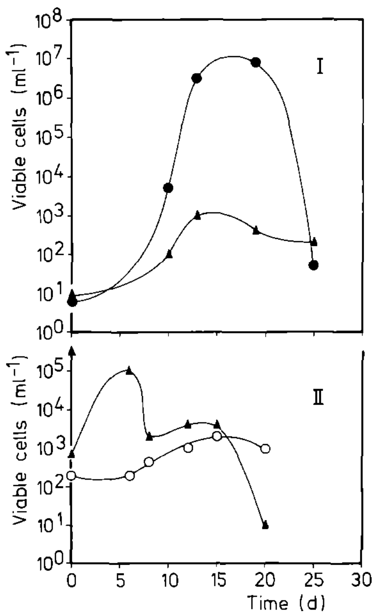

Fig. 2. Growth of *Thiobacillus ferrooxidans* (●), *Leptospirillum*-like bacteria (○), and *Thiobacillus thiooxidans* (▲) during discontinuous leaching with mixed cultures

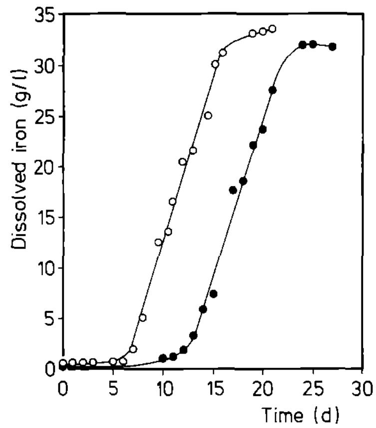

Fig. 3. Discontinuous leaching of pyrite by mixed cultures containing *Thiobacillus ferrooxidans* (●) or *Leptospirillum*-like bacteria (○) as sole iron oxidizing organisms

Though determination of MPN indicated only slow growth of *Leptospirillum*-like organisms, dissolution of iron was about as fast as that of *Thiobacillus ferrooxidans* (Fig. 3). Maximum iron leaching rates of 131 mg/l h (culture I) and 134 mg/l h (culture II) were achieved, resulting in final dissolution of 82% (culture I) and 85% (culture II) iron from the concentrate. Oxidation rates for copper and zinc with different iron-oxidizing bacteria did not differ significantly either (data not shown).

Continuous leaching by Leptospirillum-free cultures

Continuous leaching experiments were performed at various dilution rates using a mixed culture of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. At a pulp density of 240 g/l and dilution rates of 0.010 h^{-1}, 0.015 h^{-1} and 0.020 h^{-1} dissolved metal concentrations shown in Fig. 4 were achieved at steady-state conditions.

Leaching rates for copper and zinc increased with increasing dilution rate resulting in constant dissolved zinc concentration and only slightly decreasing dissolved copper concentration. At all dilution rates the percentage of leached metals was about 60% of the total zinc and about 20% of the copper content of the flotation concentrate. The

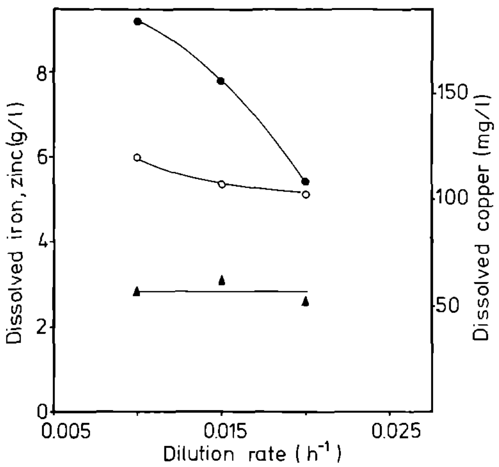

Fig. 4. Influence of dilution rate on steady-state concentration of dissolved iron (●), copper (○), and zinc (▲)

iron leaching rate was independent of dilution rate within the operating range of experiments. The observed decrease of dissolved iron concentration with increasing dilution rate is congruent with a constant steady-state leaching rate of about 100 mg/l h.

Continuous leaching by Leptospirillum-containing cultures

Continuous operation was started with a mixed culture of *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Leptospirillum*-like bacteria. Competition between various strains of *Thiobacillus ferrooxidans* and *Leptospirillum*-like bacteria was expected to lead to the selection of strains with high oxidizing activity at the experimental conditions chosen. Continuous cultivation has been reported to be a suitable method to select more productive mutants of *Thiobacillus ferrooxidans* (Vian et al. 1986). During continuous cultivation of *Leptospirillum*-free cultures no increase of oxidation rate due to selection could be observed, probably because efficient selection of thiobacilli had already been achieved during long-term subcultivation on pyrite concentrate in shake flasks.

Leptospirillum-like bacteria have been reported to accelerate bacterial leaching of pyrite at low pH inhibiting *Thiobacillus ferrooxidans* (Norris 1983). Therefore they were expected to be more suitable for continuous leaching at pH 1.5 than *Thiobacillus ferrooxidans*. Introduction of *Leptospirillum*-like bacteria into the continuous process resulted in coexistence of thiobacilli and *Leptospirillum*-like bacteria. After several months

Table 1. Influence of mixed culture composition on steady-state metal leaching rates. Reciprocal dilution rate (residence time) 1/D = 40 h ... 55 h

Leaching rates during a long-term continuous test inoculated with a *Leptospirillum*-poor culture compared to the results of the first steady-state after inoculation with both thiobacilli and *Leptospirillum*-like bacteria[a]

Viable cell counts (cells/ml)		Leaching rates (mg/l h)			Time (d)
T. ferrooxidans	*Leptospirillum*-like *bacteria*	Iron	Copper	Zinc	
1×10^7	$<6 \times 10^0$	108	2.0	52	11
2×10^7	8×10^2	137	1.7	45	14[a]
2×10^3	8×10^6	255	2.2	57	114
3×10^2	2×10^6	424	2.5	63	158

of continuous cultivation *Leptospirillum*-like bacteria displaced *Thiobacillus ferrooxidans*. The effect of microbial changes on steady-state leaching rates is shown in Table 1.

Leaching rates for copper and zinc at steady-state conditions were not affected significantly by *Leptospirillum*-like bacteria, whereas iron oxidation was strongly enhanced in the presence of *Leptospirillum*-like bacteria. After enrichment of *Leptospirillum*-like bacteria the iron leaching rate was by a factor of about 2.4 higher than the corresponding rate with *Leptospirillum*-free cultures. As a result of further selection during subsequent continuous leaching experiments the leaching rate was even 3.9 times higher.

Influence of pH on continuous leaching by Leptospirillum-like bacteria

In order to investigate the influence of pH on the coexistence of *Leptospirillum*-like bacteria and thiobacilli pH was varied during continuous leaching. Results are given in Table 2.

At a pH of 1.5 viable cell counts indicated predominance of *Leptospirillum*-like bacteria. By

Table 2. Influence of pH during continuous microbial leaching on mixed culture composition. Reciprocal dilution rate (residence time) 1/D = 100 h

pH	Viable cell counts (cells/ml)		
	Leptospirillum-like *bacteria*	*Thiobacillus ferrooxidans*	*Thiobacillus thiooxidans*
1.5	7×10^5	8×10^4	2×10^2
2.3	1×10^2	2×10^5	2×10^2

raising the pH to 2.3 the composition of the microbial population was significantly effected. After a period of only little more than the residence time a considerable change in the composition of the mixed culture was observed. The amount of *Thiobacillus ferrooxidans* slightly increased, whereas the viable cell number of *Leptospirillum*-like bacteria decreased to less than 0.015%. This rapid loss of oxidizing activity of *Leptospirillum*-like organisms resulted in a decrease of the dissolved iron concentration to about 20%.

The importance of pH for the establishment of the steady-state population of iron-oxidizing bacteria must be taken into account when selecting the most suitable pH for a continuous leaching process. Formation of insoluble ferric iron precipitates which may occur during microbial leaching of sulphidic iron (Vuorinen et al. 1986) can be avoided by operation at low pH. On the other hand, shake flask experiments have shown inhibition of pyrite dissolution activity of the commonly used species *Thiobacillus ferrooxidans* at pH 1.5 (Norris 1983).

In our experiments with *Leptospirillum*-free cultures, pyrite oxidizing activity of strains of *Thiobacillus ferrooxidans* was observed even at pH 1.5. But oxidation activity of *Thiobacillus ferrooxidans* is more sensitive to extremely acidic conditions than the activity of *Leptospirillum*-like bacteria. This was demonstrated by measurement of respiratory activity of both organisms. Respiratory activity of *Thiobacillus ferrooxidans* isolated from reactor leaching suspension was measured by Warburg manometry using $FeSO_4$ as energy source. Decrease of pH from 2.0 to 1.0 resulted in a decrease of respiratory activity to 15%. *Leptospirillum*-like bacteria were demonstrated to be less effected by a decrease of pH. Compared to the respiratory activity at pH 2.0, they showed at least 64% activity at pH 1.0.

Our experiments demonstrate fast bacterial leaching of pyrite under extremely acidic conditions by *Leptospirillum*-like bacteria. Continuous cultivation of mixed cultures at low pH is a suitable method for the enrichment of leptospirilla with high leaching activity.

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