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Metal Leaching of Fly Ash from Municipal Waste Incineration by *Aspergillus niger*

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Biological leaching of fly ash from municipal waste incineration by *Aspergillus niger* was examined in batch cultures and compared with chemical leaching. *A. niger* grew in the presence of up to 10% (w/v) of fly ash in the medium. In the presence of fly ash *A. niger* produced gluconate, whereas in its absence citrate was produced. Variation of the fly ash concentration in the growth medium (one-step process) resulted in different amounts of solubilized metals. A total of 3% (w/v) fly ash generally gave maximum extraction yields (in percent of the amount applied). In a two-step process *A. niger* first was cultivated in the growth medium, and subsequently the microbiologically produced citric acid was used as the leaching agent. At 6% (w/v) fly ash, the amounts of leached metals (leaching for 1 day) were 81% of Cd, 66% of Zn, 57% of Cu, 52% of Pb, 32% of Mn, 27% of Al, and less than 10% of Cr, Fe, and Ni, respectively. Chemical leaching with commercial citric acid of equal molarity was only slightly higher than microbial leaching. The environmental quality of the residues can be improved with respect to a re-use of these materials for construction purposes.

Introduction

Incineration of municipal solid waste has two main advantages: reducing the volume by about 90% and reducing the chemical reactivity by destruction of organic compounds. Fly ash from municipal waste incineration is recovered by electric filters. It represents a concentrate of a wide variety of elements. The content of certain heavy metals (i.e., Cd, Cu, Ni, Pb, Zn) makes this residue ecologically harmful. Due to its toxicity, most of the fly ash is now either immobilized with cement and deposited in controlled landfills or stored in underground repositories. This treatment is not entirely satisfactory. Some of the elements (i.e., Al, Zn) are present in concentrations that would allow an economical metal recovery. From this point of view, the fly ash could be considered as an "artificial ore".

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Bioleaching of different metals from numerous materials using microbes has been well documented. In the field of biohydrometallurgy, chemolithoautotrophic bacteria such as *Thiobacillus* species are of great industrial importance for the leaching of metals (e.g., copper, gold, uranium) mostly from ore deposits and mine tailings (*1*, *2*). In addition, heterotrophic fungi such as *Aspergillus* and *Penicillium* species show a potential for applications in biomining processes. However, only few data are available from the literature on fungal leaching. Vachon *et al.* (*3*) extracted Al from red mud with *Aspergillus niger* and *Penicillium simplicissimum*. Singer *et al.* (*4*) removed Al from fly ash of coal-burning power plants by commercial or microbiologically produced citric acid. Others have studied the fungal leaching of nickeliferous laterites (*5*, *6*). As for the mechanisms of bioleaching, in general, fungi acidify the nutrient medium during growth on organic compounds (excretion of protons, absorption of nutrients in exchange for protons, excretion of organic acids, acidification by carbon dioxide formation). Bioleaching with fungal microorganisms is thus based on the following mechanisms (*7*−*9*): (a) acidolysis, solubilization of the material on account of the acidification (the most important mechanism). (b) complexolysis, complexation of metals by the excreted organic acids or amino acids; (c) redoxolysis, for example, the reduction of ferric iron which is mediated by oxalic acid; (d) bioaccumulation, the mycelium acts as a "sink" for the metal ions.

The tendency of *Aspergillus niger* to overproduce organic acids is well known. It is possible to manufacture citric (*10*, *11*), oxalic (*12*), or gluconic (*13*) acid with *A. niger*. This paper reports for the first time bioleaching of fly ash from municipal waste incineration. It is the aim of this work to demonstrate the suitability of *A. niger* to leach the fly ash and thus to improve the environmental quality of the ash with respect to a re-use for construction purposes.

Experimental Section

Stock Cultures and Inoculum. *Aspergillus niger* was originally isolated from wood by David Fischer (Institute of Plant Biology, University of Zürich). The fungus was cultivated on 3.9% (w/v) potato dextrose agar plates (PDA, Difco Lab., Detroit). Spores were washed from 1-week-old cultures with a sterile solution of 0.2% (w/v) SDS (sodium dodecyl sulfate). After the number of spores was determined with the Neubauer counting chamber ($1.3-2.2 \times 10^7$), the culture media were inoculated with 1 mL of spore suspension/100 mL of medium.

Fly Ash. The fly ash retained by electric filters was obtained from the municipal waste incineration plant in Hinwil (Switzerland). It was washed with the double volume of water (w/v) of pH > 9 (removing water-soluble compounds), and subsequently the ash was dried in the plant on a vacuum filter. Representive samples were taken at different times during 1 day and prepared (homogenization in a cement mixer) by Sulzer Chemtech (Winterthur, Switzerland). An elemental analysis of this fly ash is given in Table 1 (*14*). For the experiments, the lumpy fly ash has been ground, lyophilized (removal of water), and dry-autoclaved.

Shake-Flask Experiments. *A. niger* was cultivated in 250-mL or 1-L Erlenmeyer flasks containing 100 or 300 mL,

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TABLE 1

Chemical Analysis of Selected Elements from Fly Ash[a]

element	g/100 g (%)	method
Al	7.0	ABC
C	4.3	D
Ca	13.2	ABC
Cd	0.049	B
Cr	0.07	AB
Cu	0.11	B
Fe	2.8	ABC
Mn	0.077	ABC
Na	1.1	ABC
Ni	0.014	AB
Pb	0.89	BC
Si	10.0	AB
Zn	3.1	BC

[a] Analysis carried out by EMPA Dübendorf. Measurement method A, XRF with glass specimen (uncertainty ±5%); B, XRF with compacted powder specimen (uncertainty ±10−20%); C, ICP-AES (uncertainty ±5%); D, oxidation at 2000 °C + IR detection (uncertainty ±5%).

respectively, of a sucrose medium. The medium consisted of (g/L) sucrose (100), $NaNO_3$ (1.5), KH_2PO_4 (0.5), $MgSO_4 \cdot 7H_2O$ (0.025), KCl (0.025), and yeast extract (1.6). Cultures were incubated at a temperature of 30 °C on a rotatory shaker at 110 rpm. Depending on the experimental setup, different amounts of fly ash were added to the cultures. During the experiments, pH, consumption of the substrate, and production of organic acids was monitored. Cell-free incubations were performed under the same conditions. At the end of the incubation period, metal concentrations were measured.

Optimal Fly Ash Concentration for Leaching. *A. niger* was incubated with various amounts of fly ash from 0 to 100 g/L (0−10% w/v). The spore solution for inoculation of the growth medium contained 2.2×10^7 spores/mL. The cultures were incubated until pH no longer decreased (382−836 h).

Biological and Chemical Metal Leaching. Spore concentration for inoculation of the growth medium was 1.3×10^7. *A. niger* was incubated either in a one-step process or in a two-step process. In the one-step process, incubation was carried out with 50 g/L of fly ash. In the two-step process *A. niger* was first cultivated in 1-L Erlenmeyer flasks containing 300 mL of sucrose medium lacking fly ash (first step). When the substrate was consumed, the suspensions were filtered. The filtrate then was used for the leaching process (second step). To compare the leaching efficiency of 100 mL of medium employed in these two processes, the filtrate (about 250 mL) was distributed among three 250-mL Erlenmeyer flasks containing 5 g of fly ash each (6% w/v). Experiments were terminated after 1, 7, and 23 d. Sterile sucrose medium was used as control. In addition, leaching efficiency of the microbiologically produced citric acid in the two-step process was compared with commercial citric acid of equal molarity (incubated for 1 d). Part of the acid produced was diluted (final concentration 10 times less) to test the influence of acid concentration (1-d incubation).

Analytical Methods. For measurement of pH, sugars, and organic acids, 0.5−1-mL samples were taken and centrifuged at 9000*g* for 10 min. The consumption of sugars and the production of organic acids were determined by HPLC [column: Aminex HPX-87H, 300 × 7.8 mm (BioRad Lab., Richmont, CA) with microguard cation H precolumn (BioRad); mobile phase: 10 mN H_2SO_4; flow rate: 0.5 mL/min; temperature: 40 °C; injection loop: 100 μL; detection: refractive index detector and UV detector at 210 nm]. Samples were diluted depending on the concentration of metabolites up to 1:10 and injected through a 0.22-μm HPLC filter. Metal analysis was performed using inductively coupled plasma atomic emission spectroscopy (Spectroflame-ICP-AES, Spectro, Analytical Instruments, Kleve, Germany) at the following wavelengths (nm): Al (396.15), Cd (228.8), Cr (267.71), Cu (324.75), Fe (261.18), Mn (257.61), Ni (231.6), Pb (283.3), Zn (206.2 nm). All samples were measured with standard addition. Samples were diluted up to 1:40.

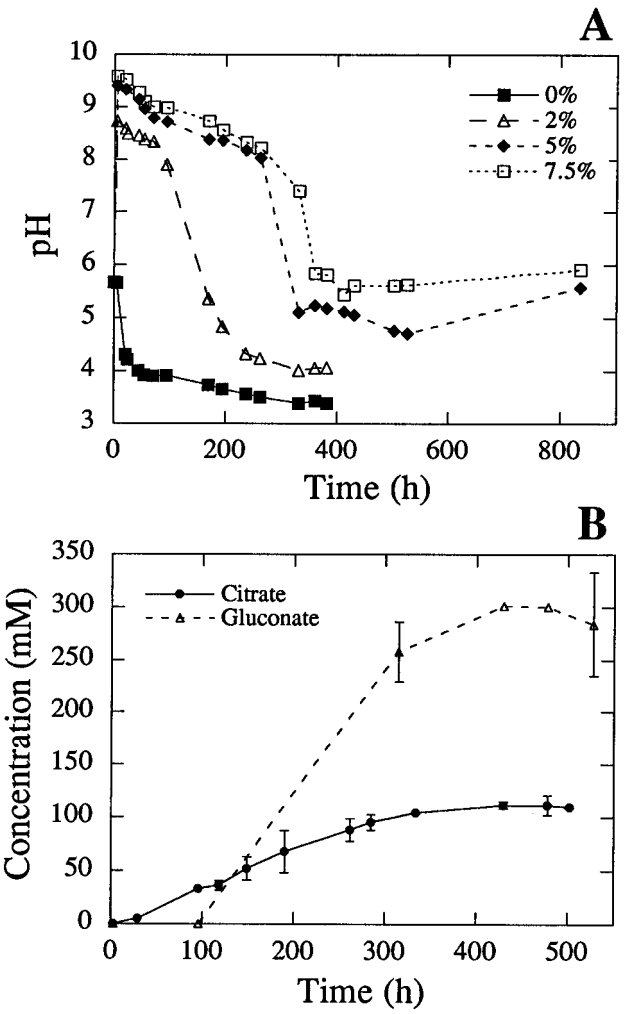

FIGURE 1. (A) pH in function of time in relation to fly ash added (w/v). (B) Organic acid concentrations in function of time. Citrate production in a medium without the addition of fly ash; gluconate production in a medium with 5% (w/v) fly ash.

Results and Discussion

Optimal Fly Ash Concentration for Leaching. *A. niger* grew with fly ash of up to 10% (w/v) in the medium. A 5% (w/v) fly ash solution resulted in a pH of about 9.3 because of the alkalinity of the ash. The higher the concentration of fly ash, the longer the lag phase lasted and the higher the final pH was (Figure 1A). pH was taken as a growth parameter. Preliminary experiments showed a good correlation ($r = 0.96$) between an increase in cellular dry weight and a decrease in pH (data not shown). Independent of the ash concentration, cultures showed similar growth rates

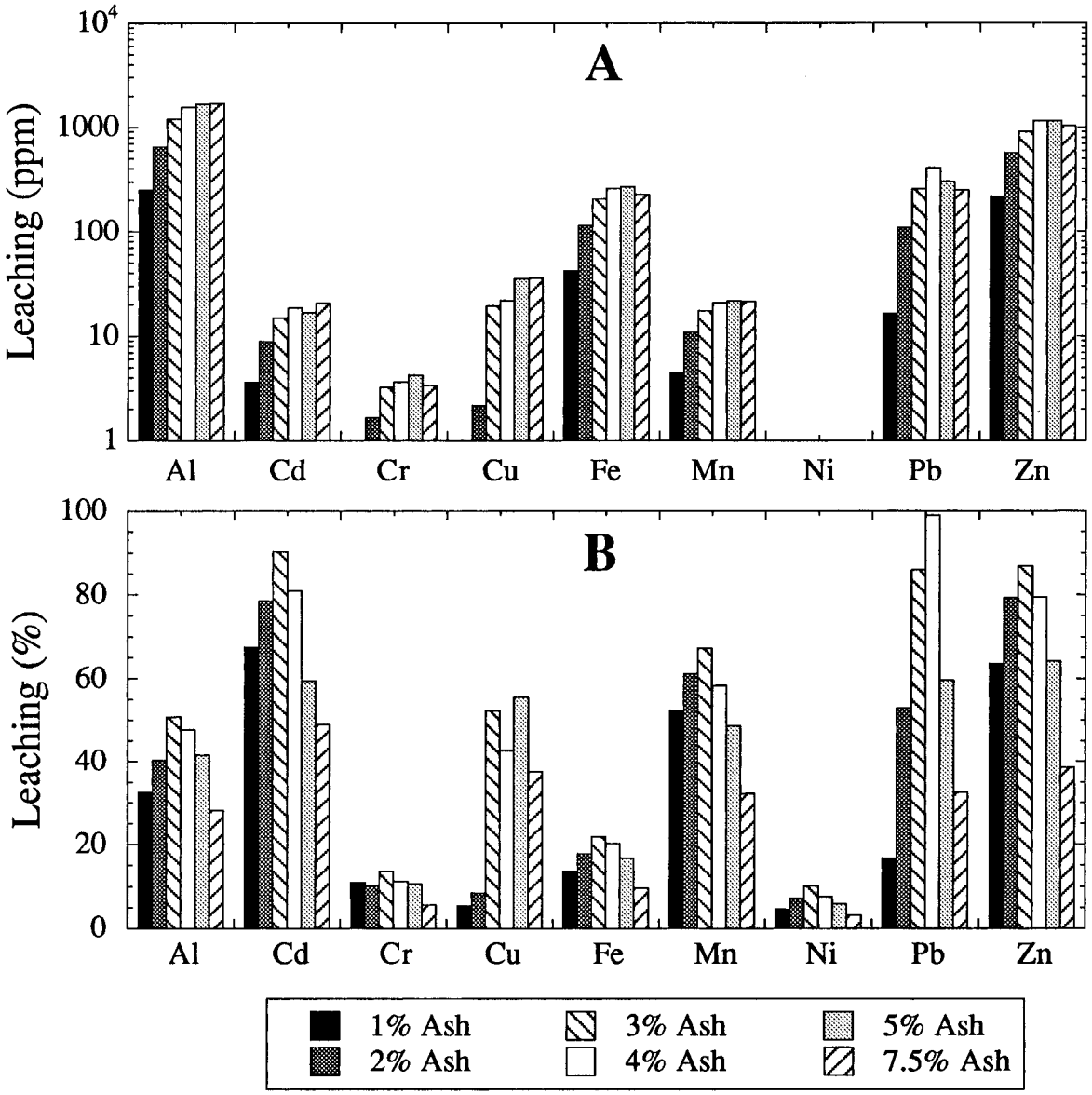

FIGURE 2. (A) Metal concentrations in the culture fluid at the end of incubation in relation to the amount of fly ash applied (1−7.5%, w/v). Cultures were incubated until pH no longer decreased (1 and 2% suspensions for 382 h; 3, 4, 5, and 7.5% suspensions for 836 h). Ni concentrations were below 1 ppm. (B) Bioleaching of different elements in percent of the amount of fly ash applied.

except that the lag phase lasted longer at higher concentrations.

In presence of fly ash, *A. niger* produced gluconate and no citrate, whereas without the addition of ash, citrate (and no gluconate) was formed (Figure 1B). Citrate production is due presumably to a lack of mainly manganese (among other elements such as Zn, Co, Fe, and Ca) in the medium without fly ash (*11*, *15*). This leads to an inhibition of the enzymes of the TCA cycle except the citrate synthase. In addition, a low pH has been reported to favor citrate production whereas higher pH values (in the presence of fly ash) stimulate oxalic and gluconic acid production (*11*, *13*). Figure 2A shows the yields of metals leached at different fly ash concentrations. In general, the highest relative solubilization values (in percent of ash added) were obtained in a 3% (w/v) fly ash suspension (Figure 2B).

Biological and Chemical Metal Leaching. In the one-step process where *A. niger* was incubated in presence of 5% (w/v) fly ash, pH decreased from 9.3 to 4.8 at the end of incubation (after 526 h). The concentration of gluconic acid produced was 283 mM. The medium used had a sucrose concentration of 289 mM. After converting sucrose in glucose and fructose, *A. niger* oxidizied glucose stochiometrically to gluconic acid. Fructose was completely consumed after 350−400 h. In the first step of the two-step process (incubation of *A. niger* in the sucrose medium without ash addition), pH decreased from 5.5 to 2.3. Citrate concentration at the end of incubation (after 500 h) was 110 mM. In the second step (cell-free incubation of the microbially produced citric acid with fly ash), pH increased from 2.3 to 6.4 due to the alkalinity of the ash.

In the one-step process where gluconate acts as the leaching agent, 57% of Cd, 52% of Pb and Zn, 41% of Mn, 33% of Cu, 30% of Al, 12% of Fe, and less than 10% of Cr and Ni were extracted (Figure 3). Leaching efficiency of gluconate in the one-step process as compared to the effect of citrate in the two-step process (Figure 3) was significantly higher for Al, Fe, Mn, and Ni (at the average 4.2%; error probability: $p \leq 0.05$) and significantly lower for Cd, Cu, and Zn (at the average 20.2%). In case of Cr, no difference was found. In the two-step process after 1 day, 81% of Cd, 66% of Zn, 57% of Cu, 52% of Pb, 32% of Mn, 27% of Al,

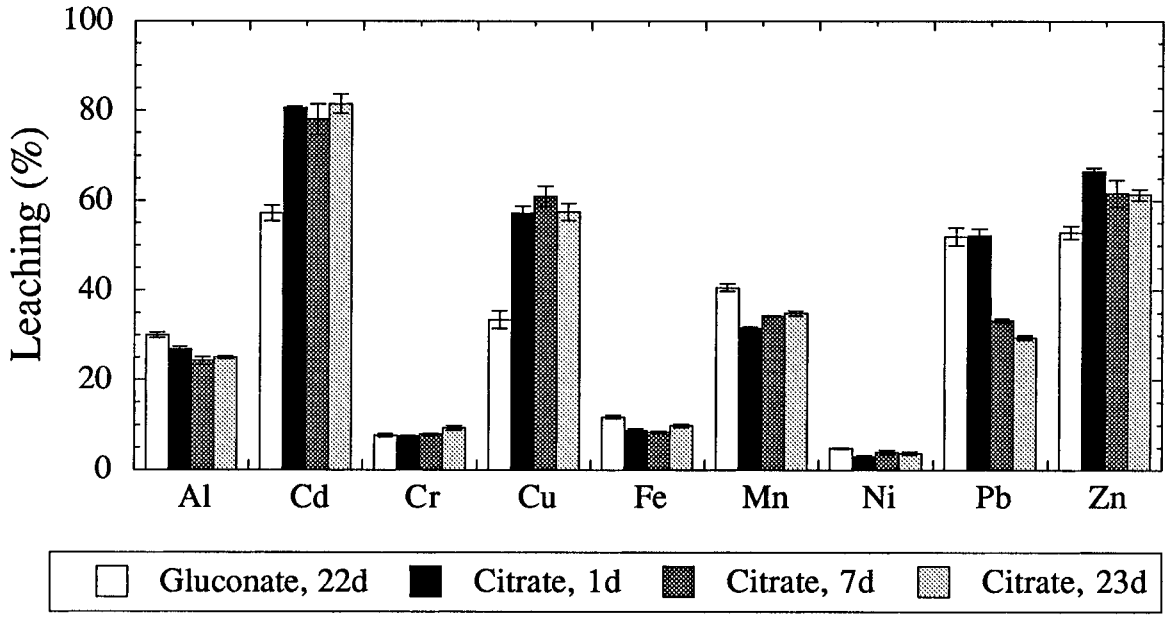

FIGURE 3. Bioleaching of different elements in percent of the amount of fly ash applied (5 g of ash/100 mL of medium employed). One-step process (22 days, gluconate as leaching agent) compared with two-step process (citrate as leaching agent) where samples were taken after 1, 7, and 23 days.

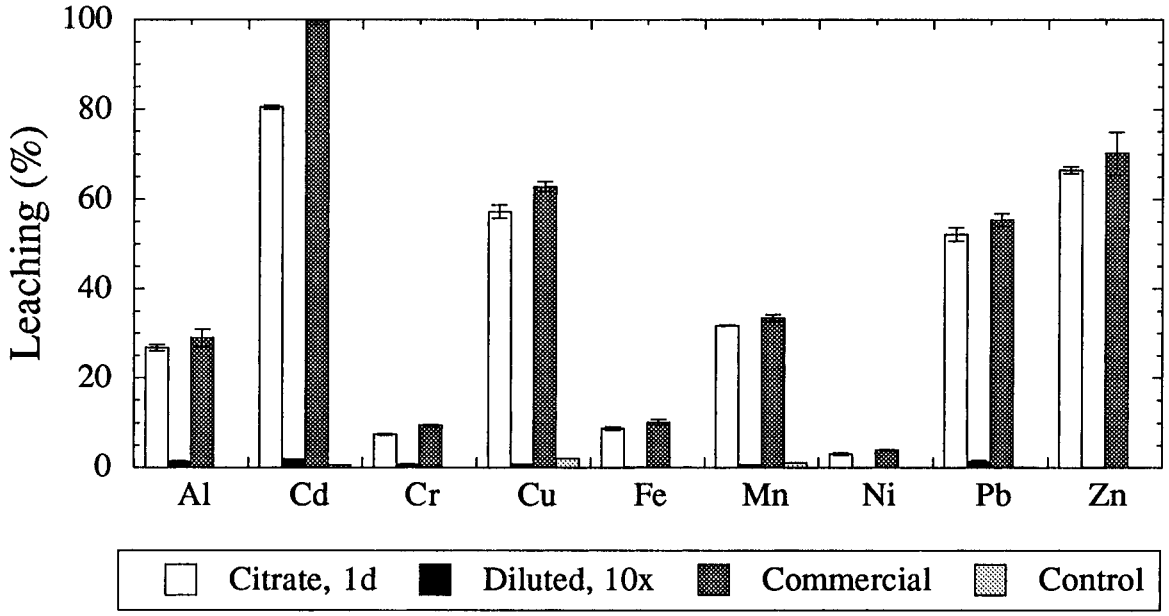

FIGURE 4. Leaching of different elements in percent of the amount of fly ash applied (60 g/L). Incubation was terminated after 24 h. Influence of acid concentration by diluting microbially produced acid (Diluted, 10x). Microbially produced citric acid compared with commercial citric acid (Commercial). Sterile sucrose medium was used as control.

and less than 10% of Cr, Fe, and Ni, respectively, were extracted. Time does not have a significant influence on the leaching efficiency in the two-step process except in the case of Pb. Solubilization of Pb decreased with time from 52% after 1 day to 29% after 23 days. This is probably due to adsorption processes on suspended fly ash. As shown in Figure 3, a prolongation of the second step did not increase the metal yield. An incubation time of 1 day was sufficient for an efficacious leaching. Chemical leaching with commercially available citric acid of equal molarity was only slightly higher (at the average 4.5%) than microbially mediated leaching (Figure 4). Cd showed the most significant difference. Close to 100% was extracted with commercial citric acid. Sterile sucrose medium was used as control. None of the tested elements was extracted by the medium in amounts higher than 2% after 1 day. Acid concentration appeared to have a pronounced effect on leaching efficiency. When the microbially produced citric acid concentration was decreased 10 times from 110 to 11 mM, the leaching efficiency was reduced to less than 2% for each element (Figure 4).

This study demonstrates the applicability of a bioleaching process using microbiologically produced organic acids for the leaching of fly ash from municipal waste incineration. For further studies, the two-step process should be preferred because of its "easier" handling, allowing optimization and control of both steps of the process. For example, the temperature of the leaching step can be different from the one in the growth phase. Singer *et al.* (*4*) have demonstrated that temperature has a significant effect on the leaching

efficiency. Furthermore, the biomass is not mixed with the residue as in the one-step process. Results show the potential metal recovery from this "artificial ore" with respect to a re-use of this material for construction purposes due to increased environmental quality.

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