Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Zinc Leaching from Fly Ash in Municipal Waste Incineration by Thermophilic Archaean *Acidianus brierleyi* Growing on Elemental Sulfur

Yasuhiro Konishi[a]; Masatoshi Matsui[a]; Hiroyuki Fujiwara[a]; Toshiyuki Nomura[a]; Keisuke Nakahara[b]
[a] Department of Chemical Engineering, Osaka Prefecture University, Osaka, Japan [b] JFE Engineering, Kawasaki, Japan

To cite this Article Konishi, Yasuhiro , Matsui, Masatoshi , Fujiwara, Hiroyuki , Nomura, Toshiyuki and Nakahara, Keisuke(2003) 'Zinc Leaching from Fly Ash in Municipal Waste Incineration by Thermophilic Archaean *Acidianus brierleyi* Growing on Elemental Sulfur', Separation Science and Technology, 38: 16, 4117 — 4130

To link to this Article: DOI: 10.1081/SS-120024722

URL: http://dx.doi.org/10.1081/SS-120024722

SEPARATION SCIENCE AND TECHNOLOGY
Vol. 38, No. 16, pp. 4117–4130, 2003

Zinc Leaching from Fly Ash in Municipal Waste Incineration by Thermophilic Archaean *Acidianus brierleyi* Growing on Elemental Sulfur

Yasuhiro Konishi,[1,*] Masatoshi Matsui,[1] Hiroyuki Fujiwara,[1] Toshiyuki Nomura,[1] and Keisuke Nakahara[2]

[1]Department of Chemical Engineering, Osaka Prefecture University,
Sakai, Osaka, Japan
[2]JFE Engineering, Kawasaki, Kanagawa, Japan

ABSTRACT

This article describes the zinc leaching of fly ash from municipal waste incineration by the thermophilic archaean *Acidianus brierleyi*. In the bioleaching system, inorganic acid, sulfuric acid, was biologically produced from elemental sulfur added to liquid medium and simultaneously used to leach zinc in the fly ash. Leaching experiments with *A. brierleyi* growing on elemental sulfur were performed to determine the effects of various process parameters on the leaching rate of zinc in the fly ash. Under the conditions determined as optimum, the bioleaching of

---

[*]Correspondence: Yasuhiro Konishi, Department of Chemical Engineering, Osaka Prefecture University, 1-1, Gakuen-cho, Sakai, Osaka 599-8531, Japan; Fax: +81-722-54-9911; E-mail: yasuhiro@chemeng.osakafu-u.ac.jp.

DOI: 10.1081/SS-120024722
0149-6395 (Print); 1520-5754 (Online)

www.dekker.com

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

fly ash by *A. brierleyi* yielded 90% extraction of zinc in 9 days of batch operation at an initial ash–liquid loading ratio of 1.0 kg/m^3 and an initial sulfur–liquid loading ratio of 5.0 kg/m^3.

Key Words: Fly ash; Zinc recovery; Bioleaching; Sulfur-oxidizing microbe; *Acidianus brierleyi*.

INTRODUCTION

Because of the gradual depletion of natural mineral resources, it is of significance to recover valuable metals from solid industrial waste materials such as fly ash, sludges, and dust. Although physical and chemical processes have been extensively developed for extracting the metals from solid wastes, another possible process is the leaching of valuable metals from solid wastes by microbial means. A number of studies have addressed microbial recovery of copper and zinc from industrial solid wastes, as discussed in reviews of the literature on applied microbiology.[1,2]

Fly ash from municipal waste incineration, which can be considered an artificial ore containing various valuable metals, contains zinc in concentration levels that allow economic recovery of the metal.[1,3] Because fly ash as a product of incineration contains heavy metals mainly as oxides, the mechanisms of the metal oxide solubilization are discussed in terms of a direct microbial attack on metal oxides (enzymatic action) and an indirect attack (nonenzymatic action). Previous work on the bioleaching of fly ash showed that the heterotrophic fungus *Aspergillus niger* produces organic acids (gluconic acid or citric acid) from sucrose medium, and that the microbially produced organic acids act as leaching agents.[3] The bioleaching with *A. niger* yielded high extractions of cadmium (81%), zinc (66%), and copper (57%) at the end of incubation after 22 days. However, it is generally accepted that heterotrophic microorganisms utilize relatively expensive organic substrates as their carbon and energy sources. Recently, the autotrophic bacteria, *Acidithiobacillus thiooxidans* (formerly *Thiobacillus thiooxidans*) and *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*), were used for leaching of fly ash.[4] In the leaching with a mixed culture of *Acidithiobacillus* strains, 81% extraction was achieved for the economically most interesting metal, zinc, in 8 days, and high extractions of copper (89%), nickel (64%), and cadmium (100%) were obtained in 8 days. The leaching of copper, which is present in the fly ash as chalcocite (Cu_2S) and cuprite (Cu_2O), was dependent on the direct action of the *Acidithiobacillus* strains. On the other hand, zinc and other metals in the fly ash were present as their fully oxidized forms, and the metal oxides were found to be solubilized by microbially produced sulfuric acid. More recently, Krebs et al.[5] have used various *Acidithiobacillus* species to leach heavy metals in fly ash from municipal solid waste incineration. In the presence of elemental sulfur (1% w/v) and sewage sludge (4% v/v), more than 80% of zinc, copper, and cadmium, and 60% of aluminum were solubilized after several weeks, depending on the amount of fly ash added. In addition, coal fly ash was microbially treated to leach aluminum and iron in the solid waste by using *A. thiooxidans*.[6] These previous studies indicate that the bioleaching of fly ash appears to be a technically feasible method. A similar system was treated by Konishi et al.,[7] who studied the leaching of manganese nodules (metal oxides) during the growth of the mesophile *A. ferrooxidans* or the extremophile *Acidianus brierleyi* using elemental sulfur as their substrate. The bioleaching mechanism was considered that sulfuric acid is biologically produced from elemental sulfur added to the solution:

$$S^0 + (3/2)O_2 + H_2O \rightarrow H_2SO_4 \qquad (1)$$

and is simultaneously used as a leaching agent. Konishi et al.[7] demonstrated that the thermophile *A. brierleyi* growing on elemental sulfur can solubilize manganese nodules (metal oxides) faster at 65°C than did the mesophile *A. ferrooxidans* at 30°C. In view of the temperature dependence of chemical reaction rate, it is preferable to realize indirect attack on fly ash by microbially produced acids at higher temperatures. Thus, the thermophilic bacterium *A. brierleyi* is a candidate microbe for accomplishing effective extractions of valuable metals from fly ash. However, no reports have appeared concerning the bioleaching of fly ash by such extremophilic microbes.

This article describes the bioleaching of zinc from fly ash by the thermophile *A. brierleyi* growing on elemental sulfur. The batch growth and consequent zinc leaching at 65°C were monitored by measuring the liquid-phase concentrations of free cells and dissolved zinc as a function of time. Rate data for the bioleaching of zinc in a batch reactor were collected to examine the dependence of leaching rate on various process parameters.

EXPERIMENTAL SECTION

Materials

Fly Ash

The fly ash used in this study was generated in melting furnace in Japan, which is a large-scale furnace to vitrify the residues from a municipal solid waste incinerator. The chemical composition of the fly ash was 1.34 wt% zinc, 0.568 wt% lead, 0.091 wt% chromium, 0.068 wt% cadmium, and 26.4 wt% SiO_2, respectively. The ash particles were smaller than 20 μm. The fly ash was washed twice at an ash–water loading ratio of 37.5 kg/m^3 with deionized water to remove water-soluble compounds. The washed ash was then dried at 70°C.

Microorganism and Medium

The extremophilic archaean *A. brierleyi* DSM 1651 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). The DSM 1651 culture was subcultured aerobically at 65°C in the *A. brierleyi* medium described in the *DSM Catalogue of Strains 1989*. The medium had the following composition (in kg/m^3 deionized water): $(NH_4)_2SO_4$, 3.0; $K_2HPO_4 \cdot 3H_2O$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.1; $Ca(NO_3)_2$, 0.01; yeast extract, 0.2; and elemental sulfur powder, 10.0. The initial pH was adjusted to 2.0 by using 6 $kmol/m^3$ H_2SO_4. After 7 days of incubation at 65°C, the culture was passed through quantitative filter paper to remove sulfur particles and then centrifuged to collect the cells. The collected cells were suspended in the fresh medium, and the cell suspension was immediately used as the inoculum in bioleaching runs of the fly ash.

The solid substrate used in this work was rhombic sulfur particles of 25 to 63 μm. The sulfur particles added to the liquid medium had previously been washed twice with 0.1 $kmol/m^3$ HCl solution and 0.1 $kmol/m^3$ NaOH solution, to remove inorganic nonsulfur elements. After that, the sulfur particles were washed twice with deionized water and then dried at room temperature.

Apparatus and Procedure

Leaching experiments with *A. brierleyi* were conducted at 65°C in Erlenmeyer flasks containing 200 cm^3 of liquid medium. A 10-cm^3 volume of the active culture of *A. brierleyi* was inoculated into the medium, and the flask contents were shaken on rotary shaker at 120 rpm. A batch bioleaching run was initiated by adding both elemental sulfur and fly ash to the liquid medium, and this was taken as zero time. In some runs, the fly ash was added at various times after the addition of elemental sulfur to the liquid medium containing *A. brierleyi* cells. The initial cell concentration was $(0.9 \text{ to } 1.2) \times 10^{14}$ cells/m^3-suspension. The initial sulfur powder–liquid loading ratio ranged from 0.5 to 40 kg/m^3, and the initial fly ash–liquid loading ratio was from 0.2 to 5.0 kg/m^3. Additional experiments were done on the microbial oxidation of elemental sulfur by *A. brierleyi* at 65°C in the absence of fly ash. A solution sample of 2 cm^3 was periodically withdrawn from the flask. The number of free cells in the liquid samples was counted using a Petroff-Hausser counting chamber. The solution samples were also analyzed for zinc by atomic absorption spectroscopy (AA-6650G, Shimadzu).

Aside from bioleaching runs, experiments were done to examine the chemical leaching of zinc from the fly ash with dilute sulfuric acid. Accurately weighed amounts of fly ash and elemental sulfur were mixed with 200 cm^3 of the liquid medium using a rotary shaker at 120 rpm. The pH of liquid medium was adjusted to either 2.5 or 1.3 by addition of 6 $kmol/m^3$ H_2SO_4. The initial ash–liquid loading ratio was 0.2 kg/m^3, and the initial sulfur–liquid loading ratio was 5.0 kg/m^3. The chemical leaching runs were performed at 65°C in the absence of the sulfur-oxidizing archaean *A. brierleyi*.

RESULTS AND DISCUSSION

Microbial Oxidation of Elemental Sulfur

Figure 1 shows a batch growth curve for *A. brierleyi* grown on elemental sulfur at an initial sulfur–liquid loading ratio of 5 kg/m^3, along with a time course of solution pH. As the liquid-phase concentration of *A. brierleyi* cells increased markedly from the initial cell concentration, the pH of solution declined from the initial value to pH 1.5 after 3 days of batch cultivation. This significant decrease in the solution pH appears to result from the microbial oxidation of elemental sulfur to sulfuric acid, Eq. (1). It is likely that the microbially produced acid acts as a leaching agent to solubilize metal oxide in the fly ash.

Bioleaching of Zinc from Fly Ash

Figure 2 shows representative leaching behavior of the fly ash at 65°C and an initial ash–liquid loading ratio of 0.2 kg/m^3. The fraction α_{Zn} of zinc leached from the fly ash was determined from the liquid-phase concentration of zinc at any time, by considering the initial zinc content in the fly ash. In a control culture containing no elemental sulfur, no growth of *A. brierleyi* cells was evident, and little zinc was leached from the fly ash. This suggests that zinc oxide in the fly ash cannot serve as a solid substrate for the leaching microbe *A. brierleyi*. In a sterile control in the absence of *A. brierleyi* cells, little zinc in the fly ash was dissolved with dilute sulfuric acid at an initial pH 2.5. In a sterile control with dilute

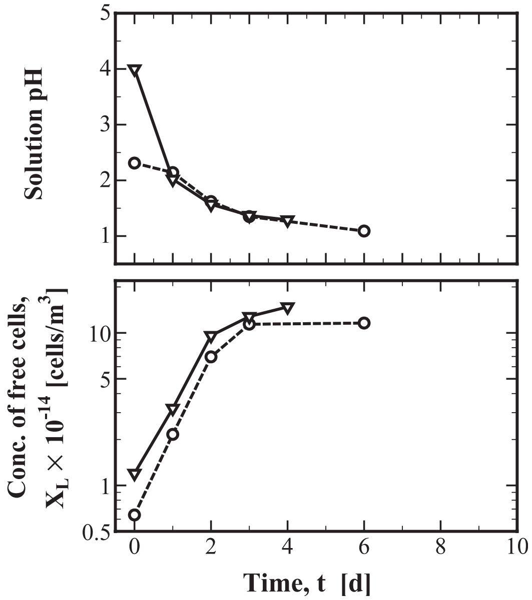

Figure 1. Batch growth of *A. brierleyi* on elemental sulfur at an initial sulfur–liquid loading ratio of 5.0 kg/m^3: (▽) initial pH 4.0, initial cell concentration $X_{L0} = 1.2 \times 10^{14}$ cells/m^3; (○) initial pH 2.3, initial cell concentration $X_{L0} = 0.64 \times 10^{14}$ cells/m^3.

sulfuric acid at pH 1.3, 22% of zinc was dissolved from the fly ash during the first 0.1 day, after which, no zinc was leached. Compared with the sterile control runs, the leaching of zinc was markedly accelerated in the presence of elemental sulfur and *A. brierleyi* cells, which involved a significant bacterial growth and a marked decrease in solution pH. When the solution pH fell to pH 1.3 after 6 days in the presence of *A. brierleyi*, 52% of zinc was solubilized by biogenic sulfuric acid, which is a final product of microbial sulfur oxidation. Comparing the zinc leaching fractions between the bioleaching and sterile runs at pH 1.3, it can be concluded that the microbially produced acid and other metabolities were more effective than abiotically produced acid. Especially, 90% leaching of zinc in the fly ash was achieved in 9 days when *A. brierleyi* cells grew on elemental sulfur at 65°C.

Figure 3 shows the effect of the initial sulfur-loading ratio W_{S0}/V at an initial ash–liquid loading ratio W_{A0}/V of 0.2 kg/m^3. The leaching rate of zinc was markedly dependent on the initial amount of elemental sulfur as solid substrate, which is reflected in the production rate of oxidized sulfur

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

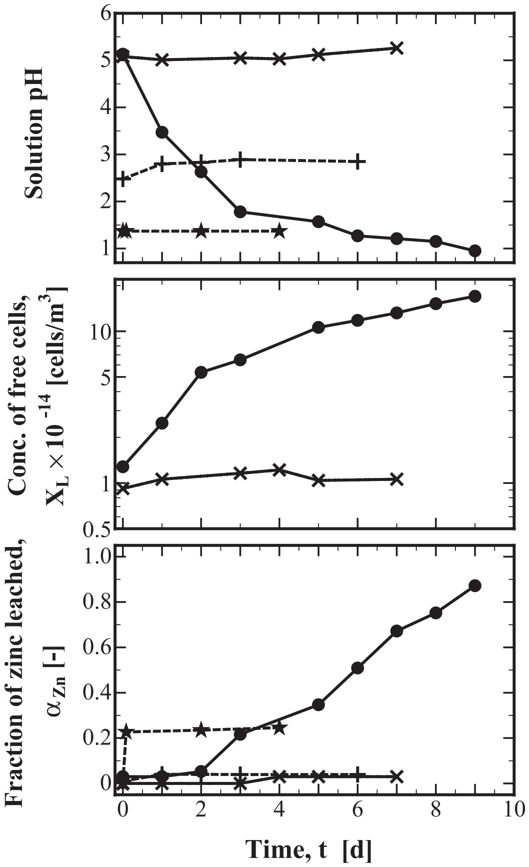

Figure 2. Typical leaching behavior of fly ash at an initial ash–liquid loading ratio of 0.2 kg/m^3: (●) initial cell concentration $X_{L0} = 1.28 \times 10^{14}$ cells/m^3, initial pH 5.0, initial sulfur–liquid loading ratio of 5 kg/m^3; (+) sterile control, initial pH 2.5, initial sulfur–liquid loading ratio of 5 kg/m^3; (☆) sterile control, initial pH 1.3, initial sulfur–liquid loading ratio of 5.0 kg/m^3; (×) initial cell concentration $X_{L0} = 0.92 \times 10^{14}$ cells/m^3, initial pH 5.0, no addition of elemental sulfur.

compounds as leaching agents. As expected, the leaching rate was enhanced as the initial sulfur–liquid loading ratio W_{S0}/V was increased from 0.5 kg/m^3 to 5 kg/m^3. However, there was an adverse effect by increasing the initial sulfur–liquid loading ratio W_{S0}/V from 5 kg/m^3 to 40 kg/m^3. The zinc leaching at $W_{S0}/V = 40$ kg/m^3 was slower than that at $W_{S0}/V = 5$ kg/m^3. This indicates that an optimum for the bioleaching of fly ash takes

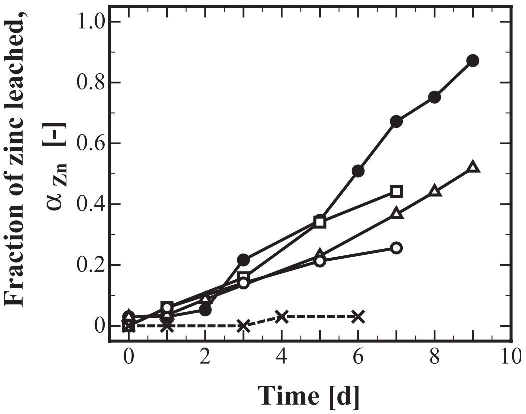

Figure 3. Effect of initial sulfur–liquid loading ratio W_{S0}/V on bioleaching rate of zinc at an initial ash–liquid loading ratio of 0.2 kg/m^3 and initial cell concentrations X_{L0} of (0.9–1.2) × 10^{14} cells/m^3: (×) $W_{S0}/V = 0$ kg/m^3; (△) $W_{S0}/V = 0.5$ kg/m^3; (●) $W_{S0}/V = 5$ kg/m^3; (□) $W_{S0}/V = 20$ kg/m^3; (○) $W_{S0}/V = 40$ kg/m^3.

place at an initial sulfur-loading ratio W_{S0}/V of 5 kg/m^3, when the initial ash–liquid loading ratio W_{A0}/V was 0.2 kg/m^3. A similar dependence on the initial sulfur amount was observed in the microbial oxidation of elemental sulfur by *A. ferrooxidans*[8] and the bioleaching of marine manganese nodules (metal oxides) by *A. brierleyi* growing on elemental sulfur.[7] On the other hand, a model approach can be adopted to confirm the observed effect of the sulfur substrate concentration. In our previous work,[7] a mathematical model was used to predict the time course of the sulfur oxidation fraction, and the simulation results indicated that there was an adverse effect by increasing the initial sulfur–liquid loading ratio. Both model simulations and experimental tests demonstrated that there exists an optimum level of initial sulfur-loading ratio.

Figure 4 shows that the initial ash–liquid loading ratio W_{A0}/V had a marked effect on the leaching rate of zinc. When the initial ash–liquid loading ratio W_{A0}/V was 1.0 kg/m^3, the solution pH was increased from the initial pH 3.9 to pH 5.5 and the leaching fraction of zinc was 40% in 8 days. The fly ash, at its loading ratio W_{A0}/V of 1.0 kg/m^3, is likely to release a significant amount of alkaline components, such as calcium hydroxide, which caused partial repression of bacterial growth and subsequent zinc leaching. Further addition of 5.0 kg/m^3 fly ash to the *A. brierleyi* culture resulted in a rapid increase in solution pH within 1 day, which strongly inhibited the growth of *A. brierleyi* and the leaching of zinc. The rapid increase in solution pH can be largely overcome by adding the fly ash after

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

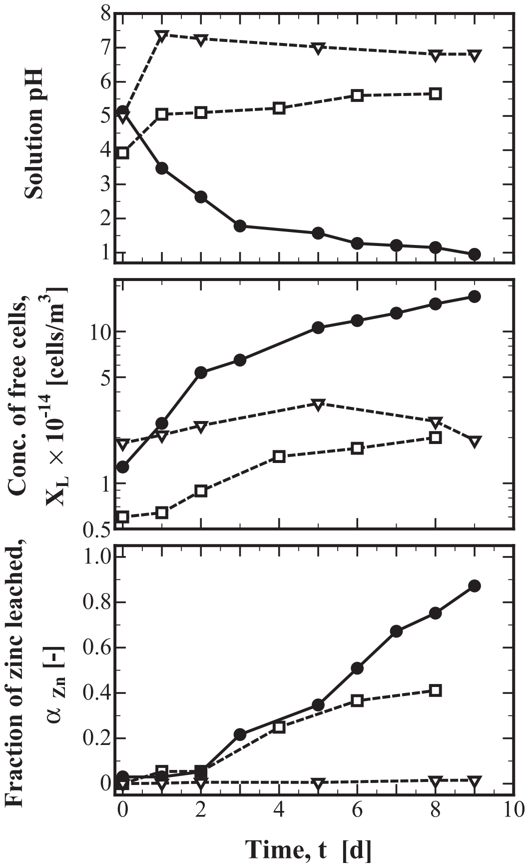

Figure 4. Effect of initial ash–liquid loading ratio W_{A0}/V on bioleaching behavior of fly ash at an initial sulfur–liquid loading ratio of 5.0 kg/m^3 and initial cell concentrations X_{L0} of (0.9–1.2) × 10^{14} cells/m^3: (●) W_{A0}/V = 0.2 kg/m^3; (□) W_{A0}/V = 1.0 kg/m^3; (▽) W_{A0}/V = 5.0 kg/m^3.

the microbial production of inorganic acids. That is to say, the biogenic acid can be partially used to suppress an increase in the solution pH by neutralizing the alkaline components released from the fly ash. Because it is accepted that sulfur oxidation by *A. brierleyi* occurs at an optimum at pH 2.0,[9] the fly ash should be added after the pH of the leach solution decreased to a low value below pH 2.0.

Figure 5 shows the effect of the time τ, at which the fly ash was added to the *A. brierleyi* cultures at an ash–liquid loading ratio W_{A0}/V of 5.0 kg/m^3.

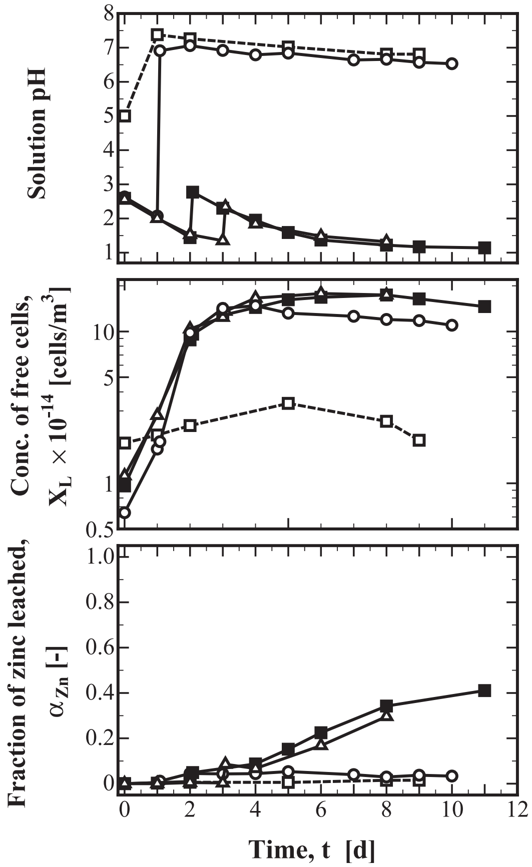

Figure 5. Effect of ash addition time τ on bioleaching behavior of fly ash at an initial ash–liquid loading ratio of 5.0 kg/m^3, an initial sulfur–liquid loading ratio of 5.0 kg/m^3, and initial cell concentrations X_{L0} of (0.9–1.2) × 10^{14} cells/m^3: (□) τ = 0 d; (○) τ = 1 d; (■) τ = 2 d; (△) τ = 3 d.

When the fly ash was added in 1 day after the start of batch growth of *A. brierleyi* on elemental sulfur, the solution pH increased sharply from pH 2.0 to pH 6.9, and then gradually declined to pH 6.5. In this case, the amount of biogenic acids produced during the first 1 day was insufficient to neutralize alkaline compounds released from the fly ash, and the zinc was scarcely leached from the fly ash for 10 days. However, there was a significant stimulation of zinc leaching at the addition time τ of 2 and 3 d, although the solution pH was increased to pH 2.5 to 2.8 immediately after the addition of fly ash. Especially at an addition time τ of 2 d, the zinc was appreciably leached beyond 40% in 11 days of batch operation, that is, in 9 days after the addition of fly ash. The moderate zinc leaching of about 40% appears to be because the loading ratio W_{A0}/V of fly ash at 5.0 kg/m^3 was large relative to the amount of inorganic acids produced from elemental sulfur.

To determine the effect of the addition time τ at higher zinc leaching rates, the ash–liquid loading ratio W_{A0}/V was decreased from 5.0 kg/m^3 to 1.0 kg/m^3 at a sulfur–liquid loading ratio W_{S0}/V of 5.0 kg/m^3. Figure 6 shows the effect of the addition time τ of fly ash at its loading ratio W_{A0}/V of 1.0 kg/m^3. When the fly ash was added in 2 and 3 days after the start of

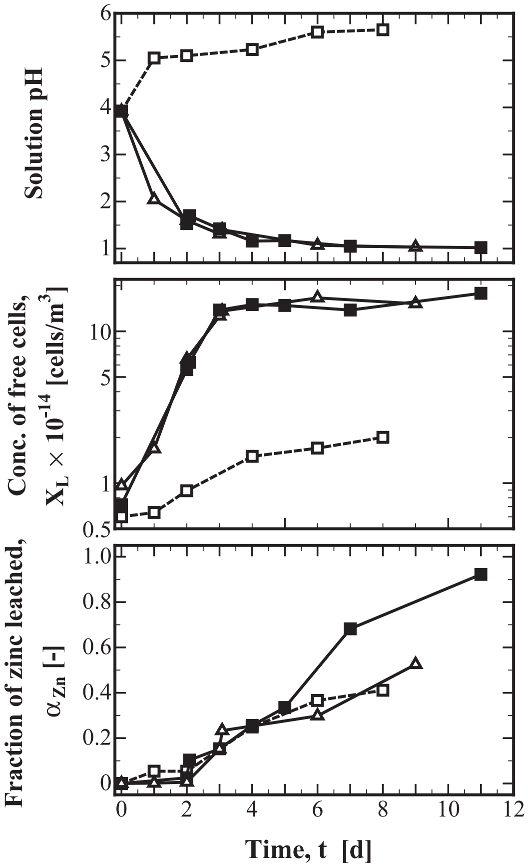

Figure 6. Effect of ash addition time τ on bioleaching behavior of fly ash at an initial ash–liquid loading ratio of 1.0 kg/m^3, an initial sulfur–liquid loading ratio of 5.0 kg/m^3, and initial cell concentrations X_{L0} of (0.9–1.2) × 10^{14} cells/m^3: (□) τ = 0 d; (■) τ = 2 d; (△) τ = 3 d.

batch cultivation, there was no increase in solution pH and a marked increase in the leaching rate of zinc. At an addition time τ of 2 d, 90% leaching of zinc was achieved in 11 days, that is, 9 days of leaching operation. When the addition of fly ash was postponed in day 3, the rate of zinc leaching is likely to be similar to the rate at an addition time τ of 2 d. Thus, these results indicate that at an ash–liquid loading ratio W_{A0}/V of 1.0 kg/m^3, the leaching of zinc was markedly accelerated when the addition time τ was set at 2 days.

General Discussion

The proposed bioleaching of fly ash involves the microbial oxidation of added elemental sulfur to sulfuric acid, and then leaching of fly ash with it. There is an advantage to the bioleaching with inexpensive elemental sulfur[10] compared to leaching with commercial sulfuric acid, which is relatively more expensive. In terms of safety, moreover, the use of biogenic acids is of greater advantage than the use of commercial hazardous chemicals (e.g., sulfuric acid). The inherent risk of bulk acid usage and storage can be minimized when the sulfur-oxidizing archaean *A. brierleyi* can continuously produce and supply the inorganic acids in situ (no need for transportation). Bioleaching with elemental sulfur as its substrate enables the leaching of fly ash. For the proposed bioleaching process, it is economically favorable to use inexpensive elemental sulfur, which is obtainable as a by-product from other industrial processes. However, when elemental sulfur is used only once for leaching zinc from fly ash and then is discarded, the bioleaching process appears to require a large amount of elemental sulfur, compared to the cost of the zinc recovered. One way to overcome the problem of high sulfur consumption is to develop a unique downstream processing that utilizes the biological activity of several bacteria associated with the cycling of sulfur in the biosphere. In biological downstream processing, sulfate-reducing bacteria can be applied to produce hydrogen sulfide from sulfate ions accumulated in the leach solution[11] so that the biogenic hydrogen sulfide is used to recover dissolved metals as sulfide precipitates. The effluent H_2S gas from the precipitation operation is biologically oxidized to elemental sulfur using phototrophic sulfur bacteria[12] and chemoautotrophic sulfur bacteria,[13] and the precipitated sulfur is collected and recycled to the bioleaching process of fly ash. Thus, the sulfur cycle ($S^0 \rightarrow SO_3{}^{2-} \rightarrow SO_4{}^{2-} \rightarrow H_2S \rightarrow S^0$) is artificially constructed by microbial means, and the microbial sulfur transformations are utilized for the recovery of valuable metals from fly ash. The problem of high sulfur consumption in upstream processing can be solved by the development of biological downstream processing.

CONCLUSION

In the proposed bioleaching with the sulfur-oxidizing archaean *A. brierleyi*, the inorganic acid produced from elemental sulfur was used to leach the zinc from the fly ash. When 0.2 kg/m^3 of the fly ash was initially added to *A. brierleyi* cultures at the initial sulfur–liquid loading ratio of 5.0 kg/m^3, the zinc in the ash sample was successfully leached to 90% in 9 days. However, fly ash at a loading ratio of 1.0 kg/m^3 caused a substantial decline in the leaching rate of zinc because the solution pH was increased by the release of alkaline components from the fly ash sample. To overcome this decline in the zinc leaching, the fly ash, with its alkalinity, was added after 2 days when *A. brierleyi* cultures had reached a pH of 1.5 by the accumulation of biogenic acid in the leach solution. By employing this procedure, over 90% leaching of zinc was achieved for 9 days at the initial ash–liquid loading ratio of 1.0 kg/m^3 and the initial sulfur–liquid loading ratio of 5.0 kg/m^3.

ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Scientific Research (B) (13555281) from the Ministry of Education, Science, Sports and Culture, Japan.

SYMBOLS

V	volume of solid–liquid mixture (m^3)
W_{A0}	initial mass of fly ash (kg)
W_{S0}	initial mass of elemental sulfur (kg)
X_L	concentration of free cells in liquid phase (cells/m^3)
X_{L0}	initial concentration of free cells in liquid phase (cells/m^3)
α_{Zn}	fraction of zinc leached from fly ash (dimensionless)
τ	time at which the fly ash was added to microbial cultures (d)

REFERENCES

1. Krebs, W.; Brombacher, C.; Bosshard, P.P.; Bachofen, R.; Brandl, H. Microbial recovery of metals from solids. FEMS Microbiol. Rev. **1997**, *20* (3–4), 605–617.

2. Bosecker, K. Microbial leaching in environmental clean-up programmes. Hydrometallurgy **2001**, *59* (2–3), 245–248.
3. Bosshard, P.P.; Bachofen, R.; Brandl, H. Metal leaching of fly ash from municipal waste incineration by *Aspergillus niger*. Environ. Sci. Technol. **1996**, *30* (10), 3066–3070.
4. Brombacher, C.; Bachofen, R.; Brandl, H. Development of a laboratory-scale leaching plant for metal extraction from fly ash by *Thiobacillus* strains. Appl. Environ. Microbiol. **1998**, *64* (4), 1237–1241.
5. Krebs, W.; Bachofen, R.; Brandl, H. Growth stimulation of sulfur oxidizing bacteria for optimization of metal leaching efficiency of fly ash from municipal solid waste incineration. Hydrometallurgy **2001**, *59* (2–3), 283–290.
6. Seidel, A.; Zimmels, Y.; Armon, R. Mechanism of bioleaching of coal fly ash by *Thiobacillus thiooxidans*. Chem. Eng. J. **2001**, *83* (2), 123–130.
7. Konishi, Y.; Asai, S.; Sawada, Y. Leaching of marine manganese nodules by acidophilic bacteria growing on elemental sulfur. Metall. Mater. Trans. B **1997**, *28B* (1), 25–32.
8. Konishi, Y.; Takasaka, Y.; Asai, S. Kinetics of growth and elemental sulfur oxidation in batch culture of *Thiobacillus ferrooxidans*. Biotechnol. Bioeng. **1994**, *44* (6), 667–673.
9. Brierley, C.L.; Brierley, J.A. A chemoautotrophic and thermophilic microorganism isolated from an acid hot spring. Can. J. Microbiol. **1973**, *19* (2), 183–188.
10. Maini, G.; Sharman, A.K.; Sunderland, G.; Knowles, C.J.; Jackman, S.A. An integrated method incorporating sulfur-oxidizing bacteria and electrokinetics to enhance removal of copper from contaminated soil. Environ. Sci. Technol. **2000**, *34* (6), 1081–1087.
11. Konishi, Y.; Yoshida, N.; Asai, S. Desorption of hydrogen sulfide during batch growth of the sulfate-reducing bacterium *Desulfovibrio desulfuricans*. Biotechnol. Prog. **1996**, *12* (3), 322–330.
12. Takashima, T.; Nishiki, T.; Konishi, Y. Anaerobic oxidation of dissolved hydrogen sulfide in continuous culture of the phototrophic bacterium *Prosthecochloris aestuarii*. J. Biosci. Bioeng. **2000**, *89* (3), 247–251.
13. Takashima, T.; Fukunishi, N.; Nishiki, T.; Konishi, Y. Anaerobic oxidation of dissolved hydrogen sulfide in continuous culture of the chemoautotrophic bacterium *Thiobacillus denitrificans*. Kagaku Kogaku Ronbunshu **2002**, *28* (1), 25–30.

Received November 2002
Revised March 2003

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016