IJBPAS, December, Special Issue, 2021, 10(12): 386-395
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'
www.ijbpas.com

EFFECT OF SUBSTRATE CONCENTRATIONS ON EXTRACTION OF METALS FROM FLY ASH BY *THIOBACILLUS FERROOXIDANS* AND *PSEUDOMONAS FLUORESCENCE*

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Received 19th Aug. 2021; Revised 20th Sept. 2021; Accepted 29th Oct. 2021; Available online 1st Dec. 2021

https://doi.org/10.31032/IJBPAS/2021/10.12.2036

INTRODUCTION-

Most of the microorganisms interact with metals. This property of microbes can be explored for metal extraction. The two important aspects, the economic and industrial are of keen interest. First includes leaching of the metals from their ores and second involves leaching from the industrial wastes. In the first case, the metal is extracted from large quantities of low grade ores and in second reaction; metals are recycled and concentrated together. The later provides the tool for the removal of toxic and heavy metals; hence it helps in bioremediation of the polluted environment.

Fly ash is the incombustible matter which is left after all the organic components of the coal have been consumed during the process of coal combustion and is collected by means of mechanical electrostatic precipitators. Fly ash is composed of mainly silt-sized spherical amorphous ferro-aluminosilicate minerals and is generally characterized as having low permeability, low bulk density as well as high specific surface area. The ash particle generally ranges in size from 0.5 to 200 µ. Fly ash particles are mainly composed of silicon (SiO_2), aluminum (Al_2O_3), iron (Fe_2O_3), titanium (TiO_2), and manganese (Mn_2O_3) (Scheetz and Earle, 1998). Fly ash

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The mechanism of bioleaching of coal fly ash (CFA) by *Thiobacillus thiooxidans* was studied by Brombacher *et al*. (1998). The study of interactions between bacteria, metabolic products, CFA particles and leaching products were studied. It was demonstrated that bacterial growth and the amount of metals leached from the CFA were compared with biological and chemical interaction, which involve various components in this system. Kinetic model of CuS oxidation by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* bacteria was proposed by Domka (2001). The CuS oxidation with *Thiobacillus ferrooxidans* bacteria was found to be the best described by the model of inhibition of the first order with respect to the substrate and product inhibitor, while the process with *Thiobacillus thiooxidans* and mixed cultures of these bacteria was best described by first order reaction with respect to the substrate.

The term, metal is applied to the substance that has a silvery luster and is a good conductor of electricity as well as heat. Some metals, like alkali metals are relatively soft, malleable and ductile. Alkaline earth metals and metalloids are the other two categories proposed by chemists. There are various methods of metal extraction from their mineral ore. Bioextraction of metal is an important process as it minimized the production cost of the metals.

MATERIALS AND METHODS

Sample collection and Physico – chemical analysis of fly ash:

The fly ash used in the experiment was collected from Parli Thermal Power station premises at moist places away from dumping sites. The 1-2 kg fly ash was collected in air tight polyethylene bags sterilized with alcohol. The collected ash was washed with water to remove water soluble compounds and dried on hot plate. The collected fly ash

was subjected to analyze the microbiological (microflora) and Physico-chemical characters such as colour of the ash, pH, Redox potential, conductivity, salinity and mineralogical characterization were analyzed as per the method of Black *et al*. (1965). The physical properties of the fly ash bulk density, maximum water holding capacity, porosity were analyzed as per the methods of soil analysis.

SEM and EDX analysis of fly ash:

The morphological changes in the surfaces of individual minerals were investigated by SEM (scanning electron microscopy) and the changes of chemical composition and element present on surface of the fly ash sample by Energy Dispersive X-ray (EDAX) analysis. Mineral samples were coated with platinum and subsequently examined in a scanning electron microscope Jeole 3500.

Chemical analysis of fly ash:

To assess the metals, present in coal fly ash according to method, developed by Francis *et al*. (1999) was used. The collected ash was washed with water to remove water soluble compounds and dried in oven at 100 ^{0}C temperature. The 2 g of oven dried fly ash was mixed with 6 ml of nitric acid (HNO_3) and kept as it is for 24 hours. After 24 hours sample was filtered. Filtrate was used for spectrophotometric analysis of aluminum iron and manganese after appropriate dilution.

Isolation of *Thiobacillus ferrooxidans:*

For isolation of bioleaching microorganisms, ore was collected from Radhanagari bauxite mine at various depths. Collected samples were transferred in the pre sterilized polyethylene bags and stored at 4 ^{0}C in refrigerator until use. From the collected soil samples 1 g of bauxite ore was ground to fine powder and mixed in 100 ml of 9K medium [composition g/ l ammonium sulphate $(NH_4)_2SO_4$ – 3.0, magnesium sulphate $MgSO_47H_2O$ -0.5, potassium hydrogen phosphate K_2HPO_4 - 0.5, potassium chloride KCl- 0.1, calcium nitrate Ca $(NO_3)_2$ -0.01, ferrous sulphate $FeSO_4.7H_2O$- 21.00] (Silverman and Lundgren, 1959). The pH of the medium was adjusted to 2.0 with 10N H_2SO_4. The culture in 9k medium was incubated for 1 to 4 weeks until growth was observed microscopically or until a chemical change occurred in the medium compared with an un-inoculated control.

The inoculated flasks were incubated at 32 ± 2 ^{0}C for 1 to 3 weeks at 140 rpm constant shaking condition. The presence of iron oxidizing bacteria in the liquid 9K medium was indicated by the formation of a characteristics ferric precipitation and orange coloring of medium. A serial dilution of

culture was spreaded on 9K solid medium. The inoculated plates were incubated at 32 ± 2 ^{0}C for 10 to 20 days residence time. To observe the colony size, shape, colour and other morphological features. Single colony was picked from the plate by using a sterile inoculation loop and streaked on newly made plates.

Isolation of *Pseudomonas fluorescens:*

To isolate efficient indigenous bioleaching microbes from bauxite ore, sample was collected and stored in refrigerator at 4 ^{0}C. To isolate dominant heterotrophic microbes LB broth was used. To isolate the indigenous microbes 2 g of bauxite ore was inoculated with 40 ml of Luria Bertani (1951) broth in aseptic conditions. Inoculated flasks were incubated at 32 ± 2 ^{0}C for 4 to 6 days at 140 rpm constant shaking conditions. The development of turbidity in the medium was assumed to be due to microbial growth. Serial diluted turbid sample was streaked on solid LB agar medium. [Composition g/l trypton -10.0, yeast extract – 5.0, NaCl-5.0, Agar -15] and incubated for 48 hours. After 48 hours, grown dominant colonies were selected for further purification and biochemical identification.

RESULTS AND DISCUSSION

The analysis of Fly was done through SEM, XRD and EDS which shows that (Fig. 1, 3 and 4). The brown colonies were obtained as a result of autotrophic growth of *Thiobacillus ferrooxidans* on 9K medium while white coloured colonies were obtained as organotrophic growth of *Thiobacillus ferrooxidans* (Fig. 2).

Effect of substrate concentration extraction of metals by *Thiobacillus ferrooxidans*:

It was noted that 5 g of the substrate (pulp) concentration gave more extraction when compared with other Fly ash concentrations. In general, 6 g Fly ash concentration was found to be better for the aluminum extraction. Iron extraction also ranged from 39.43 to 104.65 mg at 1 g of the Fly ash. Iron extraction ranged from 171 (least) to 286 mg at 10 g and 4 g Fly ash respectively. When both the aluminum and iron extraction efficiency was calculated, it was noted that it ranged from 45.74 to 81.60 at 1.0 g to 4.0 g substrate concentration respectively (Table 1 and Graph 2).

Effect of substrate concentration extraction of metals by *Pseudomonas fluorescens*:

Results in the Table 2 and Graph 2 show that aluminum extraction was higher at 4 g bauxite ore concentration. Other pulp density also gave aluminum extraction from 25.32 to 38.46 at 12 and 2 g bauxite ore

concentrations respectively. Iron extraction was also found variable at different pulp densities. The maximum iron extraction was found at 2 g and 4 g. In other cases it was found to be 46.23 percent to 67.27 percent for 12 g and 6 g respectively. Bioextraction efficiency of aluminum and iron also shows that 2 g and 4 g were most favourable for extraction of metals from bauxite ore.

Bioextraction of aluminum, manganese and iron from fly ash was studied at different temperature. It was found that there was increase in aluminum, manganese and iron with the increase of temperature up to 38 ^{0}C whereas in case of *Pseudomonas fluorescens* metal extraction was increased up to 34 ^{0}C. Many authors' investigation shows that the efficiency of bioleaching is depend on temperature (Chen and Lin, 2000) with its optimal, at 37 ^{0}C, however, the optimum incubation temperature ranging from 30 to 37 ^{0}C suitable for metal leaching from the industrial wastes, considering the increasing cost with increase in temperature (Gomez *et al*., 2000; Lee *et al*., 2001).

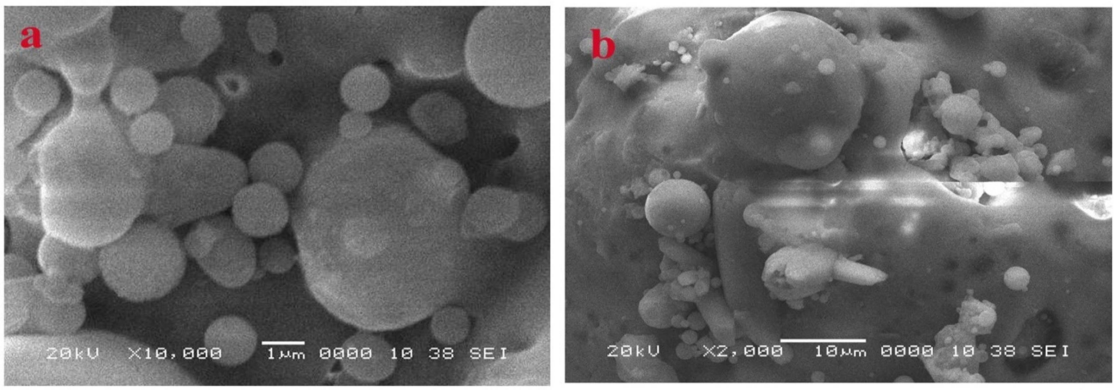
Increasing temperature in the range of 20 to 35 ^{0}C was found to be enhanced biological oxidation rate of ferrous ions by *Thiobacillus ferrooxidans* (Nemati and Webb, 1997). Deng, (2002) also studied the temperature effect in the range of 20 to 45 ^{0}C. It was observed that the biooxidation range of iron and arsenic using *Thiobacillus ferrooxidans* was highest in the range of 28 to 32 ^{0}C which leads to production of ferric iron as well as H_2SO_4; hence dissolution of metal is increased.

Table 1: Effect of substrate concentrations on extraction of metals from fly ash by *Thiobacillus ferrooxidans*

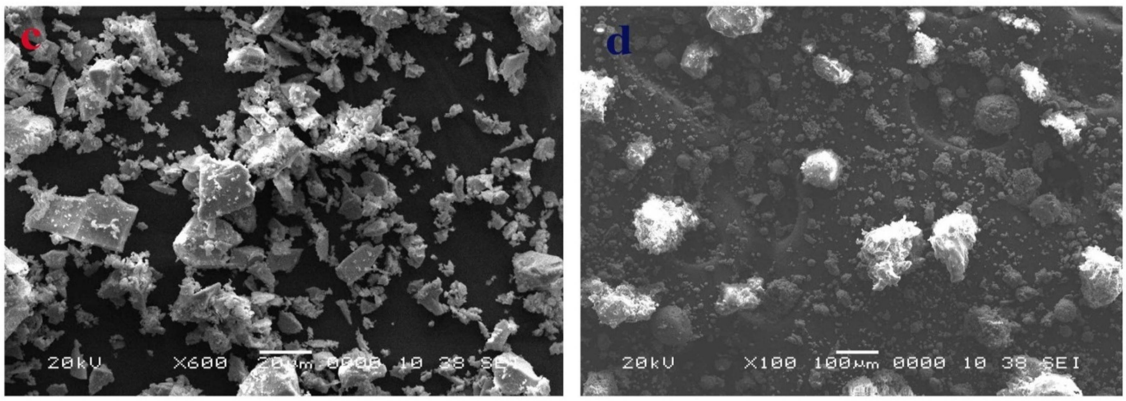
Initial wt. of fly ash in (g)	Metal solubilization in media (mg/g)			Bioextraction of metals (%)			Bioextraction Efficiency (%)
	Al	Fe	Mn	Al	Fe	Mg	
2	86.23±1.33	39.42±0.32	6.78±2.4	71.66	81.25	77.56	74.54
4	124.20±1.6	74.86±1.86	8.5±1.32	50.4	78.72	60.71	58.33
6	164.0±0.60	104.65±2.4	16.88±1.6	45.05	72.22	66.68	53.38
8	114.22±2.0	68.96±1.45	18.44±1.8	34.16	34.69	56.25	28.24
10	104.45±2.2	44.56±1.44	10.22±1.4	17.21	18.33	23.8	17.83
12	96.00±1.46	42.22±2.45	8.46±2.46	13.25	14.68	14.81	13.72
Cont-	4.2±1.34	2.34±0.45	0.66±1.23	2.39	4.83	6.74	4.071
C.D. (p=0.05)	28.84	26.70	5.12				

Table: 02 Effect of substrate concentrations on extraction of metals from fly ash by *Pseudomonas fluorescens*

Initial wt. of fly ash (g)	Metal solubilization in media (mg/g)			Bioextraction of metals (%)			Bioextraction Efficiency (%)
	Al	Fe	Mn	Al	Fe	Mg	
2	26.18±1.22	41.23±0.86	1.22±0.44	11.07	85.89	15.25	38.99
4	36.48±2.43	46.20±1.84	2.46±0.84	14.82	49.14	17.50	24.04
6	40.30±2.45	52.00±1.23	2.64±0.22	21.81	36.11	11.00	17.84
8	32.22±1.45	50.24±1.66	1.26±1.42	6.71	25.63	3.93	11.82
10	26.40±1.68	42.22±2.45	0.86±1.34	4.37	17.59	2.04	7.84
12	20.80±1.26	36.10±2.40	0.76±1.88	1.95	12.62	1.40	5.41
Con	4.26±1.45	4.89±1.56	0.86±1.68	1.73	5.14	6.14	2.82
C.D. (p=0.05)	7.63	6.27	0.85				

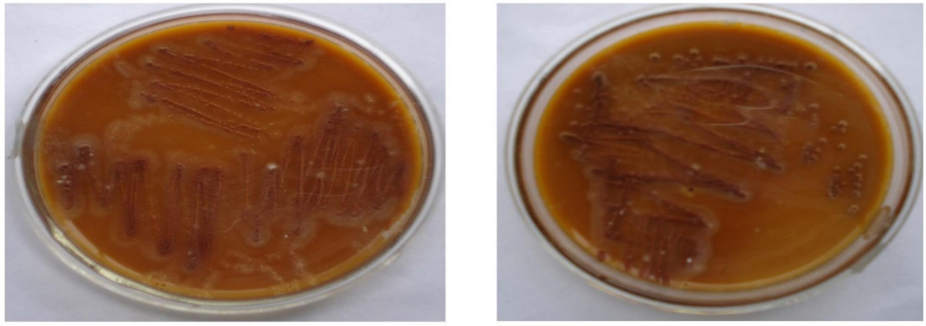


SEM images of Fly ash: a) 1µm. b) 10



SEM images of Fly ash: c) 20µm. d) 100µm.

Fig 1. SEM analysis of Fly ash



a) *Thiobacillus ferrooxidans* autotrophic growth on 9K solid medium

b) *Thiobacillus ferrooxidans* chemoorganotrophic growth on solid glucose medium

Fig. 2. Autotrophic and organotrophic growth of *Thiobacillus ferrooxidans* on 9K and solid glucose medium

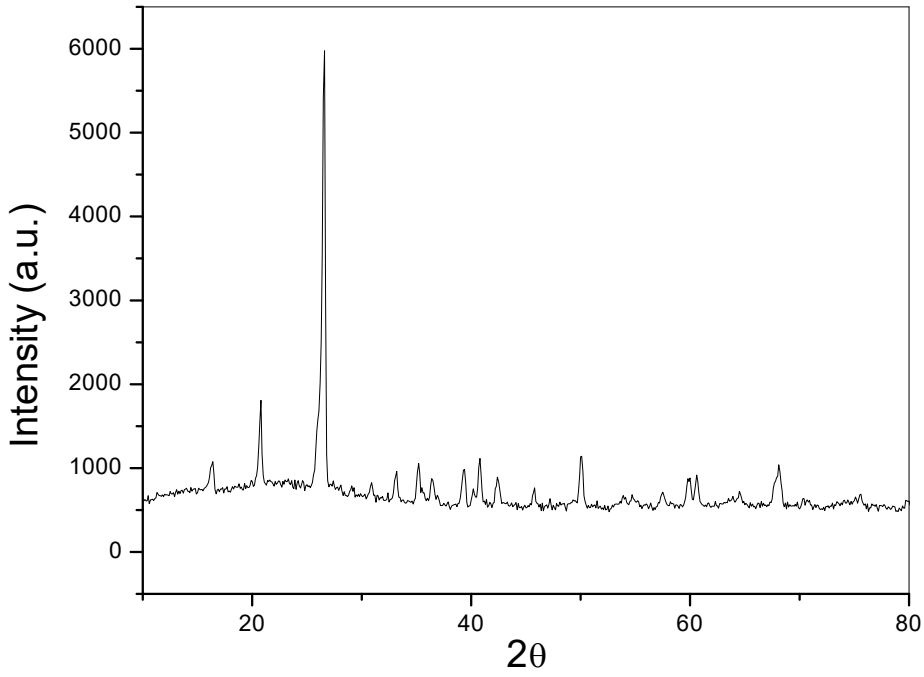


Fig. 3: XRD pattern of Fly ash

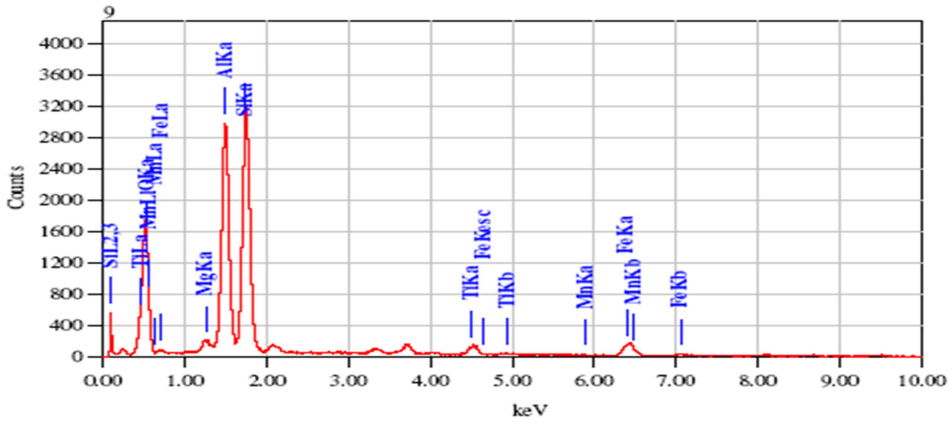
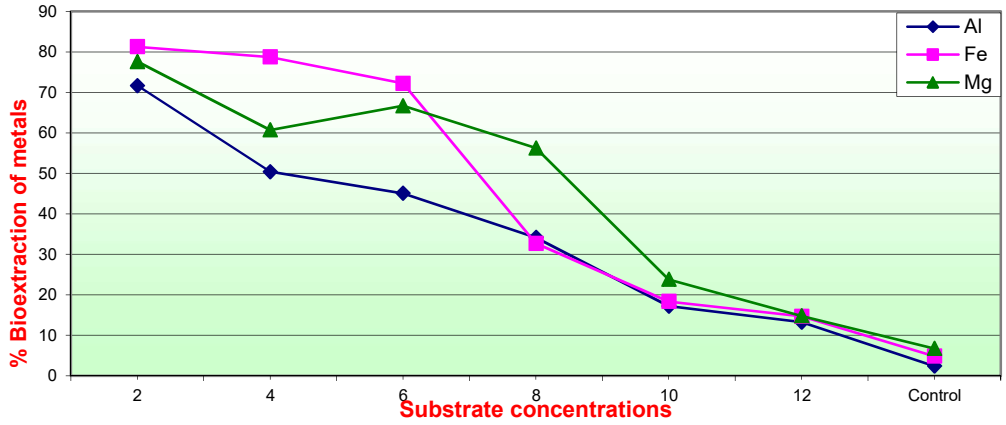
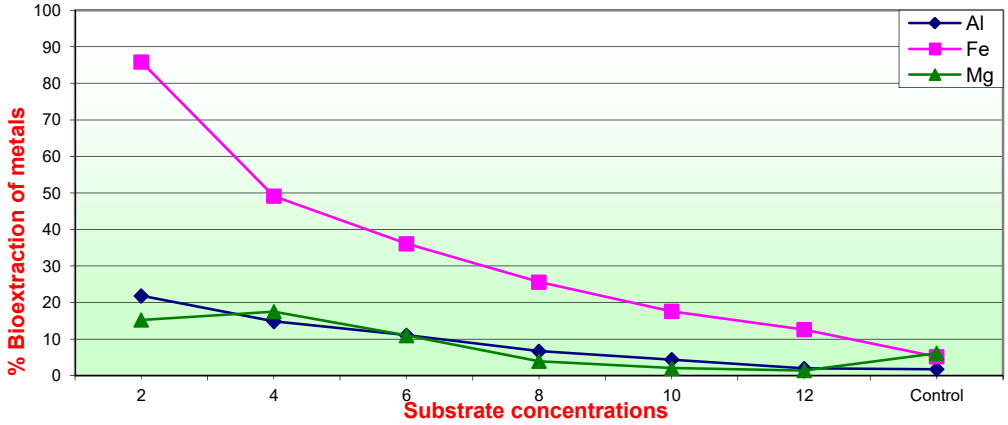


Fig. 4: EDS spectrum of Fly ash



Graph 1: Effect of substrate concentrations on extraction of metals from fly ash by *Thiobacillus ferrooxidans*



Graph 2: Effect of substrate concentrations on extraction of metals from fly ash by *Pseudomonas fluorescens*

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