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Bioleaching of Alumina from low grade Indian bauxite (41% Al_2O_3) by indigenous bacteria with reference to pH, Time and Carbon source

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Abstract

The main objective of this work is to upgrade lean ore of Bauxite. Upgradation of low grade ore by bioleaching process with microorganisms is a new area which offers economic benefits in commercial production. The present work includes studying the indigenous bacteria isolated from the ore which has capability to leach out Aluminum from the same ore sample. The work has also aim to characterize the indigenous microorganism responsible for removal of Alumina *and to find out the optimum condition for the process.*

Keywords: Low grade Bauxite, Aluminum, pH, OD, AAS, isolation, characterization.

Introduction

The process of metal extraction from low grade ores by using the microorganisims is referred as Bioleaching. It is very simple and effective technique use microorganisms like mesophiles, thermophilic and extermophiles. The bacteria are mainly *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *sulfobacillus* and *Sulfolobus* which have potential to remove the valuable metal like copper, Zinc, Nickel and Cobalt. These bacteria tolerate acids and metabolize sulfur[1]. Silicate bacteria are also able to remove silicon from the ore[1]. The process involves extraction of metals from low grade ores by using microorganisms has already been successfully performed for obtaining copper, gold and uranium. It is observed that bioleaching process fulfilled about 20% of world's copper demand. Similarly the same process has also been used to extract uranium from the Elliott Lake in Canada. Now day's bioleaching techniques have more demand globally because of constant requirement of metals in day to day life[2]. The factors like lack of skill labor, techniques and various risks occurs in mining like smelting crushing are the cause of low production. So a novel idea for leaching has come to play an important role to fulfill the metal demands of the world. The process of Bioleaching does not require lots of energy but it is slow process. in this technique High temperature roasting and smelting is not required, so there are decided benefits in addition to the fact that bioleaching can get metals from low grade ores[3].

Bauxite: Bauxite is an ore which is used for extraction of Aluminum by Bayer's process. It is the main source of Aluminum. The ore is first chemically processed to produce aluminum oxide (alumina); then it is smelted to produce the pure aluminum metal. In this process only high grade ore are used for production of aluminum. The ore is a heterogeneous material which contains gibbsite and diaspora[4]. It is a valuable mineral commonly used for extraction of Aluminum, manufacture of refractories, chemical and ceramics. Aluminum is an abundant metallic chemical element widely used throughout the world for variety of product. Aluminum is widely used in five major industries like transportation, construction, electrical, container packing and mechanical equipments[5].

Microorganisms used: In Bioleaching process both autotrophic and heterotrophic bacteria and some fungal species are used for different ores[6]. The bacterial species like acidophiles are specifically used in refectory gold ore leaching for removal of pyrite matrix. Whereas the bacteria species of *Thiobacillus* are used for bioleaching of metals from sulphidic minerals. The *Thiobacillus* species are aerobic and acidophilic autotrophs[7]. These bacteria derive their energy requirement from oxidation of iron and sulfur compound. Fe^{2+} and H_2SO_4 bring about metal solubilisation which is produced in the system[8]. The Thiobacillus species have potentials to oxidize Fe^{2}+ and S that determine the efficiency of Bioleaching. The microorganisms has potential to leach and mobilize metals from solid materials comprises of three principles namely: i. Redox reactions, ii. Formation of organic or inorganic acids, iii. Excretion of complexing agents.

Both autotrophic and heterotrophic microorganisims are tested for metal recovery by species of *Pseudomonas, Thiobacillus,* Bacillus*, Sulpholobus, Leptospirillum, Acidophillum, Aspergillus, etc.* Specifically, a consortium of microorganisms namely*, Acidithiobacillus ferrooxidans, Acidithiobacillus*

thiooxidans, Leptospirillum ferrooxidans, Sulpholobus spp. and *thermophilic* bacteria including *Sulpholobus hermosulphidoxidans and Sulpholobus brierleyi* are known to be involved in bioleaching. Anaerobes would also be found in leaching areas. The same experiment has been conducted by taking fungus species *Aspergillus niger* and *Penicillium* spp, for nickel recovery from chromit[9,10].

Heap Leaching: Bio-heap leaching is an emerging technology with significant potentials to add value to the mining industries so as to deliver attractive environmental and social benefits to all the associates[11]. The modest nutritional requirements of bioleaching organisms may be provided with the aeration of iron and/or Sulphur containing mineral suspensions in water or the irrigation of a heap, while working in a large scale. The emphasis is given on the factors affecting heap bioleaching. The cost of bioheap leaching in respect of some existing commercially operating heap bioleaching plants is also included. Application of chalcopyrite bioleaching in heap/dump leach processes can potentially Result in lower cost and reduced environment impact in Copper production.

In-situ leaching: In This process leaching occurs in the natural condition of ore where the microorganism in aqueous solution is pumped through a hole within the ore[12]. Then lechate liquid is collected for extraction of metal at the bottom of ore. In this process the ore remains in its original position in earth. Surface blasting of earth is done to increase the permeability of water. Water containing thiobacillus is pumped through drilled passage to the ores. Acidic water seeps through the rock and collect at bottom.

Slope leaching: In this process the ore first finely grounded and kept in large pile in slope then it is subjected to continuous sprinkling of aqueous solution of microorganisim. The lechate liquid is collected for metal extraction at the bottom of ore. Here the ores are first ground to get fine pieces and then dumped into large leaching dump. Water contain inoculums of *Thiobacillus* is continuously sprinkled over the ore. Water is collected from the bottom and used to extract metals and generate bacteria in an oxidation pond.

Future aspects on bioleaching: In our days, at the beginning of the XXI century, bioleaching occupies an increasingly important place among the available mining technologies. The situation is now quite different than was 25 years ago, when the first international meeting on the subject took place in Socorro, New Mexico[8], initiating the now traditional International Bio hydrometallurgy Symposia. Today bioleaching is no longer a promising technology but an actual economical alternative for treating specific mineral ores. Examination of the current large-scale bioleaching operations reveals that an important number of them are located in developing countries. This is not purely accidental but the necessary result of two important factors: for one thing, many developing countries have significant mineral reserves and mining constitutes one of their main sources of income; on the other hand, bioleaching is a technique especially suitable for developing countries because of its simplicity and low capital cost requirement. This is the case for countries like Chile, Indonesia, Mexico, Peru and Zambia. The current panorama of bioleaching in developing countries is encouraging. It is expected that in the coming years several new commercial-size the bioleaching plants will be installed. The work will open up a new era in beneficiation of bauxite through biotechnological route. Up gradation of low grade ore by bioleaching process with microorganisms is a new area and studied as more cheaply than commercial production[13].

Environmental Consequences: The potential benefits of bioleaching in context with environmental consequences are as follows: i. Bioleaching process is very simple, inexpensive technology and employed for collecting the metals from waste and drainage. ii. Bioleaching is a relatively simple technology in terms of its equipment requirements and conditions of operation at ambient pressure and non-excessive temperatures close to ambient. iii. The process of extraction of metal using mechanical and chemical procedure is difficult and expensive where as the biological procedure are more cost-effective, use little energy and can work even if low concentration of metal. iv. Usually the bioleaching process does not produce harmful emission and it reduces the pollution of metal containing waste. v. Bioleaching process applied for both small and large projects for concentrate treatment. vi. It can accept a variety of feed types over a period of time and is a more forgiving process due to the longer residence time compared to other treatment methods.

Aims and objective: The main objective of this study is to upgrade lean ore of Bauxite. Upgradation of low grade ore by bioleaching process with microorganisms is a new area and offers economic benefits in commercial production. Present study includes various media required for the growth of microorganism isolated from bauxite samples analyzing 41% of alumina followed by isolation and characterization of the unknown microorganisms. PH and optical density was measured at definite interval of time and chemical analysis of the bioleached bauxite has been conducted by Atomic Absorption Spectrophotometer to assess the extent of up gradation of the bauxite samples. XRD and gravimetric analysis have been done to assess the purity of the produced alumina by combination of Heap leaching and solvent extraction process. The present piece of work has following objectives: i. To study the potential of isolated indigenous bacteria to leach out Aluminum from bauxite ore. ii. Performance study of indigenous bacteria in terms of Aluminum removal. iii. To study the Optimum condition for maximum Aluminum removal. iv. Characterization of the isolated indigenous bacteria.

Materials and methods

Materials: The bauxite ore was obtained from NALCO-region of Orissa; these bauxite ore contain 41% of alumina for the leaching experiment as shown in Table-1.

Table-1: Mineralogical and Chemical Composition of Bauxite ore containing 41% of alumina.

Chemical ingredients	Percentage (%)
Al_2O_3	41%
SiO_2	2.4%
Fe_2O_3	24.7%
TiO_2	2.6%
CaO	-----
MgO	-----

Particle Size analysis of Bauxite Ore: The bulk ore samples were grounded and sieved to -240 BSS (British standard size) size sieve particle and pan particle size are taken for bioleaching experiment.

Isolation of Microorganisms: In natural habitats micro organisms usually grow in complex mixed populations containing several species. This presents a problem for microbial because a single type of micro organism cannot be studied adequately in a mixed culture. One need a pure culture, a population of cells arising from a single cell, to characterized an individual species. The collected ore is diluted several times to reduce the microbial population sufficiently to separate colonies when plating. Then small volumes of several diluted samples contain around 30 to 300 cells is transfer to the center of an agar plate and spread evenly over the surface with a sterile bent glass rod on agar surface to that every cell grows into a completely separate colony, a macroscopically visible growth or cluster of micro organism on a solid medium, each colony represent a pure culture for the leaching experiment.

Microbial growth media: The microorganisms once isolated in pure form were further used for leaching experiment; sub cultured on microbial growth media. a microbial growth medium is a solid or liquid preparation used to grow, transport and store micro organisms. The media composition for growth kinetics studies of isolated micro organisms taken is Nutrient Agar Media with various concentrations of the Carbon- sources as shown in Table-2.

Methodology: The above mentioned medium ingredients were collected, weighed the components in an exact composition using electronic or beam balances. 100ml distilled water was added and swirled to dissolve the peptone and beef extract in a conical flask (250ml). pH was adjusted to 7.0 with the help of pH meter. Agar was added for solidification. After the preparation of medium it was then autoclaved at 15lb pressure for 15min. Experiment was carried out in 250ml conical flasks containing 100ml of metabolite having bauxite pulp density of 2% i.e. 2g of ore in 100ml of Nutrient medium. The initial pH of the metabolite was measured in contrast to Chemical sterile control flask were incubated on shaker at 100 rpm. In the time course, samples were removed at intervals and centrifuged to remove solid suspension. Supernatants were analyzed for monitoring the pH and dissolve element. The leached suspensions were centrifuged after filtration and Metal concentration in the filtrate was analyzed after suitable dilution by using Perkin Elmer atomic absorption spectrophotometer.

Table-2: Composition of Nutrient agar media varying in C source.

Composition	Nutrient Agar Media (For microbial growth)		
	Media-I	Media-II	Media-III
Peptone	0.5 g	0.5 g	0.5 g
Beef Extract	0.3 g	0.6 g	0.9 g
NaCl	0.5 g	0.5 g	0.5 g
Distilled water	100ml	100ml	100ml
Agar	1.5 g	1.5 g	1.5 g

Microscopic Examination: Gram Staining for the Bacteria: A clean slide was taken with a drop of sterilized distilled water on the middle of it. Then, a loop full of bacterial suspension was taken and mixed with the sterilized water drop for making a thin film on the slide by spreading uniformly. The film was fixed by passing it over the gentle flame for two or three times. After heat fixed the slide was flooded with crystal violet solution and allowed to stand for 30sec after that the excess stain was removed by washing with gentle stream of tap water. Then the slide was immersed in iodine solution for 1 minute and washed thoroughly with 95% alcohol for 10 sec. alcoholic was drained off and washed thoroughly with gentle stream of tap water. Then the slide was counterstained with safranin and the excess stain removed by washing with tap water. Finally the slide was examined under microscope.

Motility Test: Hanging drop preparation is useful for microscopic examination of living microorganisms, especially bacteria without staining them and to see their motility due to flagella. Cleaned and flamed a hanging drop slide was taken and placed it on the table with the depression uppermost. A little Vaseline or petroleum jelly was spreaded around cavity of the slide. Cleaned cover slips were taken and petroleum jelly was applied on each of the four corners of the cover slip, using a match stick. Cover slip was placed on a clean paper with the petroleum jelly slide up. One loop full of culture was transferred to the center of the cover slip. The depression slide was placed on to the cover slip, with the cavity facing down so that the depression covers the suspension. Press the slide gently to form a seal between the cover slip and the slide. The preparation was lifted and quickly turns the hanging drop preparation cover slip

up so that the culture drop is suspended. The preparation was examined under low power objective with reduced light. A drop of oil was placed on the cover sip and examined the preparation under oil immersion objective.

Results and discussion

For the Bioleaching of alumina experiment 100 ml of the broth media containing varied concentration of carbon sources i.e. 0.3g, 0.6g, 0.9g of beef extract was taken in 250 ml conical flask. 2g of the ore containing 41% of alumina was added in the media. A loop full of bacterial colonies was also added in the media. The nutrient broth containing 2g ore and bacteria was then kept in the orbital shaker for leaching experiment. Initial pH was recorded and pH was observed at every 3 days interval of time.

Observation of Alumina percentage (Al_2O_3%): In case of 0.3g carbon source it is seen that there is gradual rise in pH from 7 to 9.48 after 21days of incubation. The highest % of Aluminum is observed in 15 days that is 15.50% in pH 9.22. But after 15days the % of Al_2O_3 gradually decreases. In case of 0.6g carbon source it is seen that pH increased from 7 to 9.45 after 21days of incubation. The highest % of Aluminum is observed in 12 days that is 2.41% in pH 9.34. But after 12days the % gradually decreases to 1.39 after 21 days of incubation. In case of 0.9g carbon source it is seen that pH increased from 7 to 9.64 after 21 days of incubation where Al_2O_3 % being 1.29. The highest % of Aluminum are observed in 9days that is 2.46% in pH 8.49. But after 9 days the % gradually decreases to 1.29 after 21 Days of incubation as show in Table-3, Figure-3.

Discussion: Bauxite is commonly used in the commercial production of Al_2O_3 in the Bayer's process[5]. It is the main source of Aluminum. The ore is first chemically processed to produce aluminum oxide (alumina); then it is smelted to produce the pure aluminum metal. In this process only high grade ore are used for production of Aluminum[14]. Large amount of Aluminum used in five major industries like transportation, construction, electrical, container packing and mechanical equipment. So the demand of this valuable metal increasing day by day. Therefore uses of bioleaching process under this condition seem promising to fulfill this scarcity[15]. Bioleaching process is mediated due to the chemical attack by the extract organic acids on the ores. The acid increasing metal dissolution by lowering the pH as well as soluble the metal by chelating into soluble organic metabolic complexes. The effect of particle size on the experiment of metal using different size fraction may be due to high porosity which allows easy diffusion of the metabolites inside the ore body which in turn helps more metal solubilization[16]. The pre heating of ore enhances the leaching potential of microorganisims[17].

Table-3: Variation of Al_2O_3 % with respect to Carbon source, pH and Time.

Leaching Days	0.3 g of C Source		0.6 g of C Source		0.9 g of C Source	
	pH	Al_2O_3 %	pH	Al_2O_3 %	pH	Al_2O_3 %
1	7	0	7	0	7	0
03	7.67	0.56	7.634	0.36	7.631	0.59
06	8.35	1.58	8.37	1.86	8.30	1.98
09	8.72	2.36	9.01	2.29	8.49	2.46
12	9.01	4.92	9.34	2.41	8.74	2.45
15	9.22	15.50	9.11	1.30	8.92	1.59
18	9.45	4.02	9.36	1.62	9.33	1.31
21	9.48	1.27	9.45	1.39	9.64	1.29

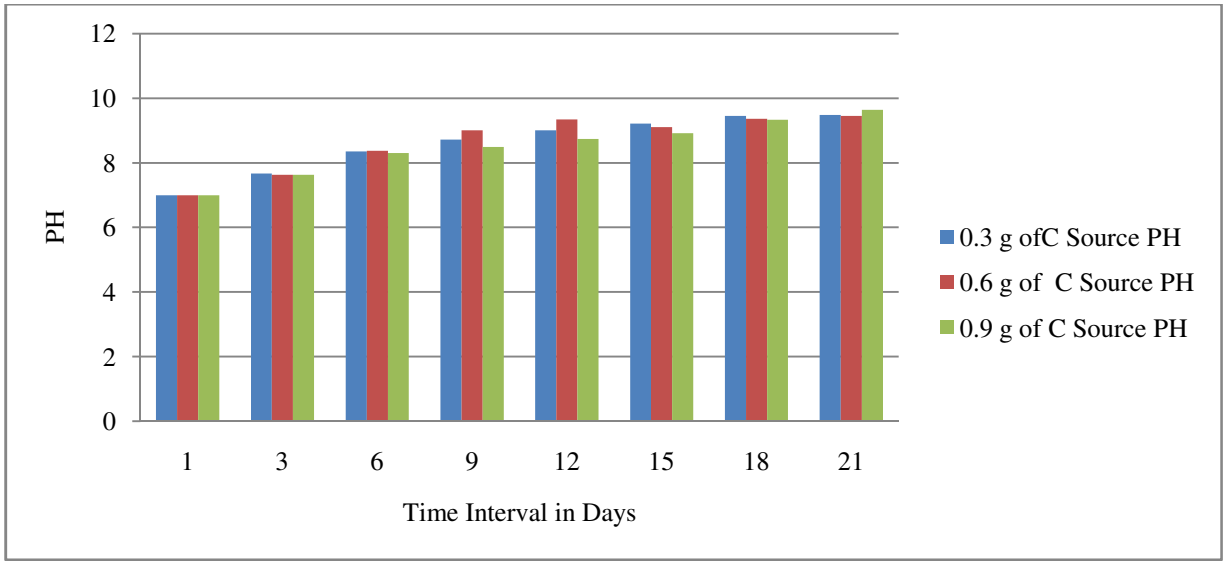

Figure-1: Variation of PH with respect to different Conc. of Carbon Source and Time interval.

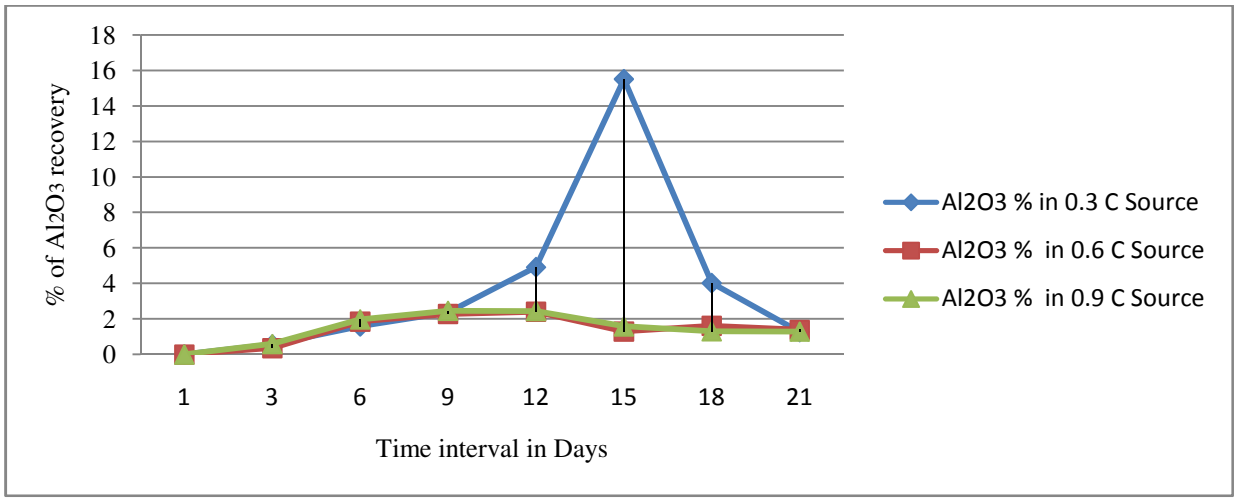

Figure-2: Variation of % Al_2O_3 recovery with respect to time and C- source.

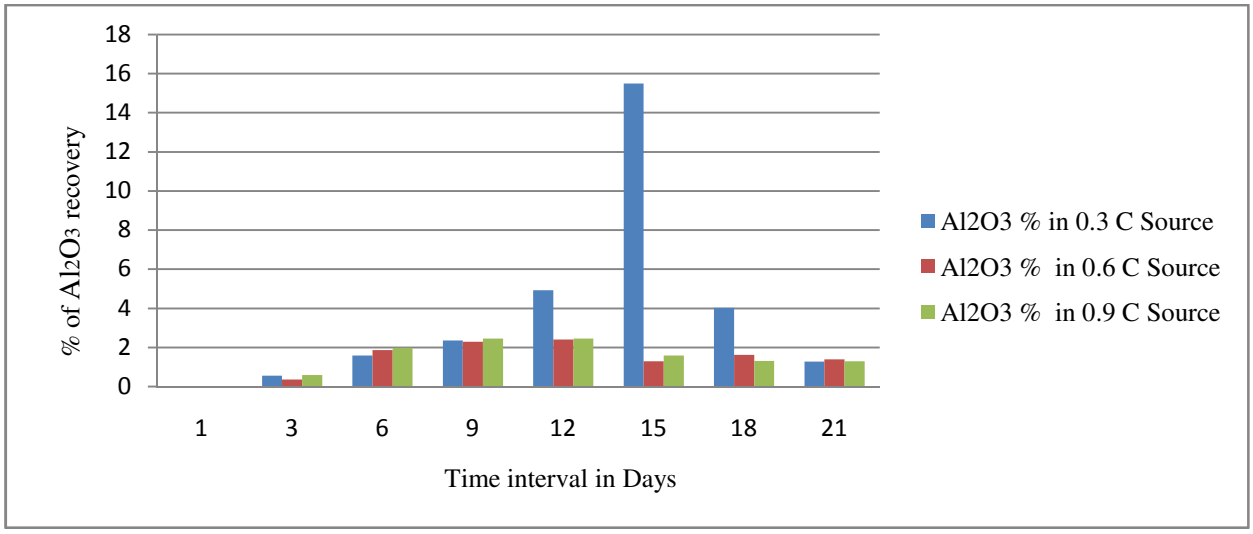

Figure-3: Variation of % Al_2O_3 recovery with respect to time and C- source.

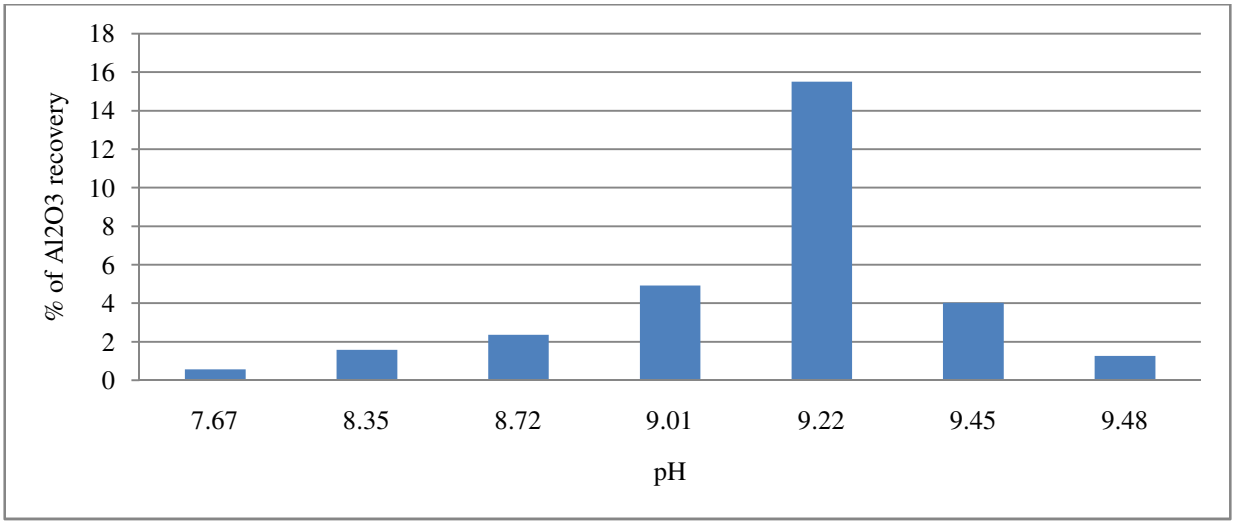

Figure-4: Variation of % of Al_2O_3 with respect to pH in 0.3g C source.

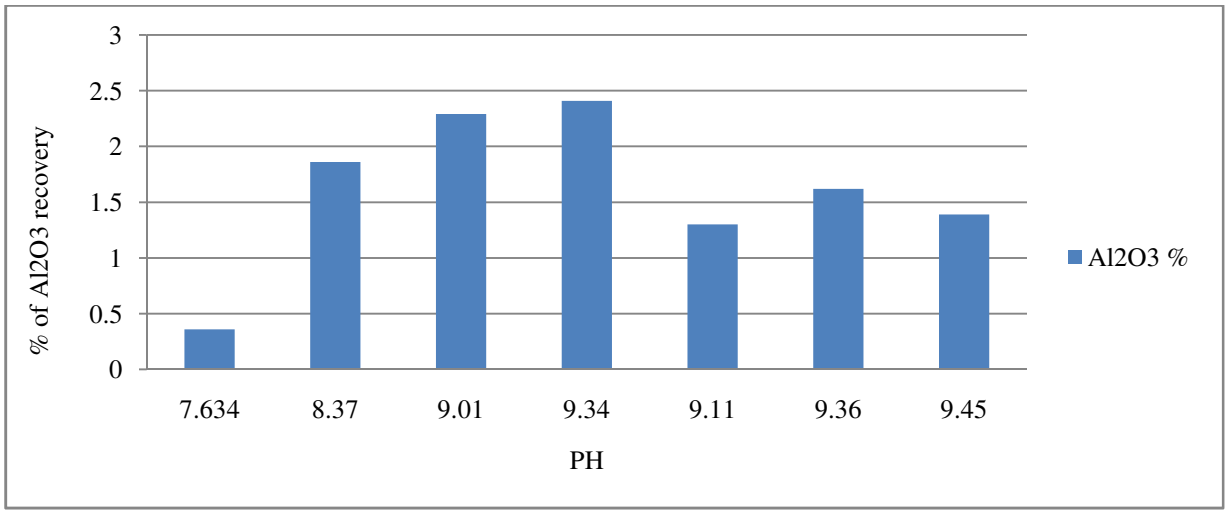

Figure-5: Variation of % of Al_2O_3 with respect to pH in 0.6g C source.

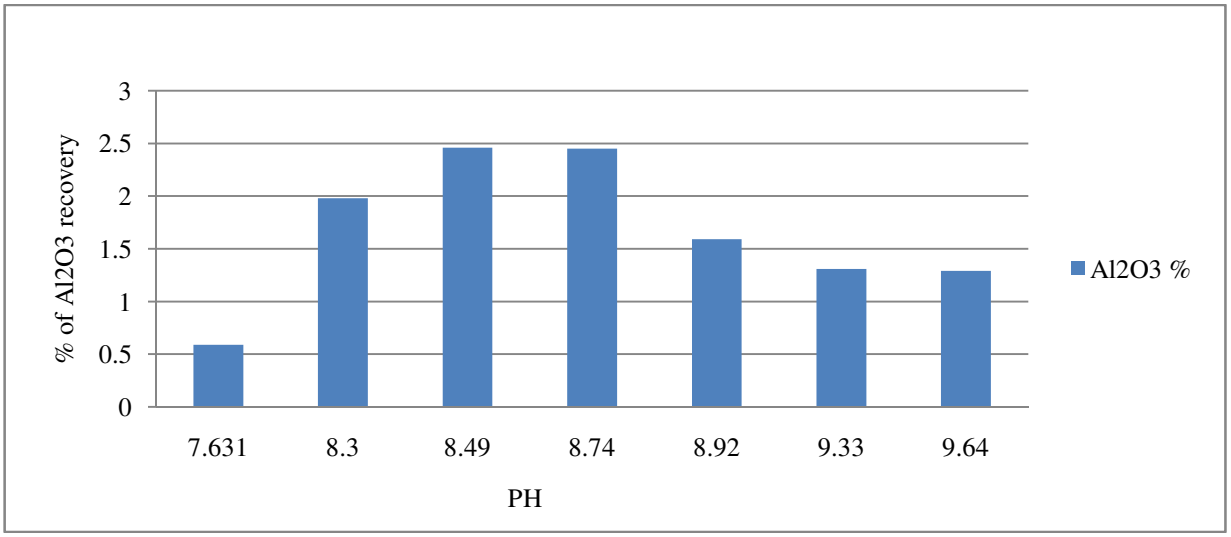

Figure-6: Variation of % of Al_2O_3 with respect to pH in 0.9g C source.

Observation of alumina percentage (Al_2O_3%) for 0.3 g carbon source: In case of 0.3g carbon source it is seen that there is gradual rise in pH form 7 to 9.45 .but the highest % of Al_2O_3 are observed in 15 days which is 15.50%. After 15 days the pH gradually increased to 9.48 but the % of Al_2O_3 recovery decreases to 1.27. Therefore the 15 days of incubation and PH 9.22 can be considering the optimum value for 0.3g c source.

Observation of alumina percentage (Al_2O_3%) for 0.6 g carbon source: In case of 0.6g carbon source it is seen that there is gradual rise in pH form 7 to 9.48.but the highest % of Al_2O_3 are observed in 12 days which is 2.41%. After 12 days the pH gradually increased to 9.45 but the % of Al_2O_3 recovery decreases to 1.39. Therefore the 12 days of incubation and PH 9.34 can be considering the optimum value for 0.6g c source.

Observation of alumina percentage (Al_2O_3%) for 0.9 g carbon source: In case of 0.9g carbon source it is seen that there is gradual rise in pH from 7 to 9.64 .but the highest % of Al_2O_3 are observed in 9 days which is 2.64%. After 9 days the pH gradually increased to 9.64 but the % of Al_2O_3 recovery decreases to 1.29. Therefore the 9 days of incubation and PH 8.49 can be considering the optimum value for 0.9g c source.

From the above observation it clears that 0.3g carbon source have better result in comparison to 0.6g and 0.9g. The suitable time required for leaching is about 15 days and the optimum PH is 9.22.

Conclusion

During the bioleaching studies of low grade bauxite ore, the pH of sample progressively increased due to alumina solubilization and reached maximum at 9.48, 9.45, 9.64 in case of 0.3g, 0.6g, 0.9g carbon source of ore sample. The leached suspensions were centrifuged after filtration tested for % of Al_2O_3%. Metal concentration in the filtrate In the case of 0.3g carbon source is more. After 15 days incubation considering it as optimum level. Al_2O_3 % showed a negligible increase in case of 0.6g and 0.9g. Hence 0.3g carbon source is considered as suitable Carbon source at PH 9.22 for maximum recovery % of Al_2O_3. The isolated microorganisms was found to be Gram–ve, rod shaped and motile. The reaming studies of bacterial screening and identification followed by creating a database and optimizing a bioreactor to create the bacterial leaching at a faster rate is going on

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