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BIORECOVERY OF METAL IONS FROM BROWN SHALE USING ORTHOGONAL EXPERIMENTAL ARRAY DESIGN

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Abstract

Aspergillus fumigatus has been selected to bioleach the metals ion from brown shale. Process parameters like pH (5.0, 5.5, 5.8, 6.0, and 6.5), substrate concentration (2, 4, 6, 8, and 10%), pulp density (1, 5, 10, 15, and 20%), temperature (30, 35, 40, 45, 50°C) and agitation (100, 150, 200, 250, 300 rpm) were optimized according to L25 Taguchi orthogonal experimental array design that resulted in the twenty five batch bio recovery experiments. After biorecovery experiments, optimum value of pH, substrate, pulp density, temperature and agitation was detected 6.0, 5%, 10%, 45°C & 300 rpm respectively. Maximum boileached metals analyzed by Atomic Absorption Spectrophotometry (AAS) were Al 85.00%, Mg 81.30%, Cu 68.10%, Mn 71.50%, and Ni 62.10% with an inoculum size of 3.10 $\times 10^6$ spores /mL.

Key words: Aspergillus fumigatus, biorecovery, brown shale, process optimization

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1. Introduction

In the contemporary world, low grade and rejected ores, mine tailings and wastes has become the blessing of god due to continuous exhaustion of high grade ore deposits. Number of valuable metals are housed in these rejected resources that yet to be mined. It is more difficult to extract metals from these rejected resources by using classical methods that require huge outlay at the cost of environment. Substitute of classical methods is the bioleaching that used to extract metals from discarded ores and wastes to consumable. Bioleaching is a green process based on the capability of microbial activities to convert insoluble compounds in to dissolvable elements that can be separated easily. Millions of tonnes of low-grade ores containing Al, Cu, Zn, Mg, Mn, Fe, Pb, As, Au, Ag, U and other valuable rare earth metals are present in different geographical regions of the world (Yanting et al., 2005). These precious metals can be bioleached to economic level by microbial activities thus, the depleted ores are valuable (Lavalle et al., 2008; Paldyna et al., 2017). Slight reaction conditions, less energy input and simple processes makes bioleaching a valuable technology for treating discarded ores, shales, schists, metallurgy residues and electronic wastes that have positive impact on the environment (Amiri et al., 2011; Liu et al., 2008).

Shales are fine-grained rocks that are in the form of sediments. These are mainly composed of

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minerals along with fine quartz and mice. These shales have originated in Precambrian era.

Dead remains of algae, bacteria and other life forms that lived in the sea constituted the color of the shales. These dead remains also constitute the organic matter in the form of laminas, interlayers and small inclusions. In Pakistan, it occur in Foothills of Himalayas at Lagarbon, Korakoram valley at Shishi Gol and South of Mirkhani, In Kakul in Hazara District, Chitral areas and Turbela areas (Ahmad, 1969). Undoubtedly, the most successful microbes are chemolithoautotrophic bacteria, that bioleach the precious metals from discarded ores, shales and schist. Fungal bioleaching has taken an important role in bioleaching of the precious metals from low grade discarded ores, shales and schists etc. Generally, microorganisms extract metals by redox mechanism, bioaccumulation and soluble complex salt formation during the bioleaching (Gentina and Acevedo, 2013). Microbes have direct attachment to the minerals, resulting in the formation of soluble complex salts (Rezza et al., 2001; Vardanyan et al., 2018).

In case of fungi, bioleaching mechanism is related to the excretion of metabolites, mainly organic and inorganic acids. These metabolites form soluble complex salts with minerals. Recently, different metals like Al, Fe, Ni, Cu, Co, Mn extracted microbialy from low grade discarded ores and mine tailings (Anjum et al., 2010; Yang et al., 2011; Yanting et al., 2005; Watling et al., 2009).

According to our knowledge, bioleaching of metals from low-grade brown shale has not been reported yet. In the current study, brown shale might be a good source of precious metals in order to strengthen the economy. Hence, the present research planned to extract Al, Mg, Cu, Mn, and Ni from brown shale by applying the orthogonal array design that might be helpful in implementing the process commercially. Different process parameters such as temperature, pH, substrate, pulp density and agitation have been optimized by using this design.

2. Experimental

2.1. Brown shale sample

Mineralogical Centre Chakwal, Pakistan provided the representative sample that was partially soluble in water having a pH of 7.5. The sample was dried in oven at 103^{o}C and ground to 200 mesh sizes by sieving machine.

2.2. Metallogeny of brown shale

Mineralogical analysis of raw as well as bioleached sample was performed by X-ray Diffraction Spectroscopy (XRD) whereas metal ions determination was performed by atomic absorption spectrophotometry (AAS) (Perkin Elmer, A Analyst 300) as reported by Anjum et al. (2010).

2.3. Fungal growth and bioleaching study

Aspergillus fumigates was collected from microbial laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. To obtain sufficient number of spores, it was multiplied on Potato Dextrose Agar (PDA 3.9%) slants (Bosshard et al., 1996) and incubated in growth chamber (Incubator, Sanyo, Germany) for 3 to 4 days at 45°C. The isolates were also characterized and authenticated by the Mycology Research Laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. Further, microbial growth took place in liquid media of one liter consisting of nutrients of vogel, as medium (Anjum et al., 2010) .This liquid medium (100 mL each) was further introduced into ten conical flasks and subjected to sterilization. After sterilization, the inoculation of spores of *Aspergillus fumigatus* as well as addition of 5% of given substrate (Molasses, 50% v/v) was done. Flasks were cotton plugged and agitation at 150 rpm was done using orbital shaker (Gellen Kamp, England) at 45°C for the growth period of seven days. Out of ten, one flask containing 2.04 x10^7spores mL^{-1} was selected for further study.

Present research work consisted of Twenty-five experimental flasks containing 100mL of culture medium as mentioned above under the experimental conditions as reported in Table 1. Inoculation with the spores of *Aspergillus fumigatus* (approximately 2.04 x 10^7 spores mL^{-1} of spore suspension) was done after sterilization. Substrate as well as pulp density was added according to the orthogonal design given in Table 1. All the flasks were incubated for shaking after sealing with cotton plug under the experimental conditions as reported in Table 2. Flasks were shaken for the period of fourteen days. Throughout the course of the leaching, pH was noted periodically. Then the sample was withdrawn from each flask at the end of the fourteenth day. The sample was sterilized to stop further microbial growth. Solid particles as well as fungal cells were removed by using the filtration as well as by centrifugation (10,000 rpm, 10 min) technique. The supernatant was further subjected to elemental analysis by Atomic Absorption Spectrophotometry (AAS).

2.4. Orthogonal experimental array

Taguchi method was used to construct the experimental design of L25 (5^5) (Dehghan et al., 2009) that was based on the factors previously reported (Anjum et al., 2010). The degree of influence of individual parameters as well as their optimization in combined form was studied for biorecovery of metal ions using *Aspergillus fumigatus*. Preliminary tests were performed for the optimization of the time period of bioleaching and selection of experimental parameters, and their levels by keeping inoculum size constant at one mL (approximately 2.04 x 10^7spores mL^{-1}).

Furthermore, some changes of experimental conditions were also adopted as reported for the bioleaching of low-grade ores (Dehghan et al., 2009; Sadegh Safarzadeh et al., 2008). Different factors like initial pH, ore density, substrate concentration, temperature and shaking speed were identified after performing preliminary tests that influenced the bioleaching performance significantly. These five parameters with five levels were used to construct the Orthogonal array design with L25 (5^5) matrix (Table 2). During the biorecovery experiments, these parameters were repeated twice for each level to ensure the absence of determinate as well as indeterminate errors. Therefore, the order of experiments was randomly selected. The integer values of L25 are present in the Tables 1 and 2.

Table 1. L_{25} (55) experimental work plan

Experiment No.	***Parameters and their level***				
	A	***B***	***C***	***D***	***E***
1	1	1	1	1	1
2	1	2	2	2	2
3	1	3	3	3	3
4	1	4	4	4	4
5	1	5	5	5	5
6	2	1	2	3	4
7	2	2	3	4	5
8	2	3	4	5	1
9	2	4	5	1	2
10	2	5	1	2	3
11	3	1	3	5	2
12	3	2	4	1	3
13	3	3	5	2	4
14	3	4	1	3	5
15	3	5	2	4	1
16	4	1	4	2	5
17	4	2	5	3	1
18	4	3	1	4	2
19	4	4	2	5	3
20	4	5	3	1	4
21	5	1	5	4	3
22	5	2	1	5	4
23	5	3	2	1	5
24	5	4	3	2	1
25	5	5	4	3	2

Table 2. L_{25} 5^5 Experimental Work Plan with quantitative values

Experiment No.	***Parameters and their level***				
	A	***B***	***C***	***D***	***E***
1	30.0	5.0	1.0	100.0	1.0
2	30.0	5.5	5.0	120.0	5.0
3	30.0	5.8	10.0	150.0	10.0
4	30.0	6.0	15.0	180.0	15.0
5	30.0	6.5	20.0	200.0	20.0
6	35.0	5.0	5.0	150.0	15.0
7	35.0	5.5	10.0	180.0	20.0
8	35.0	5.8	15.0	200.0	1.0
9	35.0	6.0	20.0	100.0	5.0
10	35.0	6.5	1.0	120.0	10.0
11	40.0	5.0	10.0	200.0	5.0
12	40.0	5.5	15.0	100.0	10.0
13	40.0	5.8	20.0	120.0	15.0
14	40.0	6.0	1.0	150.0	20.0
15	40.0	6.5	5.0	180.0	1.0
16	45.0	5.0	15.0	120.0	20.0
17	45.0	5.5	20.0	150.0	1.0
18	45.0	5.8	1.0	180.0	5.0
19	45.0	6.0	5.0	200.0	10.0
20	45.0	6.5	10.0	100.0	15.0
21	50.0	5.0	20.0	180.0	10.0
22	50.0	5.5	1.0	200.0	15.0
23	50	5.8	5	100	20
24	50	6.0	10	120	1
25	50	6.5	15	150	5

Note: Coded factors A= Temperature (ºC), B= pH, C= Substrate (%), D= Agitation (RPM), E= Pulp density (%)

Low, high and their average values in the column is representative of the column factors. Each row in the orthogonal array design having specific set of factors have been tested independently (Sadegh Safarzadeh et al., 2008). Experiments were performed to optimize these process parameters. Then these optimized parameters were used to recover maximum metal ions along with control experiments. Periodically, metabolites of *A. fumigatus*, were analyzed by HPLC using C18column (250 mm × 4.6 mm) equipped with diode array detector at 210 nm. Acetic acid was used as mobile phase at a flow rate of 0.5 µL/min. Organic acids were quantified by using an external standard method.

Chemical leaching was done to assess the affinity of different organic acids towards the formation of metals complexes. 0.5% (w/v) of organic acid (Oxalic, citric, malic, tartaric acid) was added separately into flasks containing brown shale (5% (w/v) and agitated for 24 hours in a shaker at 150 rpm with a temperature of 30°C. pH was noted periodically. After 24 hours, samples were centrifuged, filtered and withdrawn and then analyzed by AAS.

3. Results and Discussion

3.1. Metallogeny of brown shale

Metallogeny of ore was determined by AAS and results are reported in Table 3.

Table 3. Metallogeny of brown shale ore

Metals	*Conc (mg/Kg)*
Ca	852.07 ± 2.05
Al	401.45 ± 3.11
Mg	239.11 ± 0.84
Cu	161.03 ± 0.87
Mn	88.41 ± 0.06
Ni	71.25 ± 0.07

Al, Mg, Cu, Mn, and Ni were detected in the ore as major metal ions whereas Monticellite (Ca Mg SiO_4),Calcite ($CaCO_3$), Quartz (SiO_2), Ni-sulfide (NiFe)xS, Kaolinite ($Al_2SiO_2O_5$ (OH), Wollastonite ($CaSiO_3$), Aluminum Oxide (Al_2O_3), Dolomite CaMg $(CO_3)_2$, (NiFe) S_2 and Montmorillonite (Na, Ca)0.3(Al, Mg)$_2$Si$_4$O$_{10}$(OH)$_2$ nH$_2$O) minerals were detected by XRD (Fig. 1).

3.2. Bioleaching studies

For maximum metal ions dissolution, the bioleaching process was optimized for the period of fourteen days according to an orthogonal experimental array design while keeping the size of inoculum constant (approximately 2.04 x 10^7spores mL^{-1}). Corresponding bio recovery efficiencies with two replications obtained under the applied conditions as reported in Table 4. For each cycle, optimized value of each factor was determined.

The collected data were then analyzed by Origin pro 7.5 software to evaluate the effect of each parameter on maximum amount of metal ions dissolution. To achieve the definite goals, mean response calculation was performed. Corresponding conditions were taken in each row of that table related to its response. Then mean response was analyzed for each run as recommended by Taguchi and graphs of the marginal means of each factor. Mean response of each factor was determined to make the trend and reported in Figs. 2-6.

Mean responses for metal ions of brown shale ore affected by pH are given in Fig 2. Maximum metal ions dissolution was detected in the sample having initial pH 6.0 (Al 67.14, Mg 42.98, Cu 50.84, Mn 45.04 and Ni 35.88%). Microbial activities and their excreted metabolites govern pH of leaching medium whereas metal ions acidification, complexation and dissolution are mainly affected directly by the pH of the leaching medium. Buffering capacity of the leaching medium causes the chronological change of pH. Therefore, the optimum pH for bioleaching with *Aspergillus fumigatus* was found to be 6.0 as shown in Fig. 2.

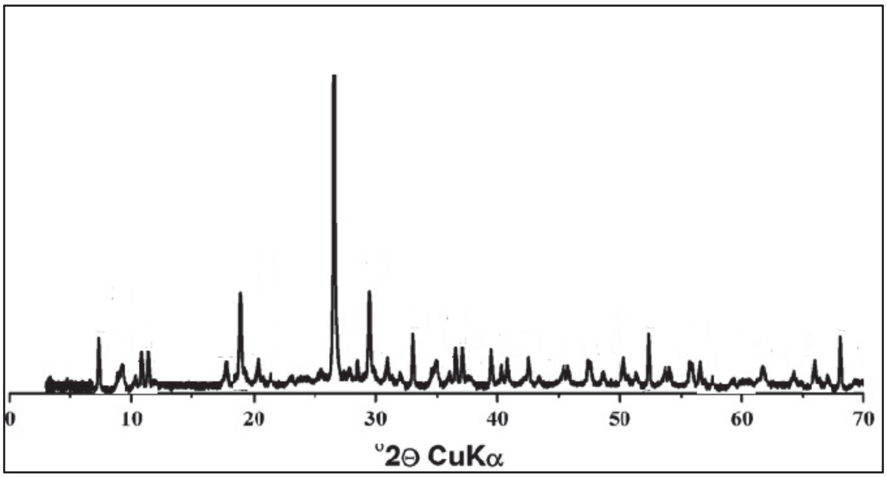

Fig. 1. X-Ray diffractogram of brown shale ore

Table 4. Orthogonal Array Design for bioleaching efficiency of metals

Runs.	***Parameters and their level***					***Bioleaching Efficiency (%)***				
	A	B	C	D	E	Al	Mg	Cu	Mn	Ni
1	30	5.0	1	100	1	7.2±0.01	14.7±0.02	11.4±0.01	11.4±0.01	8.3±0.01
2	30	5.5	5	120	5	5.6±0.01	14.6±0.01	13.4±0.01	10.5±0.01	14.3±0.02
3	30	5.8	10	150	10	13.3±0.03	15.1±0.01	21.3±0.02	13.5±0.02	21.4±0.03
4	30	6.0	15	180	15	14.6±0.02	14.7±0.02	14.2±0.01	11.4±0.01	11.3±0.01
5	30	6.5	20	200	20	15.8±0.01	15.8±0.01	11.3±0.01	21.4±0.02	10.5±0.01
6	35	5.0	5	150	15	26.3±0.03	27.1±0.02	18.0±0.01	35.9±0.03	6.8±0.01
7	35	5.5	10	180	20	24.7±0.02	26.3±0.01	25.3±0.02	29.1±0.03	15.2±0.02
8	35	5.8	15	200	1	13.9±0.04	25.6±0.01	42.1±0.03	31.4±0.04	23.5±0.03
9	35	6.0	20	100	5	15.5±0.01	15.9±0.01	47.9±0.05	23.4±0.01	21.1±0.02
10	35	6.5	1	120	10	25.1±0.01	17.1±0.01	43.1±0.04	25.3±0.03	12.4±0.01
11	40	5.0	10	200	5	49.5±0.05	36.9±0.04	65.1±0.04	45.2±0.05	11.2±0.01
12	40	5.5	15	100	10	53.4±0.03	44.8±0.02	56.3±0.04	51.8±0.03	22.5±0.02
13	40	5.8	20	120	15	66.6±0.03	43.3±0.05	52.8±0.03	62.3±0.07	32.1±0.02
14	40	6.0	1	150	20	61.3±0.05	55.6±0.03	61.5±0.04	66.3±0.05	33.4±0.02
15	40	6.5	5	180	1	47.4±0.07	45.1±0.05	42.5±0.03	58.2±0.07	24.3±0.02
16	45	5.0	15	120	20	65.2±0.04	72.0±0.08	41.6±0.04	35.1±0.05	12.5±0.01
17	45	5.5	20	150	1	72.5±0.05	82.1±0.07	61.4±0.03	66.3±0.07	46.7±0.04
18	45	5.8	1	180	5	86.2±0.04	67.4±0.07	56.2±0.06	62.4±0.08	51.4±0.05
19	45	6.0	5	200	10	85.0±0.06	81.3±0.04	68.1±0.05	71.5±0.09	62.1±0.07
20	45	6.5	10	100	15	51.3±0.02	73.5±0.06	47.0±0.07	63.2±0.08	55.6±0.05
21	50	5.0	20	180	10	56.1±0.02	56.1±0.07	41.3±0.05	49.7±0.07	39.3±0.04
22	50	5.5	1	200	15	32.1±0.02	47.3±0.04	56.2±0.04	33.5±0.01	42.5±0.05
23	50	5.8	5	100	20	47.9±0.04	57.2±0.07	53.1±0.07	48.9±0.04	53.1±0.06
24	50	6.0	10	120	1	59.3±0.03	47.3±0.04	62.5±0.06	52.6±0.07	51.5±0.07
25	50	6.5	15	200	5	41.6±0.05	36.2±0.02	59.2±0.07	61.5±0.05	49.3±0.03

Note: Values for bioleaching efficiency (%) are mean ± SD of duplicate samples analyzed individually in triplicate

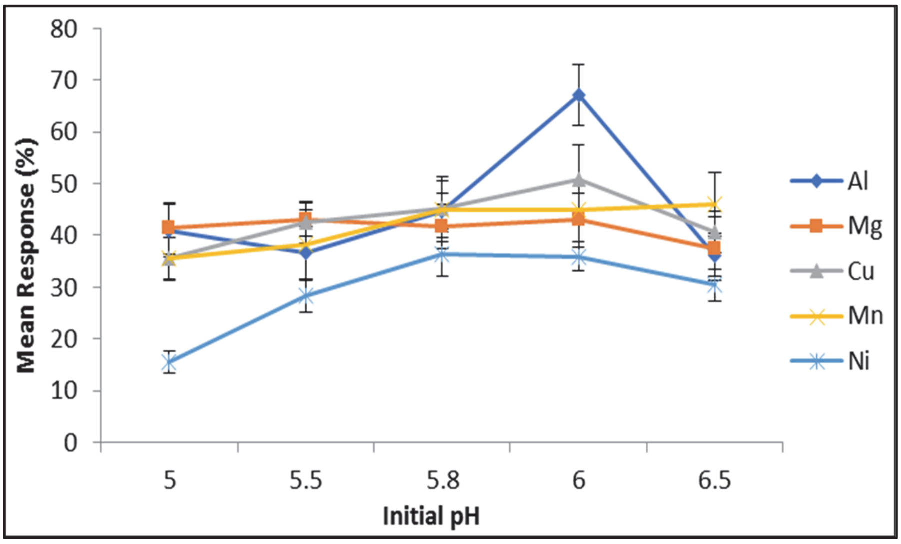

Fig. 2. Effects of Initial pH on mean responses for metal ions dissolution

At higher pH than 6, less metal ions dissolution occurs, that might be due to less microbial growth activities. Thus, less production of metabolites was observed (Shen and Jih, 2001).

In all samples, as the leaching process was going on, pH was decreased due to microbial activities or log phase of fungi which resulted in the production of acid metabolites as reported by Bosshard et al. (1996). pH was decreased to their minimum values on the 3rd day of bioleaching indicating the maximum production of acidic metabolites. Moreover, pH went on increasing up to fourteenth day of bioleaching as acid metabolites were consumed for mobility of metal ions (Table 5). Present research trend is in accordance with the results reported by Ilyas et al (2012).

The substrate is another parameter which influences the microbial growth. Microbes produce more organic acid metabolites, subsequently more metal ions are dissolved. The optimum level of substrate was found to be 5% at which optimum pH of 6.0 was detected indicating the production of acid metabolites (Table 6).

Table 5. pH profile of samples under the L25 (5^5) experimental work plan

Days Runs.	***1st***	***2nd***	***3rd***	***4th***	***5th***	***6th***	***7th***	***8th***	***9th***	***10th***	***11th***	***12th***	***13th***	***14th***
1	5.0	3.2	3.1	3.3	5.6	5.9	6.7	6.7	6.8	7.3	7.0	7.4	7.5	7.5
2	5.5	3.1	3.1	3.5	5.0	6.1	6.3	7.2	7.2	7.4	7.7	7.4	7.4	7.4
3	5.8	2.9	3.0	3.1	4.1	6.0	5.8	6.7	6.8	7.0	7.7	7.5	7.7	7.7
4	6.0	2.8	2.9	4.1	5.7	6.4	6.6	7.1	7.4	7.3	7.8	7.5	7.7	7.7
5	6.5	3.5	2.5	3.2	3.3	3.7	5.3	6.0	6.6	6.9	7.2	7.5	7.8	7.9
6	5.0	3.1	3.0	4.2	4.7	6.7	6.8	7.4	7.6	7.9	7.8	7.9	7.5	7.5
7	5.5	2.9	2.8	3.2	3.5	4.2	4.7	5.5	6.1	6.3	6.9	6.9	7.2	7.2
8	5.8	3.4	3.5	3.4	3.7	4.3	4.6	5.1	5.7	6.4	6.5	6.9	7.1	7.1
9	6.0	3.1	3.0	4.1	4.5	5.1	5.3	5.7	6.4	6.6	6.9	7.3	7.2	7.3
10	6.5	3.0	2.9	3.4	3.7	4.1	4.4	5.0	5.5	6.3	6.6	6.9	7.2	7.1
11	5.0	3.0	3.0	3.6	3.9	4.5	4.6	5.8	6.2	6.6	6.9	7.4	7.3	7.3
12	5.5	3.3	2.8	3.4	3.7	4.1	4.5	6.0	6.6	6.7	6.8	7.4	7.5	7.5
13	5.8	3.1	3.0	3,5	3,4	3.7	4.0	4.7	4.8	5.7	5.8	6.5	6.8	6.9
14	6.0	2.5	2.6	3.5	3.6	4.0	4.5	4.9	5.7	6.1	6.6	6.9	6.9	7.0
15	6.5	3.7	2.8	3.7	4.0	4.4	5.3	5.4	6.1	6.7	7.5	7.6	7.5	7.6
16	5.0	3.0	3.0	3.5	3.8	4.2	5.7	6.0	6.6	6.9	7.1	7.7	7.8	7.5
17	5.5	2.8	2.9	3.4	3.6	4.1	4.5	4.9	5.6	5.7	6.4	6.8	6.8	7.0
18	5.8	2.9	2.9	3.6	3.9	4.65	5.5	6.0	6.6	6.5	6.8	7.0	7.1	7.2
19	6.0	3.1	3.2	3.6	3.5	5.0	5.6	5.7	6.3	6.5	6.7	7.4	7.3	7.4
20	6.5	3.4	3.1	3.5	3.7	4.6	4.9	5.8	6.0	6.7	6.7	6.8	7.0	7.1
21	5.0	3.1	3.0	4.3	4.4	5.1	5.7	6.2	6.3	6.7	6.8	6.9	7.0	7.0
22	5.5	2.9	2.8	3.0	3.5	3.6	4.4	5.1	5.3	6.0	6.7	6.6	6.9	6.9
23	5.8	2.7	2.8	3.1	4.1	4.7	5.2	5.5	6.3	6.8	6.9	7.3	7.3	7.3
24	6.0	3.1	3.0	3.4	4.2	4.4	5.0	5.7	6.7	6.8	7.1	7.7	7.6	7.6
25	6.5	3.0	2.9	3.4	3.6	5.1	5.7	6.0	6.6	6.7	6.9	7.4	7.2	7.2

Table 6. Organic acid production on 3rd day of incubation

Runs.	***ORGANIC ACIDS (mg/L)***						
	Oxalic	***Citric***	***Malic***	***Gluconic***	***Acetic***	***Succinic***	***pH***
1	12.01±0.05	5.35±0.01	102.03±0.03	45.01±0.02	11.02±0.01	20.01±0.03	3.1
2	14.11±0.06	11.21±0.03	28.31±0.05	23.40±0.04	32.23±0.1	9.13±0.04	3.1
3	25.02±0.2	2.01±0.03	54.52±0.06	31.31±0.01	45.21±0.02	41.12±0.01	3.0
4	44.23±0.06	5.61±0.04	67.69±0.04	50.35±0.04	51.31±0.01	26.05±0.03	2.9
5	58.31±0.01	21.21±0.01	88.01±0.12	61.15±0.05	16.41±0.01	35.80±0.03	2.5
6	17.02±0.02	3.57±0.04	91.59±0.11	13.31±0.01	62.25±0.03	33.07±0.03	3.0
7	37.14±0.02	6.82±0.02	101.32±0.14	41.35±0.01	36.62±0.01	41.03±0.04	2.8
8	29.22±0.02	1.02±0.06	26.55±0.01	36.51±0.01	58.62±0.02	25.33±0.01	3.5
9	51.57±0.05	3.17±0.04	38.68±0.03	44.26±0.02	71.14±0.05	38.98±0.01	3.0
10	37.01±0.07	5.12±0.02	67.49±0.03	53.61±0.01	50.92±0.01	19.17±0.01	2.9
11	29.05±0.01	31.61±0.01	58.50±0.03	27.25±0.03	37.38±0.01	37.22±0.03	3.0
12	42.18±0.06	13.40±0.02	77.14±0.05	68.80±0.05	44.69±0.02	44.15±0.03	2.8
13	38.51±0.08	21.65±0.01	59.33±0.01	81.13±0.05	34.46±0.02	23.52±0.01	3.0
14	49.00±0.03	9.78±0.04	82.14±0.04	92.21±0.06	21.81±0.02	25.46±0.01	2.6
15	44.03±0.03	2.90±0.05	88.13±0.05	26.43±0.02	15.10±0.01	61.34±0.01	2.8
16	41.15±0.06	5.01±0.04	79.30±0.05	61.61±0.01	26.57±0.01	27.30±0.01	3.0
17	38.11±0.05	3.04±0.02	104.45±0.06	27.40±0.02	38.62±0.01	17.51±0.02	2.9
18	26.21±0.05	7.01±0.01	89.12±0.06	82.18±0.05	45.24±0.02	36.01±0.02	2.9
19	21.01±0.04	10.44±0.05	67.09±0.04	37.89±0.02	61.55±0.05	51.22±0.03	3.2
20	16.08±0.08	9.41±0.06	99.01±0.05	77.71±0.06	24.31±0.01	38.01±0.02	3.1
21	20.15±0.01	3.60±0.07	93.70±0.11	58.35±0.04	15.25±0.01	33.80±0.03	3.0
22	42.58±0.03	4.15±0.01	54.68±0.03	38.57±0.01	32.55±0.02	42.86±0.03	2.8
23	47.31±0.01	8.27±0.05	64.46±0.05	29.22±0.01	17.62±0.01	51.34±0.01	2.8
24	31.03±0.1	4.52±0.01	77.13±0.06	41.15±0.03	27.34±0.02	25.15±0.01	3.0
25	39.51±0.01	6.61±0.04	85.25±0.04	55.34±0.04	33.12±0.02	55.38±0.01	2.9
Experimental	23.01±0.04	8.15±0.02	73.01±0.05	43.19±0.02	53.17±0.03	47.62±0.03	3.1

Thus, maximum dissolution of minerals occurred (Fig. 3). Subsequently, the metals dissolution is influenced by pulp density that affects the kinetics of leaching reactions (Ahonen and Tuovinen, 1995). By increasing the pulp density, contact area increase between fungi, liquid phase and substrate. In the present research, optimum pulp density was detected in a percentage of 10% as shown in Fig. 4 and a yield optimum dissolved minerals i.e. Al 46.58, Mg 42.88, Cu 46.02, Mn 42.36 and Ni 31.54%.

However, a further increase in pulp density adversely affected the activity of the microbial cells thus, ultimately decline in metals dissolution.

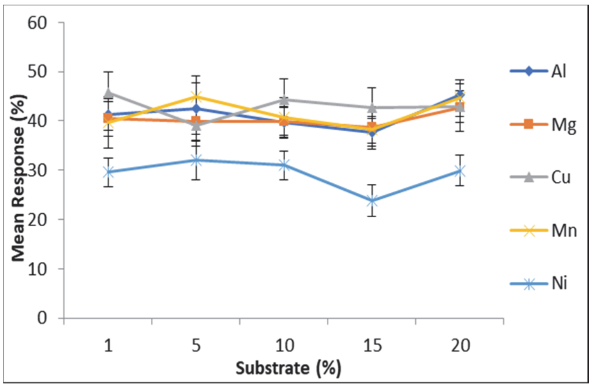

Fig. 3. Effects of substrate on mean responses for metal ions dissolution

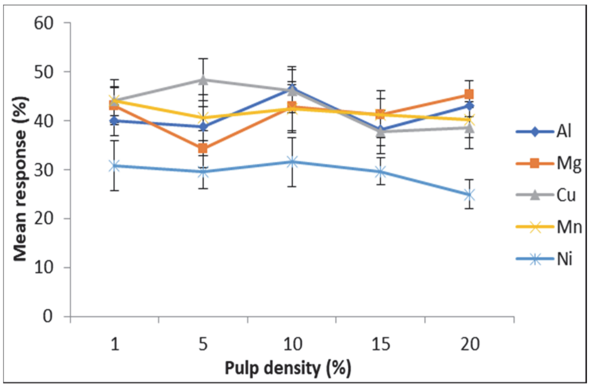

Fig. 4. Effects of pulp density mean responses for metal ions dissolution

As fine ore particles penetrate into microbial cells and stuck their growth activities, thereby resulting in the failure to oxidize ore particles. In addition to insufficient stirring, high pulp density is the major factor for the failure of industrial operation. Frequently, these factors decrease the efficiency of bioleaching at high pulp density (Xinhui et al., 2013). High pulp density cause reagent hunger, as well as resistance in liquid and mass transfer whereas smaller pulp densities that results in low metal ions solubilization, are accredited to low accessibility of substrate.

Like biochemical and chemical processes, temperature is another important environmental factor affecting the leaching operations. Metal ions leaching were smooth at a temperature of 45°C for a maximum microbial growth, above and below this temperature, oxidation reaction was stopped. Thus, the optimum temperature was 45°C as shown in Fig. 5 (Ahonen and Tuovinen, 1990).

Bioleaching kinetics is also affected by agitation. Proper aeration of growth medium, homogeneity in solid suspension (substrate and ore density), heat and mass transfer, pH and temperature uniformity are dependent on agitation with optimum velocity. Low agitation velocity leads to less aeration of medium and mass transfer effect which ultimately resulted in decreased metal ions dissolution. Hence, maximum metal ions dissolution was found at an agitation velocity of 200 rpm as depicted in Fig. 6.

Microbial growth adversely affected by agitation velocity higher than 200rpm. At higher agitation speed microbial cells collide with the metal ions present in the brown shale then wear and tear process resulted in the breakdown of the microbial cells. Thus, ultimately resulted in the decreased levels of metal ions dissolution (Witne and Phillips, 2001; Liu et al., 2008). So, the maximum metal ions dissolution was found to be at optimum pH 6.0, substrate 5.0, pulp density 10.0, temperature 45^{o}C, and agitation speed of 200 rpm.

In order to find the significance of process parameters, statistical analysis of variance (ANOVA) was performed. A significant effect of each parameter on leaching efficiency was determined by F-value. Taken together, these results show that the mean response for metal ions extraction has been significantly affected by the temperature, initial pH, substrate and ore density.

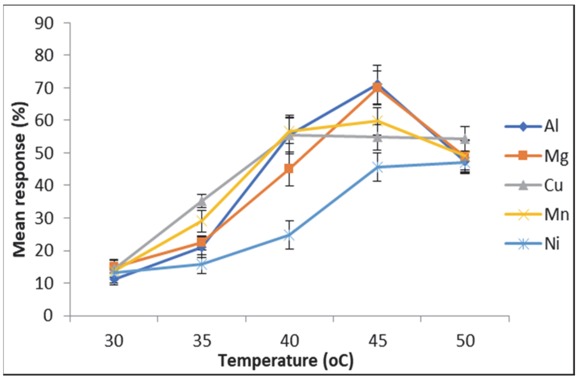

Fig. 5. Effects of temperature on mean responses for metal ions dissolution

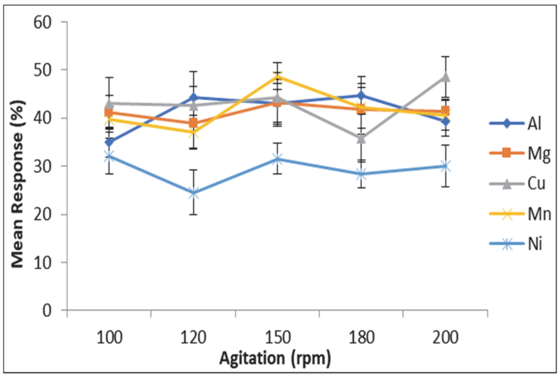

Fig. 6. Effects of Agitation on mean responses for metal ions dissolution

These factors attain F-value greater than the value given in F-distribution table at 95.0% confidence interval. All these factors have significant effects on responses for metal ions recovery. It validates that the variance of all these factors is significant as compared with the variance of error. Finally, the results and other outcomes were estimated for all combination of significant effects modelled by Taguchi method. Then, confirmation of these estimated values under optimized conditions was done by experimental procedure of bioleaching. A control experiment was also run under standard conditions.

In the current research, bio recovery of metal ions was done in a medium having initial a pH of 6.0. Then pH was noted after every 24 hours to ensure the growth as well as the biorecovery of the metal ions in the medium. At the third day, when pH was dropped to its minimum of 3.1, the sample was withdrawn to be analyzed by HPLC for the confirmation of microbial metabolites. Afterward, pH of the media fluctuated due to consumption of microbial metabolites by metal ions complex formation. pH increased day by day up to the fourteenth day whereas in the case of control samples, with no microbes, pH was increase from 6.0-7.5 as shown in Fig. 7.

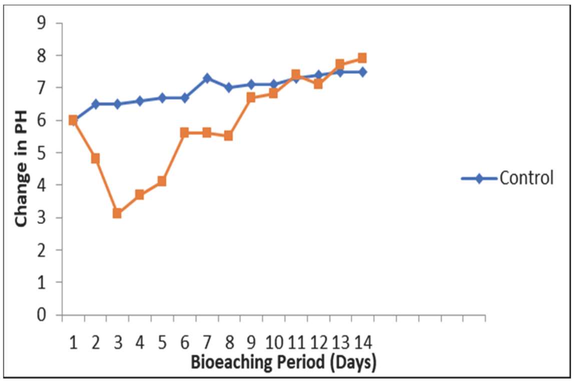

Fig. 7. pH changes during metals bio recovery studies. All values are means of duplicates with an error of ± 0.05

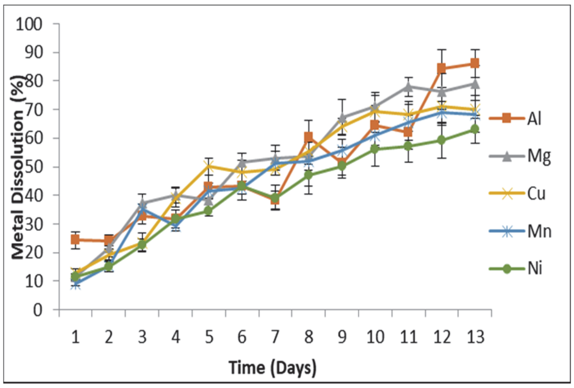

Fig. 8. Percent metal ions solubilization under optimized conditions of bio recovery

In control flask, an increase in pH is the indication of non-organic acid metabolites formation due to absence of microbes, whereas in case of experimental flask, a decrease in pH up to 3.7 indicates the maximum production of organic and inorganic acid metabolites due to healthy growth of microbes under optimal conditions. Subsequently, pH increased linearly due to consumption of metabolites for metals solubilization. During this whole period, recovery of metal ions was about 86.11% of Al, 79.25% (Mg), 70.14% (Cu), 68.31% (Mn) and 63.02% (Ni) (Fig. 8) whereas in control flask, no significant recovery of metals ions was noted (Al 11.14%, Mg 10.12, Cu 8.01, Mn 7.14% and Ni 11.36%).

3.3. Mass balances

A mass balance was determined for aluminum, magnesium, copper manganese and nickel from residual solids as well as microbial cells. The amount of these elements was then added to maximum amount of respective metals that were dissolved in supernatants at the end of leaching period. The resultant was then compared with initial amount of aluminum, magnesium, copper, manganese and nickel present in untreated brown shale.

On average, some of aluminium (5.08%), magnesium (7.39%), copper (10.04%), manganese (5.91%) and nickel (10.59%) have not been reported. These values of metal ions might be mislaid during the different preparatory steps of bio recovery. Filtration might be an important step that causes loss of analytes as a result of adsorption of metal ions on the surface of filter paper, etc.

4. Conclusions

Optimization of process parameters for metal ions bio-extraction from brown shale was done according to orthogonal array design by choosing L25 and the process parameters (pH, temperature, substrate, ore density and agitation) were evaluated on the responses for metal ions dissolution. The optimum conditions at which maximum metal dissolved, was chosen. Therefore, for maximum biorecovery of metal ions, the values recommended of proposed parameters were pH 6.0, pulp density 5%, substrate 10%, temperature 45°C and agitation 200 rpm.

Thus, 86.11% of Al, 79.25% (Mg), 70.14% (Cu), 68.31% (Mn) and 63.02% (Ni) metal ions were leached out under optimized conditions.

Taken together, our optimized conditions for biorecovery will enable us to implement the process on an industrial scale. Furthermore, percent yield of metal ions recovery from low grade ores will be maximum, thus it will be cost effective.

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