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Feasibility of bio-mobilization of rare earth elements from bauxite residual red mud

Sadia Ilyas [a,*], Hyunjung Kim [a,b,*] and Rajiv R Srivastava [c,d,*]

[a] Mineral Resources and Energy Engineering, Jeonbuk National University, Jeonju, Jeonbuk 54896, Republic of Korea

[b] Department of Environment and Energy, Jeonbuk National University, Jeonju, Jeonbuk 54896, Republic of Korea

[c] Center for Advanced Chemistry, Institute of Research and Development, Duy Tan University, Da Nang 550000, Vietnam

[d] Faculty of Natural Sciences, Duy Tan University, Da Nang 550000, Vietnam

* Correspondence: **authors:** sadiailyas1@yahoo.com (S. Ilyas); kshjkim@jbnu.ac.kr (H. Kim); r2.srivastava@gmail.com (R.R. Srivastava)

Abstract: Current work was conducted to evaluate bioleaching feasibility of red mud with *Penicillium chrysogenum strain KBS3* in the presence of glucose, sawdust, and molasses as substrate and in various leaching modes. One-step bioleaching involving 12 mM citric acid, 2.5 mM oxalic acid, 1.8 mM tartaric acid, and 1162 mM gluconic acid with glucose as substrate. Whereas, the respective biogenic acid production was observed to be 15 mM, 1 mM, 0.5 mM, and 152 mM in two-step bioleaching, which were 63 mM, 29 mM, 23 mM, and 3 mM in the spent medium bioleaching while using glucose as the substrate and pulp density at 3%. Concomitant bio-mobilization was analyzed to be 79% Y, 28% La, and 28% Ce in a one-step bioleaching system. In the spent medium bioleaching 63% Y, 28% La, and 28% Ce were mobilized, which was 67% Y, 20% La, and 15% Ce in a two-step leaching mode. Using molasses as the substrate, bio-mobilization was analyzed to be 57% Y, 13.5% La, and 12.77% Ce in one-step; 57% Y, 14% La, and 12% Ce in a two-step, and 49% Y, 6.3% La, and 2.9% Ce in the spent-medium bioleaching system. While insignificant results were observed with sawdust as substrate.

Keywords: Bauxite processing; Red mud; Rare earth elements; Bio-hydrometallurgy

1. Introduction

Red Mud is the residual mass resultant from NaOH digestion of bauxite ore ($Al_2O_3.2SiO_2.2H_2O$), following the Bayer processes the major metallurgical operation in aluminum extraction. The pH of the wet red mud slurry is about 12. The global stockpile of red mud is estimated to be about 3 billion tons that either goes to marine disposal or as the dry stack in open space (Kumar et al., 2006; Klauber et al., 2011).In both cases, it has a great environmental threat (Power et al., 2011). However, red mud contains a significant amount of rare earth elements (REEs).The rare earth elements exhibit pronounced chemical similarities as a group while individually expressing distinctive and varied chemical properties. These atomistic electronic properties are extraordinarily useful and motivate the application of REs in many modern technologies and devices. In spite of all their contribution to mitigate environmental impacts, their production is resource and pollution intensive, creating a dissonance between the environmentally damaging supply of rare earths and their use in environmentally friendly technologies. In current scenario, an improved extraction/separation

strategies to achieve a sustainable and green circular economy of rare earths from residual wastes like red mud is an absolute necessity of time (Binnemans et al., 2015; Akcil et al., 2017). The chemical composition of red mud varied widely that might be attributed to origin of native ore and/or operational conditions during Bayer process (Evans, 2016; Mongelli, 1997; Ochsenkuhn-Petropulu et al., 1994).

The extraction of REEs from red mud by pyro and hydrometallurgical approaches are labor and energy intensive, have high reagent and processing cost and are not environment friendly (Klauber et al., 2011).Henceforth, considerable efforts have been made to develop the environmentally-friendly biotechnological processing of residual waste. Besides the use of less-hazardous biogenic lixiviants in a cost-effective manner, the operational flexibility, self-sustainability and lower energy consumption are potential advantages with bio-based approaches (Srivastava et al., 2020; Ghorbani et al., 2008; Vachon et al., 1994).

Among potential bioleaching microbes, autotrophs are not suitable for processing red mud due to growth pH constrains and scarcity of required energy sources (sulfur or reduced iron) for the growth of chemolithoautotrophic bacteria like *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Sulfobacillusthermosulfidooxidans etc.* (Burgstaller and Schinner, 1993; Srivastava et al., 2020). In contrast, heterotrophs can survive in comparatively high alkaline conditions and can excrete various metabolites (organic acids, amino acids and proteins) for solubilization of metals from various spent sources (Wu and Ting, 2006; Bosshard et al., 1996; Amiri et al., 2011; Santhiya and Ting, 2005; Brandl et al., 2001). However, limited data exists pertaining to bioleaching of REEs from these residual wastes (Qu and Lian, 2013). So the specific objective of this work was;

- Utilizing the excreted metabolites of isolated heterotrophic culture as a potential source for extraction of REEs in environmentally friendly manner
- Investigate the amenability of bioleaching by using glucose, molasses and saw dust as substrate.
- Comparative study of all leaching modes and elucidate the interplay between biogenic metabolites and rare earth elements present in red mud.

2. Materials and Methods

2.1. Collection of red mud and fungal sample

The fine aggregates of bauxite red mud sample was further dried at 105 °C for 12 h and passed through 500 μm sieve before leaching studies. For analyzing the chemical composition of red mud, the process according to US EPA SW 846 Method 3050B was used to totally digest the samples (Wu and Ting, 2006). The metal ions in supernatant were analyzed using an inductively coupled plasma optical emission spectrometer (iCAP 7400 Duo, Thermo Fisher Scientific, USA). The morphology was investigated by scanning electron microscopy (SUPRA40VP, Carl zeiss, Germany). The pH value of red mud samples were determined by using a digital pH meter (Orion, Thermo Scientific, USA).

Samples for the isolation of fungal culture were collected from iron ore deposits (Kalabagh). The serially diluted sample solution was plated on 3.9% potato dextrose agar (Becton Dickinson, USA) plates and kept in an incubator for 3 days at 30°C. To culture in liquid medium, 1 mL of spore suspension was added to 100 mL of growth medium with composition (g/L) 100 glucose or required amount of pretreated substrate, 0.5 KH_2PO_4, 1.5 $NaNO_3$, 0.025 KCl, 0.025 $MgSO_4.7H_2O$, and 1.6 yeast extract. The cultures, in 250 mL Erlenmeyer flasks were maintained at 30°C in a shaking incubator at 120 rpm. The 18S rRNA genes of the isolate were amplified, sequenced, analyzed, and submitted to Gen Bank (accession number; GQ228447).

2.2. Pretreatment of Substrate and leaching experiments

Before the addition of substrates in the media, substrates were subjected to pretreatment as shown in Table, 1.

Table 1. Pretreatment of substrates for leaching experiment.

Substrates	Pretreatment	Quantity used (g/L)
Glucose	Filter sterilized	100
Molasses	Autoclaved	100
Saw dust	Soaked in sulfuric acid, dried, homogenized	100

Leaching experiments were performed in different modes as described in Table, 2. It was observed during preliminary studies that biomass production start to decrease sharply with prolonged lag phase while moving from 3 to 10% pulp density so 3% pulp density was chosen optimum in current study.

Effluents of bioleaching were first filtered through Whatman No. 1 filter paper to remove solid particles and then centrifuged at 10,000 rpm for 10 min to remove microbial mass and supernatant was analyzed for metal ions and pH values. For comparison with chemical leaching, commercial organic acid equivalent to biogenic acids (at optimum conditions) were chosen.

Table 2. Experimental design matrix of the test regime.

Leaching modes	Experimental set up
Mode-1	Incubating the fungus with red mud using glucose as substrate
Mode-2	pre-culturing the fungus using glucose as substrate and adding the red mud after 3 days of incubation
Mode-3	using the cell free spent medium which was obtained after 10 days of fungal incubation while using glucose as substrate
Mode-4	Incubating the fungus with red mud using pretreated molasses as substrate
Mode-5	pre-culturing the fungus using pretreated molasses as substrate and adding the red mud after 3 days of incubation
Mode-6	using the cell free spent medium which was obtained after 10 days of fungal incubation using pretreated molasses as substrate
Mode-7	Incubating the fungus with red mud using pretreated saw dust as substrate
Mode-8	pre-culturing the fungus using pretreated saw dust as substrate and adding the red mud after 3 days of incubation
Mode-9	using the cell free spent medium which was obtained after 10 days of fungal incubation using pretreated saw dust as substrate
Mode-10	Leaching with synthetic acid mixture (equivalent to biogenic acids at optimum conditions)
Mode-11	Leaching with sterile growth media

2.3. Analysis of Organic Acids

Analysis of organic acid metabolites was carried out by modified Escobal *et al* (1996) method. The organic acid components were investigated using high performance liquid chromatography (HPLC, Sykam GmbH, Kleinostheim, Germany) equipped with S-1121 dual piston solvent delivery system and S-3210 UV/VIS diode array detector and C18 column, mobile phase consisted of acetic acid solution (0.25 %), and flow rate of1.0 mL min^{-1} at 30 °C. Detection was performed at a wavelength of 254 nm. The organic acids were identified by comparing the retention times and quantified on the basis of peak areas.

3. Results and discussion

3.1. Characterization of residual waste

The fine aggregates of bauxite red mud sample mainly contains hematite, gibbsite, goethite, diaspore, and calcite minerals along with the significant amount of rare earths therein. Thermal analysis showed that a good amount of water loss with temperature confirms the existence of hydrated minerals in red mud. Table, 3 provided the concentrations of various metal ions present in the material.

3.2. Extraction of metals during various leaching modes

Prior to bioleaching studies, the native culture of *Penicillium chrysogenum strain KBS3* was grown in liquid growth media supplemented with various substrates until it reached the stationary phase in order to determine the optimum time period for incubation with red mud in leaching mode-2,3,5, 6, 8 and 9.

The maximum hydrolysis of substrates at the 3rdday indicated that the *Penicillium chrysogenum strain KBS3* is in the active growth phase. Therefore after 3 days incubation, the red mud was added into the fungal culture in leaching mode-2, 5and 8. The maximum biomass and minimum pH value were reached at the 10th day of incubation. Therefore the cell-free medium was obtained through filtering the culture after 10th day of incubation for leaching mode-3, 6and 9.

Metal solubilization from red mud by heterotrophic microorganisms can be due to enzymatic reduction of highly oxidized metal compounds or by the production of organic acids (acidolysis) and by compounds with hydrophilic reactive groups (Srivastava et al., 2020; Burgstaller and Schinner, 1993).However the most important mechanism is the acidolysis. The related reactions between the different organic acids and metal ions are depicted as below (M^{n+} represents the metal ions with variant valence);

$$C6H12O7 \rightarrow C6H11O7- + H+ (\text{pk}a=3.86) \quad (1)$$

$$n[C_6H_{11}O_7^-] + M^{n+} \rightarrow M[C_6H_{11}O_7]_n \quad (2)$$

$$C_2H_2O_4 \rightarrow C_2HO_4^- + H^+ (\text{Pka} = 1.25) \quad (3)$$

$$C_2HO_4^- \rightarrow C_2O_4^{2-} + H^+ (\text{Pka} = 4.14) \quad (4)$$

$$n[C_2HO_4^-] + M^{n+} \rightarrow M[C_2HO_4]_n \quad (5)$$

$$n[C_2O_4^{2-}] + 2M^{n+} \rightarrow M_2[C_2O_4]_n \quad (6)$$

$$C_6H_8O_7 \rightarrow C_6H_7O_7^- + H^+ (\text{Pka} = 3.09) \quad (7)$$

$$C_6H_7O_7^- \rightarrow C_6H_6O_7^{2-} + H^+ (\text{Pka} = 4.75) \quad (8)$$

$$C_6H_6O_7^{2-} \rightarrow C_6H_5O_7^{3-} + H^+ (\text{Pka} = 6.40) \quad (9)$$

$$n[C_6H_7O_7^-] + M^{n+} \rightarrow M[C_6H_7O_7]_n \quad (10)$$

$$n[C_6H_6O_7^{2-}] + 2M^{n+} \rightarrow M_2[C_6H_6O_7]_n \quad (11)$$

$$n[C_6H_5O_7^{3-}] + 3M^{n+} \rightarrow M_3[C_6H_5O_7]_n \quad (12)$$

$$C_4H_6O_6 \rightarrow C_4H_5O_6^- + H^+ (\text{Pka} = 2.98) \quad (13)$$

$$C_4H_5O_6^- \rightarrow C_4H_4O_6^{2-} + H^+ (\text{Pka} = 4.34) \quad (14)$$

$$n[C_4H_5O_6^-] + M^{n+} \rightarrow M[C_4H_5O_6]_n \quad (15)$$

$$n[C_4H_4O_6^{2-}] + 2M^{n+} \rightarrow M_2[C_4H_4O_6]_n \quad (16)$$

Fig.1 indicated the solubilization of REEs in various leaching modes. The leaching efficiency was lowest in leaching mode-3 (63% Y, 28% Ln, 28% Ce) using glucose as substrate and in leaching mode-6 (49 % Y, 6.3% Ln, 2.9 % Ce) using molasses as substrate while highest leaching efficiencies were observed in leaching mode-1 (79% Y, 29% Ln, 29% Ce) and 4 (57 % Y, 13.5 % Ln, 12.77 % Ce) with glucose and molasses as substrate. That was probably due to stimulating effect of REEs on microbial growth and enzyme activity (d'Aquino et al., 2009; Santhiya and Ting, 2005).

Compared to leaching mode-1 (79% Y, 29% Ln, 29% Ce) and 4(57 % Y, 13.5 % Ln, 12.77 % Ce), the metal extraction efficiency in commercial acid mixture of equivalent concentration (leaching mode-10) was lower (53% Y, 11% Ln, 25% Ce) indicating that beside biogenic organic acids some other metabolites like amino acids and proteins form complexes with the metals and facilitate their solubilization (Srivastava et al., 2020).

Insignificant leaching of metal in leaching mode-7 (13% Y, 7% Ln, and 7% Ce), mode-8 (12% Y, 9% Ln, and 7% Ce) and mode-9 (17% Y, 3.5 % Ln, and 3% Ce) was observed due to incomplete hydrolysis of saw dust as substrate.

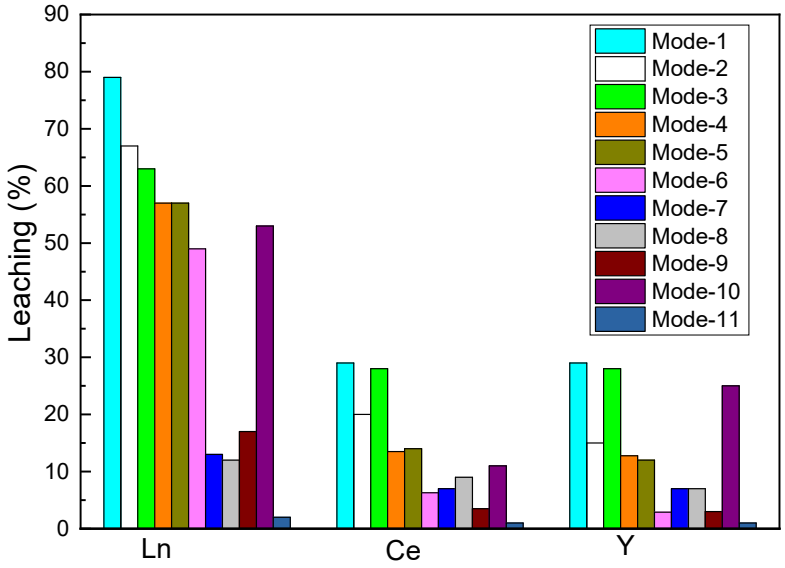

Figure 1. Mobilization of rare earth elements in various leaching modes.

Figs. 2-7 indicated the changes in pH profile with various pulp densities during various leaching modes by using glucose and molasses as substrate. With increasing pulp density, a slow decrease in pH was observed in all leaching modes. This can be due to higher toxicity of metals ions at higher pulp densities limiting microbial metabolic activities by inactivating enzymes. At 6% or higher pulp

density, the pH value increased at the beginning of bioleaching before the strain grew abundantly. That can be due to continual dissolution of substantial alkaline minerals in red mud taking more time to reach chemical equilibrium under laboratory conditions (Khaitan et al., 2009;Vachon et al., 1994; Amiri et al., 2011, Brandl et al., 2001, Wu and Ting, 2006; Pagano et al., 2002).

At optimum leaching condition (3% pulp density and 1-step leaching) biomass production rate was higher while using glucose as substrate (25g/L) compared to molasses (20 g/L) and saw dust (10g/L). High biomass production in leaching mode-1 can be due to rapid hydrolysis of substrate and easy availability of carbon source required for heterotrophic growth compared to molasses and saw dust as substrate.

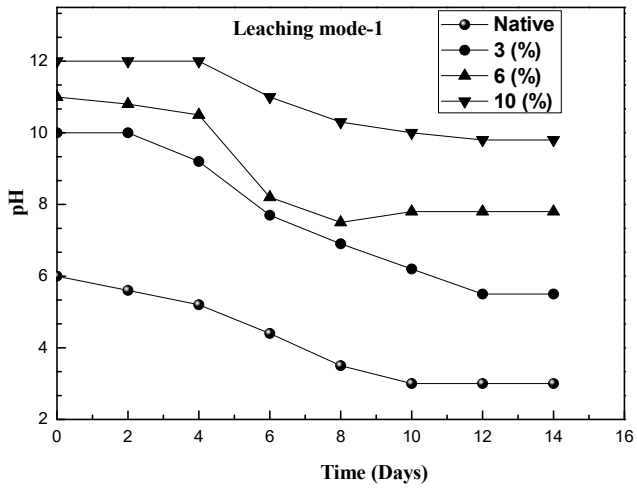

Figure 2. pH profile at various pulp densities for leaching mode-1.

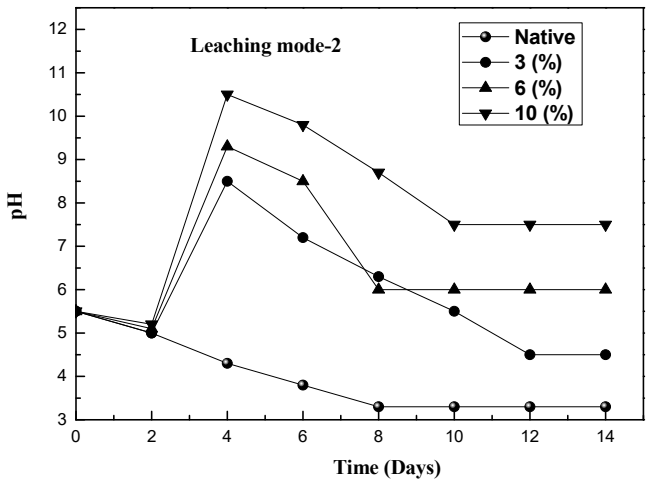

Figure 3. pH profile at various pulp densities for leaching mode-2.

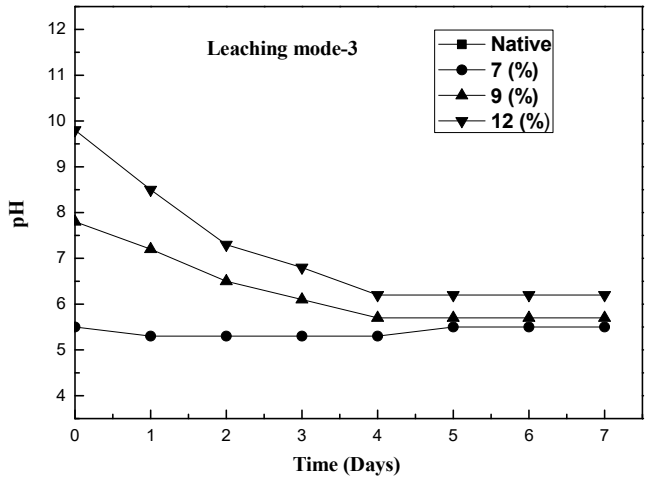

Figure 4. pH profile at various pulp densities for leaching mode-3.

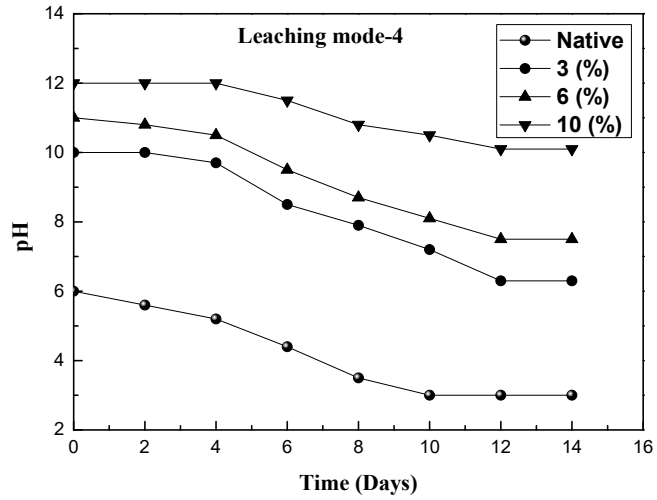

Figure 5. pH profile at various pulp densities for leaching mode-4.

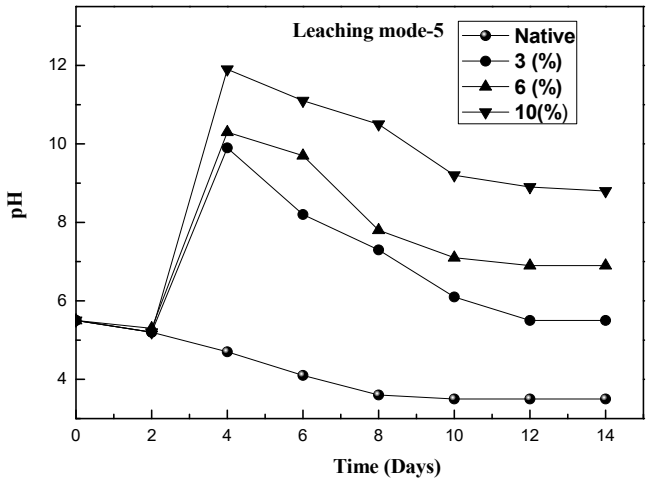

Figure 6. pH profile at various pulp densities for leaching mode-5.

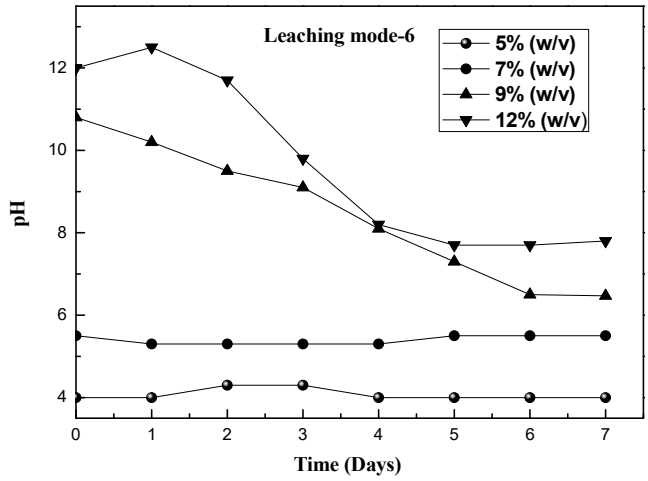

Figure 7. pH profile at various pulp densities for leaching mode-6.

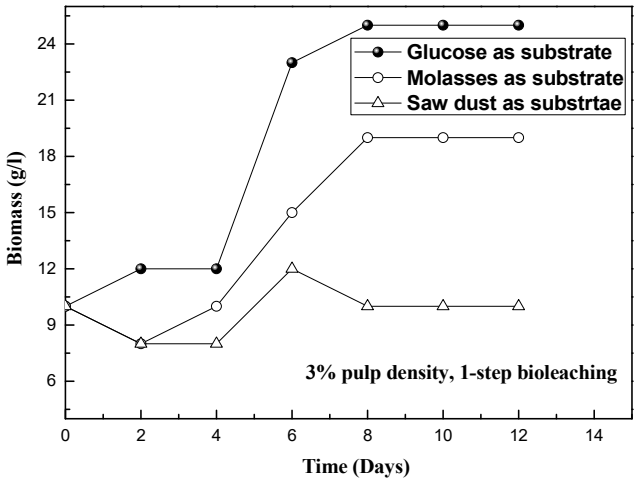

Figure 8. Biomass concentration with different substrates.

3.3. Production of Organic Acids

Organic acids have two main functions that are very important in bioleaching as they can facilitate the dissolution of metals ions via chelation and also destabilize the bonds between the surface metal and bulk leaching materials (Gräfe et al., 2011).

The organic acids secreted by *Penicillium chrysogenum strain KBS-3*were analyzed at various pulp densities of red mud in leaching mode 1-6 and compared with native system supplemented with required substrate but without feed material (**Table. 3**).

Table 3. Concentration of organic acids in fermented media (after 10 days).

Organic acid production by using glucose as substrate			
Organic acid production (mM)	**mode-1**	**mode-2**	mode-3
Citric acid	12	15	63
Oxalic acid	2.5	1	29
Tartaric acid	1.8	0.5	24.5
Gluconic acid	1162	152	123
Organic acid production by using molasses as substrate			
Organic acid production (mM)	mode-4	mode-5	mode-6
Citric acid	4.21	3.57	44.8
Oxalic acid	1.55	1.0	15.0
Tartaric acid	1.18	0.95	14.8
Gluconic acid	210.19	52.5	11
Organic acid production in absence of red mud			
Organic acid production (mM)	Glucose	Molasses	Saw dust
Citric acid	63	45	0.67
Oxalic acid	28	15	07
Tartaric acid	25	15	03
Gluconic acid	122	11	0.75

In the absence of the red mud, the fungus secreted 28 mM oxalic acid, 63 mM citric acid, 25 mM tartaric acid and 122 mM gluconic acid with glucose as substrate while 15 mM oxalic acid, 45mM citric acid, 15mM tartaric acid and 11mM gluconic acid was observed using molasses as substrate after 7 days of incubation. Insignificant biogenic organic acid production (0.67mM citric acid, 7mM oxalic acid, 3mM tartaric acid and 0.75mM gluconic acid) was observed with saw dust as substrate during 7 days of incubation. At optimum pulp density (3%) the concentration of citric, oxalic and tartaric acid produced during leaching mode-1, 2, 4, 5, and 6 was lower than leaching mode-3 where it was comparable to native one. It is noteworthy that a significant increase in the concentration of gluconic acid occurred at 3% pulp density in leaching mode-1 and 4 compared to native. It has been reported that glucose oxidase has an optimum pH above 5 and inactivated at pH below 4.0 and above 7 (Fiedurek et al. 1986) that is ideal in leaching mode-1 and 6. Additionally, the presence of glucose as substrate activates the enzyme glucose oxidase which converts glucose to hydrogen peroxide, and finally hydrolyzes it to gluconic acid. Therefore, gluconate was sufficiently produced in the presence of red mud/ residual waste and with glucose as substrate in leaching mode-1 (Wu and Ting 2006).

4. Conclusions

The present study elucidated the potential of bioleaching for REEs' extraction from its secondary reservoir red mud residue generated during the Bayer process of bauxite.

One step bioleaching yielded a higher extraction efficiency of REEs from red mud as compared to the two step and spent medium bioleaching.

Bioleaching is strongly influenced by the pulp density. The inhibition of fungal growth at higher pulp densities is due to the higher concentration of toxic components as well as an increase in the initial pH of the medium.

The reverse solubility of REEs in the presence of metabolite organic acid excreted by *Penicillium chrysogenum strain KBS3* and glucose substrate with other than one step bioleaching can be the possible reason behind the exhibited phenomenon.

More fundamental studies on metabolites and REEs' solubility is required

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