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Fungal bioleaching of incineration fly ash: Metal extraction and modeling growth kinetics

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ABSTRACT

Aspergillus niger is known to be capable of bioleaching heavy metal ions from municipal solid waste (MSW) incineration fly ash. The objective of this study was to investigate the bioleaching kinetics of the fungus in the presence of the fly ash at various pulp densities (1–6%) in a batch system. The growth of the fungus was modeled using the modified Gompertz model. Since the metals present in the fly ash are toxic and inhibit microbial growth, an inhibition kinetic model using the generalized Monod growth kinetics was evaluated. In a two-step bioleaching system where the fly ash was introduced into the culture two days after inoculation, citric acid production and the leaching of the metals aluminium, iron and zinc from the fly ash were examined. The kinetic parameters in the system were estimated using the least square method. Results showed that the modified Gompertz model fit the experimental data well. The specific growth rate decreased with increasing pulp density, with a maximum specific growth rate (μ_{max}) of 0.115 day^{-1} for the control. The critical inhibitor (i.e. fly ash) concentration (C_I^*) above which no growth occurred was found to be 6.0%. Results also showed an increase in metal concentration leached with a concomitant increase in the citric acid production at various pulp densities.

1. Introduction

Municipal solid waste (MSW) incineration fly ash is a granular material that contains many hazardous constituents, amongst which are heavy metals. In an aqueous environment, these hazardous constituents may potentially be leached [1]. Due to its toxicity, the disposal of the fly ash requires the wastes to be either immobilized with cement and deposited in controlled landfills or stored in underground repositories. In fact, some of the metal elements (e.g. Al and Zn) are present in concentrations that would allow an economic recovery. From this viewpoint, the fly ash may indeed be considered as an "artificial ore" [2].

Bioleaching processes are based on the ability of microorganisms (bacteria and fungi) to transform solid compounds, via the production of organic or inorganic acids which results in soluble and extractable elements that can be recovered [3]. Heterotrophic fungi such as *Aspergillus* and *Penicillium* species have shown potential for applications in biomining processes. For instance, Al was extracted from red mud and from fly ash of coal-burning power plants using commercial or microbiologically produced citric acid. Fungus has been used for the bioleaching of nickeliferous laterites, electronic scrap wastes, and municipal waste incineration fly ash [2,4]. The use of central composite design to optimize several factors important in the bioleaching of a municipal solid waste incineration fly ash has been reported [5].

In order to exploit this intrinsic capability of some microorganisms for metal winning and recycling, more efforts are needed to understand the behavior of microorganisms during bioleaching. The objective of this study is to examine the kinetics of fungal growth, organic acid production and metal ion solubility in the presence of the fly ash.

2. Materials and methods

2.1. Fly ash

The municipal solid wastes (MSW) incineration fly ash (mean particle size: 26.3 μm) used in this study was obtained from Tuas South Incineration Plant in Singapore. The fly ash was autoclaved at 121 °C for 15 min prior to use.

2.2. Fungi inoculum preparation

Aspergillus niger was obtained from Dr. H. Brandl (University of Zürich, Switzerland). 7-day old conidia were harvested from the surface of potato dextrose agar (Becton Dickinson Co.) using sterile deionized (DI) water [2]. The number of spores was enumerated under a microscope (Olympus CX40) at 400× magnification using a Superior Marienfeld 0.1 mm depth haemocytometer. The spore suspension was diluted with deionized water to the desired spore suspension concentration (10^7 spores/ml).

2.3. Shake flask pure culture

1 ml of spore suspension was added to 100 ml of standard sucrose medium with the following composition (g/l): sucrose (100), $NaNO_3$ (1.5), KH_2PO_4 (0.5),

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Table 1
Modified growth model.

Model	Equation		Reference
Modified Gompertz	$y = A\exp\left\{-\exp\left[\frac{\mu \cdot e}{A}(\lambda - t) + 1\right]\right\}$	(1)	Zwietering et al. [8]; Gompertz [31]
Modified Logistic	$y = \frac{A}{\left\{1 + \exp[(4\mu_m/A)(\lambda - t) + 2]\right\}}$	(2)	Zwietering et al. [8]; Ricker [32]
Modified Richards	$y = A\left\{1 + \nu \cdot \exp(1+\nu) \cdot \exp\left[\frac{\mu_m}{A} \cdot (1+\nu)^{(1+1/\nu)} \cdot (\lambda - t)\right]\right\}^{(-1/\nu)}$	(3)	Zwietering et al. [8]; Richards [33]
Modified Schnute	$y = \left(\mu_m \frac{1-b}{a}\right)\left[\frac{1 - b \cdot \exp(a \cdot \lambda + 1 - b - at)}{1-b}\right]^{1/b}$	(4)	Zwietering et al. [8]; Schnute [28]

$MgSO_4{\cdot}7H_2O$ (0.025), KCl (0.025), yeast extract (1.6), and incubated at 30 °C with rotary shaking at 120 rpm. All reagents used were of analytical grade. The liquid medium was autoclaved at 121 °C for 15 min prior to inoculation.

2.4. Shake flask two-step bioleaching

In two-step bioleaching, the fungus was first cultured in sucrose medium (as in pure culture) and incubated at 30 °C with rotary shaking at 120 rpm without the fly ash. When a large pH drop occurred after two days, sterile fly ash at 1%, 2%, 3%, 4%, 5% or 6% pulp density was added to the culture and the incubation was continued [5,6]. The fly ash addition corresponded to the time $t = 0$.

2.5. Biomass determination

In the pure culture, the biomass dry weight was determined gravimetrically after the mycelium was dried at 80 °C for 24 h. For biomass determination in the bioleaching experiments, the mycelium together with fly ash was filtered and dried at 80 °C for 24 h, followed by ashing at 500 °C for 4 h in a Carbolite CWF 1100 furnace. The biomass dry weight was calculated gravimetrically after cooling in a desiccator [7].

2.6. Analytical method

Heavy metal concentration was analyzed using an inductive coupled plasma-optical emission spectrometry (ICP-OES). The concentration of glucose was measured using YSI 2700 Biochemistry analyzer. Fructose, sucrose, citrate, oxalate and gluconate were analyzed using HP 1100 series high performance liquid chromatography (HPLC) with variable wavelengths detector (VWD) at 210 nm for organic acids detection and refractive index detector (RID) for fructose and sucrose detection. Operating conditions for HPLC consisted of a 30 cm × 7.8 mm i.d. Biorad Aminex HPX-87H hydrogen resin ionic form analytical column (9 μm particle size). The analysis was carried out at 30 °C. The mobile phase used was 5 mM sulfuric acid (Merck, analytical grade) at flow rate of 0.5 ml/min [5].

3. Results and discussion

In the two-step bioleaching, the fungus was first cultured in sucrose medium (as in pure culture) for two days before the addition of fly ash to the culture at various pulp densities. *A. niger* completely hydrolyzed sucrose to glucose and fructose within the two days of incubation. By that time, the biomass reached about 6 g/l and the pH of the medium decreased from 5.7 to 2.8.

3.1. Fungal growth model

In the quantification of the growth of organisms, some models consider only the relationship between the number of organisms and time (i.e. under non-substrate limiting conditions). These include the Gompertz, Logistic, Richards and Schnute models which involved three or four parameters. Zwietering et al. [8] recognized that these models are described by mathematical parameters which have no biological meaning, and were thus re-parameterized as shown in Table 1. Using various bacterial systems, Zwietering et al. [8] showed that the modified Gompertz model was the best in describing microbial growth according to statistical *t* test and *F* test. The modified Gompertz model has also been used to model biomass concentration of *A. niger* and citric acid production [9].

In the modified Gompertz model (Eq. (1) in Table 1)

$$y = A\exp\left\{-\exp\left[\frac{\mu \cdot e}{A}(\lambda - t) + 1\right]\right\}$$

where y is the relative biomass concentration [i.e. ln(biomass at time t/biomass at time $t = 0$)]. A is the relative maximum biomass concentration. μ is specific growth rate, λ is lag time and t is time course.

Fig. 1 shows the experimental data and the modified Gompertz model in the two-step bioleaching of fly ash in a batch system. The experimental data fit the model very well over the entire range of the fly ash pulp density. It was also noted that the fungal growth rate (i.e. slope of the curve) gradually decreased as the density of the fly ash increased (over the range 1–5%), possibly due to the inhibition effect from toxic fly ash. The threshold appears to occur at 6% pulp density as no growth was observed. It is recognized that many factors may cause toxicity to the fungus during bioleaching; these include the blocking of essential functional groups of enzymes, the conformational changes of polymers in the cells, as well as the displacement of essential metals and the modification in membrane integrity and transport processes [10]. Although this present study showed that the fungal growth rate decreased as fly ash pulp density increased, the growth rate of *A. niger* was reported to be independent of the pulp density in two-step fly ash bioleaching [2]. Other factors, such as high shear forces arising from high solids concentration and agitation speed, were not considered due to the low agitation in the shake flask in this study. Strong agitation is likely to produce high shear stresses or strong abrasion between the particles and the microorganisms, especially in stirred tank reactor in which high agitation was required for high oxygen mass transfer coefficient and for keeping the solids in suspension [11].

The parameters in the modified Gompertz model was derived from the experimental data, and shown in Table 2. At lower pulp densities (from 1% to 5%), *A. niger* attained a final maximum biomass concentration of 23–25 g/l, a value similar with the

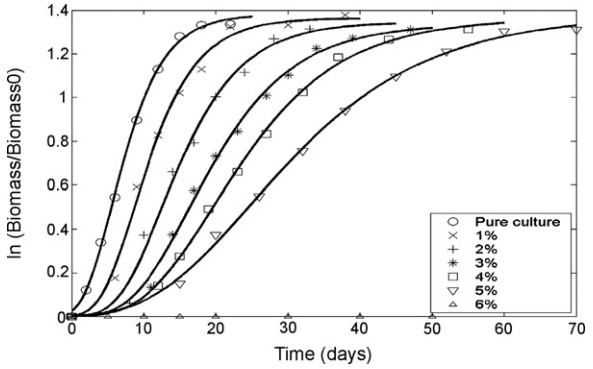

Fig. 1. Modified Gompertz model for the growth of *A. niger* in the presence of fly ash. (The model is shown as a solid line). The pure culture (i.e. in the absence of fly ash) shows the highest growth rate, while no growth was seen at 6% pulp density.