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Leaching of petroleum refinery ash by acidophilic sulfur-oxidizing microbial cultures

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ABSTRACT

Sulfur-oxidising acidophilic bacteria were obtained from weathered sulfur piles produced by a petroleum refinery. When grown on commercial sulfur the yield was 10^{10} cell/g S. Sulfur conversion to sulfate was about 95% after 17 days. Cultures were also grown together with ash obtained from incinerated refinery sludge, which contained high amounts of iron. Cultures grown in ash plus sulfur gave somewhat higher values for the growth parameters ($Y = 1.6 \times 10^{10}$ cell/g S). The sulfur conversion was about 70% (after 17 days) and more than 90% of the iron present in the ash was also leached. The sulfur-reduced compound thiosulfate, determined using ion pair HPLC, was found in the presence and absence of ash although the profile was different in each case. Sulfite was only found in non-ash containing cultures, whereas tetrathionate was only formed in the presence of ash. These results are discussed with reference to the substrates used by the micro-organisms.

1. Introduction

Petroleum refineries generate large quantities of wastewater, which after treatment produce biological sludge containing metals, oil, and suspended solids. The most common way to dispose of this sludge is to deposit it in controlled landfills. However, landfilling is becoming increasingly expensive and is not sustainable (Brombacher et al., 1998). Incineration is an alternate disposal method (World Bank, 1998) but leads to the undesirable accumulation of ashes from such systems. Ashes are produced in large quantities (85% of the input) and their disposal is difficult because of the high toxic metal content.

A complementary strategy involves desulfurization of refinery raw materials. However, this results in the production of large piles of high purity elemental sulfur. Over a period of time, this sulfur is colonised by sulfur-oxidising, acidophilic micro-organisms. These micro-organisms can be used for leaching complex raw materials such as ash if elemental sulfur is added as substrate for microbial growth giving rise to sulfuric acid formation which is well known to be the main leaching agent (Krebs et al., 1997). The main thrust of work has been done with sulfur-oxidizing micro-organisms, such as *Acidithiobacillus thiooxidans*, and *Acidithiobacillus ferrooxidans* and *Acidianus brierley* as summarised in Table 1.

Such studies using pure cultures have provided important information of metal leaching from a variety of substrates such as coal fly ash and municipal waste fly ash (Krebs et al., 2001; Seidel et al., 2001; Ishigaki et al., 2005) but these studies are typically of short duration. Not withstanding the importance of such studies, they invariably deal with the leaching of a single substrate. In this respect, mixed cultures have the advantage of being more amenable to studying multi-substrate leaching (Filali-Meknassi et al., 2000; Krebs et al., 2001). Recently, Seidel et al. (2006) used a mixed culture from contaminated sediment to oxidise sulfur as well as for iron leaching but this required more than 20 days.

In any case, regardless of the origin or composition of the culture, information regarding the nature of the intermediates formed can be useful to exploit these micro-organisms for metal leaching. Formation of compounds ranging from thiosulfate (Shrihari et al., 1993), tetrathionate (Rohwerder and Sand, 2003; Masau et al., 2001; Cheng et al., 1999) to sulfite (Takeuchi and Suzuki, 1994) has been reported.

The aim of this research is to use an acidophilic sulfur-oxidising bacterial consortium to leach sulfur and iron from ash obtained from the incineration of petroleum refinery waste sludge. Sulfur oxyanions namely, thiosulfate, sulfite and tetrathionate formation was monitored using sulfur alone as the growth substrate as well as sulfur together with ash.

2. Methods

2.1. Bacterial culture and substrates

The acidophilic sulfur-oxidising culture (pH 1.54) was collected from an acidic pool formed by the weathering of sulfur piles and was maintained in media described below. The gram test under

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Table 1
Literature values for elemental sulfur oxidation and bioleaching in the presence of elemental sulfur

Strain	S^0 ($g\ l^{-1}$)	Growth rate (μd^{-1})	Growth yield (Y cells $g\ S^{-1}$)	Sulfur conversion (η%)	Metals Leaching (η_{metals}%)	Reference
A. ferrooxidans	10	1.68	3.40×10^{11}	nd	–	Ceskova et al. (2002)
	40	0.5	nd	nd	–	Espejo and Romero (1987)
	10	1.6	6.25×10^{11}	25	–	Konishi et al. (1994)
A. thiooxidans	10	2.58	2.05×10^{11}	75–100	–	Konishi et al. (1995)
	10	0.6–0.9	nd	nd	22 Fe[a]	Seidel et al. (2001)
	10	0.70	nd	nd	–	Cheng et al. (1999)
	10	1.0×10^7–1.0×10^9 cell/ml (35 d)	nd	nd		Krebs et al. (2001)
	10	1.0	4.9×10^{11}	83	–	Gourdon and Funtowicz (1998)
	10	nd	nd		62.9 Zn[b]	Ishigaki et al. (2005)
A. brierley	5	1.28×10^8–1.5×10^9 cell/ml (9 d)	nd	nd	90 Zn[c]	Konishi et al. (2003)
Mixed culture from sewage sludge	10	5×10^7–10^9 cell/ml (60 d)	nd	nd	20 Fe[b]	Krebs et al. (2001)
Mixed culture from sediment	2–5	nd	nd	40–60	60–85%[d]	Seidel et al. (2006)

[a] Leaching of iron in coal fly ash.
[b] Leaching municipal waste fly ash.
[c] Thermophile growth at 65 °C, leaching municipal waste fly ash.
[d] Total of metals leached of contaminated sediment.

optical microscopy at 400-fold magnification showed that the consortium consisted of Gram-negative rod-shaped bacteria. Media was inoculated with 10% of 2.4×10^7 cells per ml.

The ash used in this study was bottom ash collected in drums outside the fluidised bed incinerator at a petroleum refinery site (GALP-Matosinhos, Portugal). Elemental sulfur of commercial grade (>95% purity) was from Barral, Portugal.

2.2. Culture media

Modified medium contained $MgSO_4 \cdot 7H_2O$ 0.5 $g\ l^{-1}$; $(NH_4)_2SO_4$ 3 $g\ l^{-1}$; KCl 0.1 $g\ l^{-1}$; K_2HPO_4 0.5 $g\ l^{-1}$ and $Ca(NO_3)_2$ 0.01 $g\ l^{-1}$ (Silverman and Lundgren, 1959) was used for cell growth together with the appropriate substrate; elemental sulfur sterilised by tyndallization 10 $g\ l^{-1}$, ash 1% (w/v), and sulfur plus ash. The pH value was adjusted to 2.0 with concentrated sulfuric acid. Erlenmeyer flasks (500 ml) containing 180 ml of medium were sterilised by autoclaving (121 °C, 20 min) and 20 ml of inoculum was then added. Cultures were incubated at 30 °C with orbital shaking (150 rpm). For all conditions tested, abiotic controls were done and analysed following similar procedures.

2.3. Sampling and analytical methods

Cells were counted with a Thoma counting chamber using a microscope (Olympus BHS) with phase contrast at 400× magnification. pH and E_H (standard electrode) were monitored by potentiometry (WTW pH 537). Samples were filtered through Millipore membranes (0.22 μm) and the filtrate was assayed for sulfate by the turbidimetric BaCl addition method (Vogel, 1978). Total dissolved iron and iron(II) were assayed by spectrophotometric absorption of 2,2′bipyridin complex (Sullivan, 1976).

2.4. HPLC analysis

Sulfur compounds (sulfite, thiosulfate, trithionate, tetrathionate) were determined by HPLC using a modification of the procedure of Steudel et al., 1987. A Waters W600E system control, automatic injector 712 WISP and UV detector were used. The compounds were determined with a Nova-Pak C18 (Waters) column (3.9 × 150 mm, 4 μm) using a mobile phase of acetonitrile:water (25:75) containing 1 mM Na_2CO_3 and 2 mM tetra-*n*-butylammonium hydroxide, pH 7. The eluent flow rate was 1 ml/min and detection was at 205 nm. Calibrations were performed for sulfite (Merck), thiosulfate (Titrisol, Merck), trithionate (prepared according to Kelly and Wood, 1994) and tetrathionate. Uracil was used for determination of void volume.

2.5. Flame atomic absorption

Ash samples were analysed using a spectrophotometer (Unicam Solaar M series) after microwave digestion (CEM MDS 2000) with acidic mixtures including HNO_3, HF and H_3BO_3 for complete digestion, in accordance with SW 846 method 3052 (US Environmental Protection Agency, 2002).

2.6. X-ray fluorescence spectra

X-ray fluorescence spectra of elemental sulfur, ash and solids remaining after 20 days bioleaching was determined in order to identify the elements present in the solid phase. The following acquisition parameters were used: 22 kV tube voltage; 0.16 mA tube current; Pd thin filter; 50 s livetime; 20 keV maximum energy and atmosphere of air.

3. Results and discussion

Flame atomic absorption spectroscopy indicated that the metal content (w/w) of the ash was 0.31% Fe, 0.0362% Zn, less than 0.05% vanadium and less than 0.009% nickel. Growth experiments conducted only 1% ash was performed, but the results obtained for the inoculated experiments were similar to those obtained in abiotic controls.

Fig. 1 shows the variation in pH during a 20 day cultivation period of cultures grown with sulfur alone or sulfur plus ash. When the ash (10 $g\ l^{-1}$) was supplemented with sulfur (10 $g\ l^{-1}$) yield was 1.6×10^{13} cells $kg\ S^{-1}$. In comparison, when grown with sulfur alone, this value was somewhat lower being 1.0×10^{13} cells $kg\ S^{-1}$ for the yield. The decrease in pH is likely to result from the formation of sulfuric acid.

Fig. 2 shows the conversion of sulfur alone or together with ash into sulfate, expressed as a percentage. Almost total conversion of