Biochemical Engineering Journal 67 (2012) 208–217

Contents lists available at SciVerse ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Bioleaching kinetics of a spent refinery catalyst using *Aspergillus niger* at optimal conditions

F. Amiri [a,b], S.M. Mousavi [c,*], S. Yaghmaei [a,**], M. Barati [b]

[a] *Department of Chemical and Petroleum Engineering, Center of Excellence, Development and Strategic Plants for Bioprocess Technology, Sharif University of Technology, Tehran, Iran*
[b] *Department of Material Science and Engineering, University of Toronto, Toronto, Canada*
[c] *Biotechnology Group, Chemical Engineering Department, Tarbiat Modares University, Tehran, Iran*

ARTICLE INFO

Article history:
Received 27 September 2011
Received in revised form 23 May 2012
Accepted 19 June 2012
Available online 28 June 2012

Keywords:
Bioleaching
Waste treatment
Extraction kinetics
Spent catalyst
Optimization
Filamentous fungi

ABSTRACT

The kinetics of bioleaching of Mo, Ni, and Al from spent hydrocracking catalyst, using *Aspergillus niger* was studied. The four most effective bioleaching variables were selected in accordance with the Plackett–Burman design and were further optimized via central composite design (CCD). The optimal values of the variables for maximum multi-metal bioleaching were as follows: particle size 150–212 μm, sucrose 93.8 g/L, pulp density 3% w/v, and pH 7. The maximum metal recoveries corresponding to these conditions were 99.5 ± 0.4% Mo, 45.8 ± 1.2% Ni, and 13.9 ± 0.1% Al. The relatively low Ni extraction was attributed to the precipitation of Ni in the presence of oxalic acid. Under the optimal conditions, the fungus growth was found to be higher in the presence of spent catalyst than that in the catalyst-free medium. Determinations of the organic acid concentration showed noticeable variation during bioleaching, particularly for gluconic acid. Accordingly, a modified form of shrinking core model was used to take these variations into account. The predictions by the model showed good consistency with the experimental results, suggesting that diffusion of bioleaching agent through the solid matrix was the rate-controlling step.

1. Introduction

Ceaseless exploitation of resources in the world has led to the exhaustion of high grade ore [1]. Heavy metals such as Mo, Ni, W, and V are strategic metals widely used in the production of special grades of steel; they are also used as catalysts in petroleum, petrochemical and chemical industries [2]. The gradual depletion of ores containing heavy metals, coupled with an increasing demand have promoted the search for secondary resources such as waste materials and byproducts, including spent catalysts [3]. In the petroleum refining operations, solid catalysts are extensively used to improve the process efficiency [1,2]. Spent hydrotreating and hydrorefining catalysts have been identified as hazardous wastes by USEPA since 1999 [3]. Hydrocracking catalysts, mainly made of molybdenum (Mo) or tungsten (W) with nickel (Ni) promoter supported on a porous Al_2O_3/SiO_2 base are commonly used in petroleum refining processes for producing light oils from the cracking of heavy crude oil. However, catalysts may be deactivated by contamination of the active surface with elements that are deposited during the cracking reactions (e.g., S, C, V, Fe, Ni, Si, Zn, and As). Deactivation of catalysts also occurs due to structural changes by thermal degradation, phase separation, or phase transformation that restricts their reactivation. In such cases, the spent catalyst is replaced with fresh catalyst [3,4]. As a result, large amounts of deactivated hydrocracking catalysts (*i.e.* spent catalysts) are generated that warrant their treatment before land disposal in order to meet the environmental regulations. This is primarily required because the predominant elements such as V, Ni, Mo, and Co, in the catalysts are toxic and can be easily leached out with water, leading to generation of a secondary pollution [3–6].

Worldwide, several companies are involved in metal reclamation from spent hydroprocessing catalysts; their technologies are based on two main approaches: hydrometallurgy or pyrometallurgy. Through the hydrometallurgical approach, the metals are leached out by means of catalysis with an acid or a base, while pyrometallurgical processing requires high temperature treatment of the materials through processes such as roasting and smelting [2,7]. Due to several drawbacks of the conventional techniques such as high energy costs and generation of environmental pollutants, bioleaching processes have been developed as an alternative method [8–10].

Bioleaching is based on the ability of microorganisms to transform solid compounds into soluble elements which can subsequently be recovered. In comparison with conventional

---

[*] Corresponding author. Tel.: +98 21 82884917; fax: +98 21 82884931.
[**] Corresponding author. Tel.: +98 21 66166430.
E-mail addresses: mousavi_m@modares.ac.ir (S.M. Mousavi), yaghmaei@sharif.edu (S. Yaghmaei).

1369-703X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bej.2012.06.011

technologies, bioleaching is associated with advantages such as simple operation, reduced operating cost, and smaller environmental footprint [11–15]. Most leaching active fungi which have been isolated and used for this purpose belong to the genera *Aspergillus* or *Penicillium* with the ability to excrete large amounts of leaching agents such as organic acids [13–16].

The nature and the amount of organic acid excreted by fungi are mainly influenced by: 1 – the pH of medium, 2 – the buffering capacity of the medium, 3 – the carbon source, 4 – the presence or absence of certain heavy metals and trace elements, 5 – balances of nitrogen and phosphate, 6 – the temperature of the medium, 7 – pre-culture period and inoculum used 8 – resistance of microorganisms to metal ions, 9 – physical and chemical states of the solid residue, 10 – liquid to solid ratio, and 11 – bioleaching period [10,17]. With the objective of maximizing the metal recovery in an industrial operation, process optimization is critical in such a system with numerous influential factors. To achieve this with the minimum number of experimental or plant trials, it is thus necessary to analyze the process with an initial screening design prior to optimization [18].

The statistical screening method offers several advantages over the conventional approaches including: 1 – being rapid and reliable, 2 – identifying the effective factors, 3 – taking into account the interactions among the factors, and 4 – reducing the total number of experiments [10,19]. Plackett–Burman (PB) design is an effective technique which screens the components that significantly influence the process and eliminates the insignificant components in order to obtain a smaller, more manageable set of factors. It has been applied to a number of processes including pre-treatment of macroalgae for Pb, Cr and Al determination by graphite furnace atomic absorption spectrometry (GF-AAS) [20], polysaccharide and ergosterol production from *Agaricus brasiliensis* by fermentation process [21] and bioethanol production [22].

Response surface methodology (RSM), one of the global optimization methods, is a collection of statistical and mathematical techniques useful for development, improvement, and optimization of multivariable processes. It also has important applications in design, development and formulation of new products, as well as improvement of existing product designs [23,24]. RSM has been successfully employed to optimize the compositions of microbiological media for biosorption of heavy metals [25], improving the fermentation process [23] and waste treatment [24]. Although the method is widely used for other processes, there are only few examples in the literature involving RSM for bioleaching of solid waste [10].

In kinetics studies of bioleaching systems, generally, it is assumed that the concentration of the leaching agent is constant [9,11]. However, the concentration of the biometabolites in the bioleaching systems varies due to the presence of microorganisms; hence such assumption may lead to inaccurate simulation of the bioleaching process using conventional kinetic models. This assumption was modified by Haghshenas et al. [26] and the variation of ferrous iron ion during sphalerite bioleaching by *Acidithiobacillus ferrooxidans* was considered in the shrinking core model.

In our previous study [27], a two-step bioleaching process was investigated in which the fungus was added first and the spent catalyst was introduced after initiation of biometabolites production (as opposed to simultaneous addition of both). It showed that increasing the pulp density does not give rise to a lower metal yield, which may be advantageously used to operate under higher pulp densities of the spent catalyst. However, the metal recoveries were generally low, warranting an optimization study to increase the recoveries.

The present study was undertaken to investigate the kinetics of two-step bioleaching of the metals from the spent catalyst under optimal conditions. We report for the first time a sequential optimization strategy for biological metal extraction from spent catalyst by *Aspergillus niger* through statistically designed experiments. First, the effective parameters were screened using PB two-level factorial design and then optimization of the significant parameters was carried out using RSM to maximize metal extraction from spent catalyst by *A. niger* efficiently. Afterward, a kinetic study was performed on bioleaching of the spent catalyst under optimal conditions and the profile of metal recovery percent, organic acid production together with pH, and fungal dry weight were discussed. Finally, the fungal leaching rate controlling step was determined for the first time using a modified shrinking core model (SCM) that takes into account the changes in the bioleaching agent concentration with time.

2. Materials and methods

2.1. Micro-organism

The micro-organism used in this study was *A. niger* BBRC-20018. This micro-organism was provided by the Biochemical and Bioenvironmental Research Center (BBRC), Sharif University of Technology, Iran.

2.2. Spent catalyst

Spent hydrocracking catalyst (Albemarle KF-1015-LH $Mo/Ni/Al_2O_3/SiO_2$) was provided by National Iranian Oil Refining & Distribution Company (NIORDC) and this material was used to prepare all the samples. The as-received spent catalyst with a black covering was first pre-treated by heating in a furnace at 600 °C for 4 h. The decoked spent catalyst was gently grinded and sieved to separate the fraction with desired particle size. Chemical digestion [16] indicated that the spent hydrocracking catalyst consisted of 6.4% Mo, 2.4% Ni, and 24% Al.

2.3. Bioleaching experiment according to the experimental design

Prior to bioleaching experiments, the fungal strain was acclimatized to metal ions and spent catalyst in a prolonged adaptation period in a procedure described elsewhere [16]. To obtain sufficient numbers of spores, the adapted fungus *A. niger* was cultured in a PDA (potato dextrose agar, 3.9% (w/v)) slant and incubated at 30 °C for 5 days. The mature conidia were then washed from the surface of the PDA medium using sterilized saline solution (9 g/L NaCl). The spores were counted using a Neubauer counting chamber and adjusted using sterilized saline to approximately 10^7 spores/mL. Specified quantity of the spore suspension by experiment design was added to a 500 mL Erlenmeyer flask containing 100 mL of sucrose medium. The concentrations of sucrose, $NaNO_3$, KH_2PO_4 and yeast extract were determined by the experimental design. The trace compounds $MgSO_4{\cdot}7H_2O$ and KCl were used at a constant concentration of 0.025 g/L. Prior to inoculation, pH of the growth medium was adjusted according to experimental design, and the medium was autoclaved at 121 °C for 15 min. The flasks were agitated in an incubator with specified orbital shaking and temperature. After the prescribed preculture time, the spent catalyst of a specific particle size was added to the medium, and the incubation was continued to various time intervals up to 30 days.

2.4. Analytical methods

After the desired bioleaching time, the culture from each flask was filtered and the filtrate was analyzed for the concentration of organic acids and various metal ions. Organic acids (*i.e.* citric, oxalic and gluconic acids) were analyzed using high performance liquid