Use of *Aspergillus niger* in the bioleaching of colemanite for the production of boric acid

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Abstract Colemanite is one of the most important underground riches of Turkey, having approximately 60% of the world boron deposits, and it has a large portion in the deposits. In this study, chemical leaching and biological leaching methods were used for production of boric acid from colemanite ($2CaO \cdot 3B_3O_3 \cdot 5H_2O$) (Emet-Kütahya, Turkey). Oxalic acid concentration, temperature, stirring time and solid-to-liquid ratio were taken as parameters in the chemical leaching process. It was found that the dissolution rate increases with increasing oxalic acid concentration and temperature but it decreases at higher solid-to-liquid ratios in the chemical leaching process. Using optimum conditions (d_{100} = 0.075 mm; 5% solids by weight; 0.55 M oxalic acid; 80 ± 2ºC leaching temperature; 150 rpm stirring speed; 90 min leaching time) for colemanite sample (28.05% B_2O_3) on chemical leaching with oxalic acid experiments, the calculated boric acid extraction efficiency from colemanite ore was 97.89%. Optimum conditions on bioleaching of Emet-Kütahya, Turkey colemanite ores using the fungus *Aspergillus niger* were found to be as follows: reaction temperature 25 ± 2ºC; solid-to-liquid ratio 5% solids by weight; d_{100} = 0.075 mm; stirring speed 150 rpm; initial the fungus populations in the inocula about 3×10^7 cells/ml and reaction time 21 days. The calculated boric acid extraction efficiency from colemanite ore was 90.18% under the optimum conditions. Bioleachate contained 12.95 g/l B_2O_3, 6.60 g/l Ca and 0.087 g/l Mg. Compared with chemical leaching at 5% pulp density, the fungus was less efficient in the extraction of B_2O_3 from colemanite but the difference in the extraction yields between the two processes was less than 10%. Although bioleaching generally requires a longer period of operation compared to chemical leaching, these results suggest that bioleaching by *A. niger* may be an alternative or adjunct to conventional physicochemical treatment processes of colemanite to produce boric acid.

Keywords: *Aspergillus niger,* bioleaching, boric acid, chemical leaching, colemanite, oxalic acid

INTRODUCTION

Boron compounds, of which Turkey has the largest reserves in the world with over 60% share, are very commonly used almost in all branches of industry in different ways. Huge portions of the Turkey's commercially recoverable boron reserves are colemanite, ulexite and tincal. Colemanite has a monoclinic crystal structure with a chemical formula of $2CaO \cdot 3B_3O \cdot 5H_2O$. Boron compounds are also widely used in detergents, fire retardants, nuclear reactors, agriculture, and material processing (Garrett, 1998). Commercially, boric acid is the most commonly used boron compound which has a surprising variety of applications in both industrial and consumer products and serves as a source of B_2O_3 in many fused products including textile fiber glass, optical and sealing glasses, heat resistant borosilicate glass, ceramic glazes, and porcelain enamels. Boric acid is produced in Turkey from the reaction of colemanite with sulfuric acid in a heterogeneous solid-liquid reaction leading to crystallization of gypsum ($CaSO_4 \cdot 2H_2O$) (Çetin et al. 2001).

The overall reaction is as follows:

$$2CaO \cdot 3B_2O_3 \cdot 5H_2O + 2H_2SO_4 + 6H_2O \longrightarrow 2CaSO_4 \cdot 2H_2O + 6H_3BO_3$$
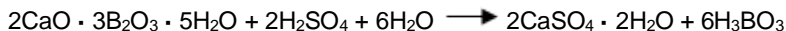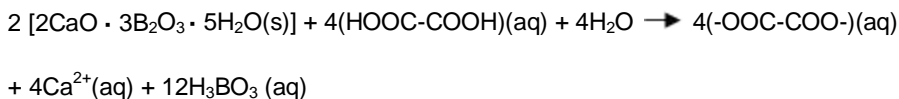
[**Equation 1**]

Calcium sulphate dehydrates, gypsum, forms as a by-product through the reaction. Gypsum is removed by filtration and boric acid is crystallized by cooling the filtrate to about 40ºC. Since filtration of gypsum affects the efficiency of the boric acid production process, and the calcium ion concentration in the boric acid solution affects the purity of boric acid crystals, filtration of gypsum crystals at the end of the reaction is a crucial process.

Some researchers studied about the dissolution of boron minerals in hydrochloric acid, sulfuric acid, oxalic acid, citric acid, chlorine saturated water, and sulfur dioxide saturated water and using carbon dioxide gas in aqueous media (Ata et al. 2000; Temur et al. 2000; Küçük et al. 2002; Çavuş and Kuşlu, 2005; Gür, 2007; Tunç et al. 2007).

When colemanite is added into the oxalic acid solution, the reactions taking place in the medium can be written as follows (Alkan and Doğan, 2004):

$$2 [2CaO \cdot 3B_2O_3 \cdot 5H_2O(s)] + 4(HOOC\text{-}COOH)(aq) + 4H_2O \longrightarrow 4(\text{-}OOC\text{-}COO\text{-})(aq)$$

$$+ 4Ca^{2+}(aq) + 12H_3BO_3 (aq)$$

[**Equation 2**]

Microbial leaching technologies have been used on an industrial scale for the recovery of copper, gold, uranium and zinc from low-grade ores, or from low-grade mineral resources (Ehrlich, 2002).

Three groups of microorganisms are used for the leaching process: autotrophic bacteria, heterotrophic bacteria and fungus. *Aspergillus niger* is one of the used fungus in bioleaching and has advantages over bacterial leaching, including the ability to grow under higher pH, and a faster leaching rate (Xu and Ting, 2004). The fungus has also been used in the production of organic acids, such as citric acid, oxalic acid and gluconic acid (Strasser et al. 1994). These acids are well known lixiviant for the leaching of heavy metals from ore materials and solid wastes (Gadd, 1999; Goyal et al. 2003; Ubaldini et al. 2010). Fungi can also be easily grown in substantial amounts using unsophisticated fermentation techniques and inexpensive growth media. Therefore, a fungal bioleaching could serve as an economical means for removal/recovery of metal ions from aqueous solutions.

In this work, chemical leaching and biological leaching methods were used for production of boric acid from colemanite (Emet-Kütahya, Turkey). In the chemical leaching tests; the effects of pulp density, temperature, leaching time and oxalic acid concentration were investigated. In the biological leaching tests, the ability of *Aspergillus niger* to production of boric acid from colemanite ore, and influence of pulp density on leaching efficiency was studied.

The purpose of this paper is to present new data on the bioleaching of boric acid from colemanite using *Aspergillus niger* in aqueous solution and to study its leaching kinetics. This bioleaching process has some advantages such as non-sulphate contamination in the product, and non-existence of gypsum that affects the purity of boric acid and also causes soil and water pollution because of its boron content.

MATERIALS AND METHODS

Colemanite sample

The colemanite sample was taken from Kütahya-Emet Boron Process Plant owned by Eti Mining A.S., Turkey. It is composed of clay minerals, crystobalite, calcite, celestite and arsenic compounds as accompanying minerals. The particle size distribution of colemanite sample was measured by the laser radiation scattering on a Laser-Particle-Sizer (Malvern Mastersizer 2000). The main particle diameter d_m (d_{90} = 0.042 mm) was calculated from granulometric data. Colemanite is basically composed of colemanite ($Ca_{0.2}B_6O_{11}5H_2O$), calcite ($CaCO_3$) and montmorillonite (($Ca0_2Al$, $Mg)_2Si_4O_{10}(OH)_24H_2O$). The chemical composition of the sample is shown in Table 1.

Table 1. Chemical composition of the colemanite ore.

Component	wt, %
B_2O_3	28.05
Al_2O_3	6.91
As_2O_3	0.79
CaO	32.48
Fe_2O_3	3.41
K_2O	4.56
MgO	12.31
SO_3	1.59
SiO_2	32.95
SrO	1.61
TiO_2	0.40
PbO	1.74

Fungal strain and growth conditions

A. niger, American Type Culture Collection (ATCC) Rockville, MD, USA as ATCC strain #11414, was obtained from Dr. S. Dincer (Department of Biology, University of Cukurova, Turkey). The fungus was cultivated on 3.9% (w/v) potato dextrose agar (Becton Dickinson, USA) plates and was kept in an incubator for 7 days at 30ºC. Sodium dodecyl sulfate (0.2% (w/v)) was used to recover the spores, which were counted under an optical microscope (Olympus CX 40) at 400 times magnification using a haemacytometer (Wolfrum et al. 2002). The spore suspension was then diluted with deionised water and standardized to 3×10^7 spores ml^{-1} of spore suspension. The spores were then added to a 250 ml flask containing 150 ml of prepared synthetic medium containing sucrose, 100 g/l; NH_4NO_3, 450 mg/l; KH_2PO_4, 100 mg/l; $MgSO_4 \cdot 7H_2O$, 300 mg/l; $FeSO_4 \cdot 7H_2O$, 0.1 mg/l; $ZnSO_4 \cdot 7H_2O$, 0.25 mg/l as culture medium in all experiments (Cameselle et al. 2003).

Determination of organic acids produced by *Aspergillus niger*

Sucrose was provided as the sole carbon source for *A. niger* and was hydrolysed to glucose and fructose. The concentration of biogenically produced organic acids (citric, gluconic and oxalic) were determined using high performance liquid chromatography (HPLC) with variable wavelength detector (CBM-20A) at 210 nm for the organic acids. A Shimadzu Prominence model high performance liquid chromatograph (HPLC) was used. The method included pump LC-20AT, column furnace SPD-M20A, communication bus module CTO-20AC, HPLC column prodigy 5 µm ODS(2) (150 mm x 4.60 mm) at a flow rate of 0.5 cm^3/min (room temperature with a mobile phase of 50 mmol/dm^3 mono-ammonium phosphate adjust to pH 2.4 with concentrated phosphoric acid). Prior to analysis, the samples were filtered with 0.45 µm nylon membrane syringe filter to protect the column from being clogged by fine particles in the samples.

Bioleaching experiments

Bioleaching tests were carried out in 250 ml Erlenmeyer flasks with 150 ml of sucrose medium at pH 5.5. Medium, colemanite samples and salts were sterilized by autoclaving for 20 min at 121ºC before spores were added. The flasks were plugged with cotton before sterilization. The flasks were inoculated with 1 ml of *Aspergillus niger* spore suspension (3 x 10^{7} spores/ml) and were agitated in an orbital shaking incubator at 150 rpm in order to keep everything in a homogenous slurry form and the temperature was kept at 30 ± 2ºC during the experiment. At regular time intervals, the culture from each flask were filtered through 0.45 µm filters and the filtrate was analyzed for pH and organic acid concentrations. The concentration of boric acid extracted by the fungus was analyzed by volumetric method. The cake (the residue/tailings) also was analyzed for B_2O_3, Ca and Mg by volumetric (titrimetric) method using a digital titrator. In all experiments, chemical grade reagents and distilled water were used, except in the chemical analysis where double distilled water was used. At the same time, each experiment was done at least in duplicate and results were reproducible within 5% and a control flask, one without the microorganisms, was used maintaining the same conditions.

Kinetic study

For the treatment with bioleaching a kinetic model (Blancarte-Zurita et al. 1987) was employed, the Equation 3 of which would be:

$$-dC_{B2O3}/dt = k (C_{B2O3\text{-}max} - C_{B2O3})$$

[**Equation 3**]

where; C_{B2O3}, dissolved boric acid concentration; *k*, kinetic coefficient; $C_{B2O3\text{-}max}$, maximum attainable boric acid concentration. Its value can be limited by the bioleaching capacity or by available boric acid in the solid; *t*, bioleaching time.

By integrating Equation 3 between the initial moment (*t* = 0, C_{B2O3} = 0) and the conditions corresponding to a time *t*, Equation 4 is obtained, from which the value of the kinetic constant can be deduced:

$$\ln[C_{B2O3\text{-}max}/(C_{B2O3\text{-}max} - C_{B2O3})] = kt$$

[**Equation 4**]

Chemical leaching experiments

Three concentrations of oxalic acid (0.1 M, 0.15 M and 0.2 M) were separately stirred and heated (30, 55 and 80ºC) in 250 ml flaks. When the solution attained the pre-required temperature, the samples in different solid rates (corresponding to 5-15% w/v solids concentration) were added and these samples were stirred three different leaching times (60, 90 and 120 min). The rate of agitation was kept constant for all the experiments. A watch glass was fitted to the flask to prevent evaporation. After each experiment, the samples were filtered and the residue was washed with distilled water. The leach liquor was analyzed for boric acid using volumetric method.

RESULTS AND DISCUSSION

Chemical leaching

The Yates technique for 2^4 experiments was used for statistical design and analysis of the results. Oxalic acid concentration, solid rate, leaching time and temperature were chosen as the major variables. The variables and levels of 2^4 full factorial designs are presented in Table 2. The higher level was designated as '+' and lower level as '-'. The models formed for boric acid concentration using the effects of variable significant at 95% or more confidence level is given below:

$$Y = 16.947 + 5.383X_1 - 3.146X_2 + 1.916X_1X_2 - 1.245X_2X_3 + 1.092X_2X_4 \quad R^2 = 0.9186$$

[**Equation 5**]

where Y is boric acid concentration.

Table 2. 24 factorial designs for boric acid production from colemanite by chemical laching.

Variables	Low level (-)	Base level	High level (+)
Pulp density (wt, %)	5	10	15
Oxalic acid concentration (M)	0.10	0.15	0.20
Temperature (ºC)	30	55	80
Leaching time (min)	60	90	120

The effect of oxalic acid concentration was highly significant and positive. This indicates that higher oxalic acid concentration is more suitable for chemical leaching. Therefore, oxalic acid concentration was preferred as a suitable parameter for the optimization tests (Akhanazarova and Kafarov, 1982). The optimized parameter (oxalic acid concentration) was evaluated using the regression equation (Equation 5) developed for boric acid production.

The best conditions for the boric acid production from colemanite after the optimization tests are as follows; particle size: $d_{100} = 0.075$ mm; pulp density 5% w/v; oxalic acid concentration: 0.55 M; temperature: 80 ± 2ºC and leaching time: 90 min. The boric acid extraction from colemanite under the optimum chemical leaching conditions was determined as 97.89%.
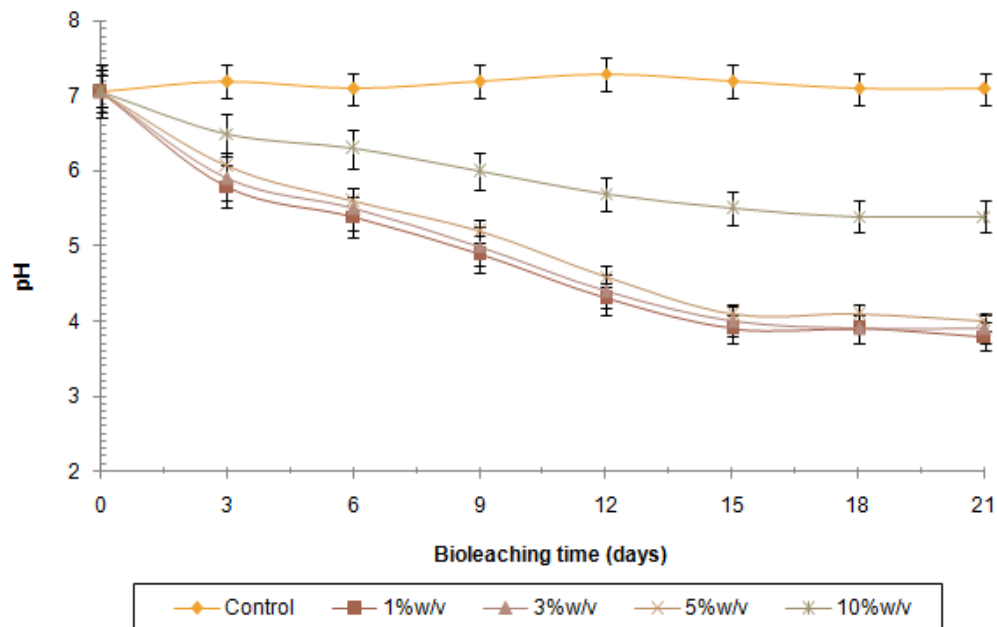

Fig. 1 Evaluation of pH during bioleaching experiments.

Bioleaching

Aspergillus niger can secrete some organic acids depending on medium composition and pH value. Oxalic acid production can be improved with culture medium rich in nitrogen source and pH close to neutrality (Terrazas et al. 2005; Ranjbar et al. 2007). During the bioleaching experiments, the initial pH was set to 7 for *Aspergillus niger*. In contrast to the control tests, changes in pH with time in the bioleaching experiments are presented in Figure 1. The solution pH decreased rapidly in first six days. The initial decrease in pH within first six days could be attributed to solubilization of boric acid. This decrease in pH was continued until 15 bioleaching days. After the 15th day, pH was stable. Solubilization of boric acid was slowed down after 15 days. At the end of bioleaching experiments (21 days), pH of the solution was determined as 4.10 at 5% w/v pulp density.
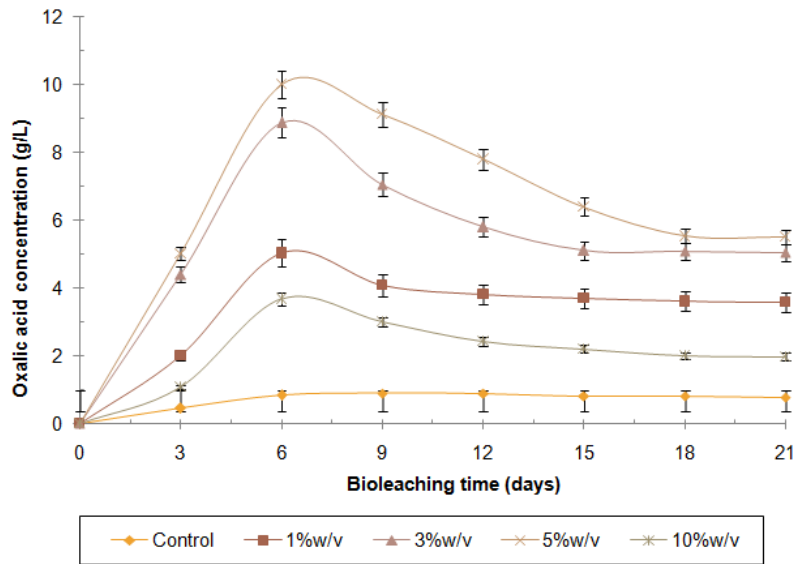

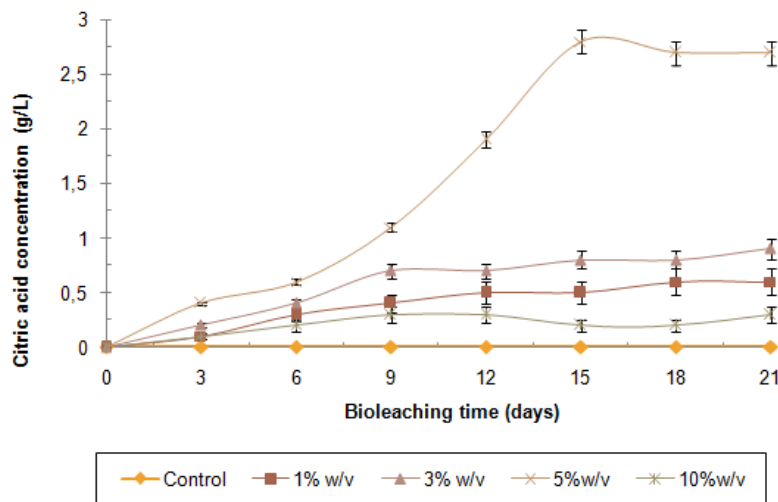**Fig. 2 Oxalic acid concentration during bioleaching experiments.**

Fig. 3 Citric acid concentration during bioelaching experiments.

Table 3. Bioleaching results of Emet-Kütahya colemanite.

Parameters				
Pulp density (%w/v)	Bioleaching time (days)	pH	ºC	B_2O_3 (%)
1	3	5.45	29.1	22.74
	6	5.38	28.1	38.19
	9	5.21	29.5	43.15
	12	3.71	29.3	52.12
	15	3.30	28.5	64.40
	18	3.25	29.7	76.41
	21	3.15	29.7	84.08
3	3	6.70	29.8	27.71
	6	5.69	28.4	41.50
	9	3.95	29.2	53.62
	12	5.05	29.7	62.05
	15	4.43	29.3	69.49
	18	4.18	29.6	79.96
	21	4.18	29.0	88.68
5	3	7.30	29.4	31.41
	6	5.60	28.6	52.22
	9	4.72	29.3	57.27
	12	4.65	29.2	71.55
	15	4.64	29.7	74.08
	18	4.60	29.3	87.75
	21	4.50	29.1	90.18
10	3	7.60	29.1	27.53
	6	5.97	28.0	31.40
	9	5.56	29.4	40.47
	12	5.37	29.1	55.70
	15	5.54	29.3	55.24
	18	5.69	29.2	54.15
	21	5.79	29.0	54.70

Fungal bioleaching is based on four mechanisms: acidolysis, complexolysis, redoxolysis and bioaccumulation (Wu and Ting, 2006). As the first three processes are mediated through the secondary metabolites produced by the fungus, it is important to determine these metabolites in order to better understand the bioleaching mechanisms. The organic acids secreted by the fungus *A. niger* were analyzed in the absence of the colemanite. The fungus secreted 12.7 mg/ml oxalic acid and 2.1 mg/ml citric acid after 14 days of incubation; no gluconic acid was produced. The oxalic and citric acid concentrations in the bioleaching experiments are presented in Figure 2 and Figure 3. These results show that for those experiments in which the colemanite was added at the beginning of cultivation the average organic acid concentration (oxalic acid: 11 g/l and citric acid: 2.8 g/l for 5% w/v pulp density). For boric acid production, oxalic acid was five times more effective than citric acid, and is capable of complexing and boric acid production from colemanite.

Bioleaching tests using *Aspergillus niger* at 1-10% w/v showed that increasing pulp density higher than 5% w/v adversely influenced the production of boric acid as illustrated in Table 3. The boric acid production rates increased as well-proportioned pulp density. The maximum boric acid production rate was 90.18% at 5% w/v pulp density. The optimum parameters after bioleaching tests from Emet-Kütahya colemanite ores are given below:

Particle Size, d_{100}:	0.075 mm
Pulp Density:	5% w/v
Initial *Aspergillus niger* spore quantities:	3×10^7 spore/ml
Temperature:	$25 \pm 2°C$
Bioleaching Time:	21 days
Initial pH:	7.06
Final pH:	4.60

Kinetic study

From the results of bioleaching experiments at optimum pulp density, the kinetics of the process with *Aspergillus niger* was studied. The highest dissolving rates were obtained at the beginning of the experiment, after which the rate decreased, and the dissolved boric acid concentration until the maximum boric acid concentration was attained. In our case, the process consists of a reaction between a solid (colemanite) and a liquid (leaching solution), and the model (Equation 4) was employed to describe the kinetics of the leaching of colemanite. Because of the great porosity of colemanite, the transport through both reacted and unreacted structures was very fast compared with the true reaction rate, and this means that the situation could be called truly homogeneous. The model was formulated with these characteristics and experimental results fitted it well. Figure 4 shows the fitting to this model of the results from experiments at optimum pulp density with *Aspergillus niger.* Under these conditions, the maximum boric acid production was determined as 90.18% at 5% w/v pulp density. The value for coefficient (k) and correlation parameter (R^2) was determined as 0.030 day^{-1} and 0.940, respectively.
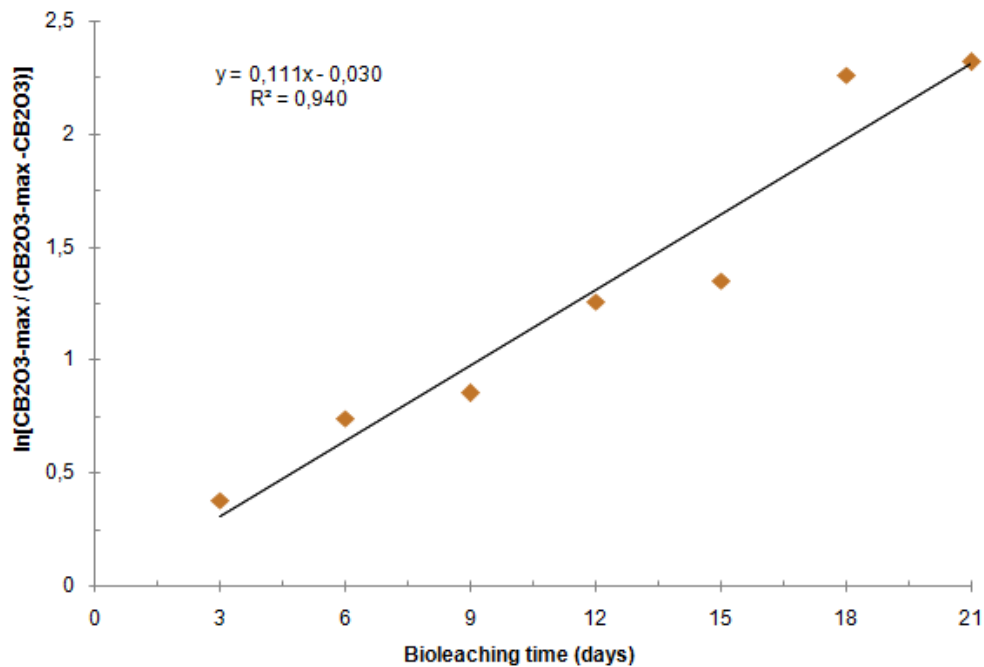

Fig. 4 Kinetic modeling to Equation 4 bioelaching at 5% w/v.

CONCLUDING REMARKS

The present investigation has shown that the highest boric acid extraction from colemanite ore (28.05% B_2O_3, 32.48% CaO and 12.31% Mg0) for particle size < 0.075 mm were obtained in bioleaching by *A. niger* at 5% w/v pulp density in 21 days. The bioleching tests indicated 90.18% of B_2O_3 in the

colemanite ores could be collected in the bioleachate (12.95 g/l B_2O_3 or 23.01 g/l H_3BO_3, 6.60 g/l Ca, 0.087 g/l Mg) and the residue/tailings after bioleaching contains 5.87% B_2O_3, 21.16% CaO and 4.52% Mg0. Further, oxalic acid was the major lixiviant among the metabolites produced by the fungus. pH decreased with time during bioleaching, but remained relatively constant in both the fresh medium and the cell-free medium leaching, thus indicating that the microorganisms play a role in effecting boric acid extraction from the colemanite ores. Bioleaching of colemanite is strongly influenced by the pulp density. The inhibition of fungal growth at higher pulp densities is probably due to the higher concentration of toxic metals in the colemanite as well as an increase in the initial pH of the suspension. Based on recent studies of the technical feasibility and amenability of the extraction of the boric acid from the colemanite ores, it seems likely that commercial scale bioleaching process operations represents a lower ecological potential risk and that even with a lower extraction yield, the economical cost of a such process would be more attractive.

ACKNOWLEDGMENTS

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How to reference this article:

BAYAT, O.; ARSLAN, V. and BAYAT, B. (2011). Use of *Aspergillus niger* in the bioleaching of colemanite for the production of boric acid. *Electronic Journal of Biotechnology*, vol. 14, no. 3. http://dx.doi.org/10.2225/vol14-issue3-fulltext-7