International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 4(2): March-April 2014

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Research Article……!!!**

Received: 11-07-2013; Revised; Accepted: 22-04-2014

STUDIES ON SOME BIOCHEMICAL CHANGES DURING BIOLEACHING OF SILICA AND IRON FROM INDIAN CHROMITE ORE BY SILICA TOLERANT *ASPERGILLUS NIGER* AB_{200}

Deblina Ball* and Ajit Kumar Banik

Department of Chemical Engineering, University of Calcutta, 92, APC Road Kolkata- 700009

Keywords:

Chromite ore, silica and iron bioleaching, biochemical changes, *Aspergillus niger*

For Correspondence:

Deblina Ball

Department of Chemical Engineering, University of Calcutta, 92, APC Road Kolkata- 700009

E-mail:

deblina.ball@gmail.com

ABSTRACT

A study was conducted to reveal the biochemical changes occurring during the process of bioleaching by *Aspergillus niger* AB_{200} responsible for the solubilization of silica and iron from the chromite ore. The organism was found to utilize 89.30% of the glucose supplied in the fermentation medium during the bioleaching process till the 9^{th} day of fermentation. Initially glucose uptake was very rapid, but it decreased on 8^{th} and 9^{th} day. Glucose was utilized by the fungus, producing of some organic acids like Gluconic acid, Oxalic acid and Citric acid responsible for leaching. Bioleaching capacity of the organism was maximum on the 7^{th} day of fermentation. But in case of nitrogen uptake by the organism, it was found that it increased upto the 9^{th} day of fermentation and maximum nitrogen in the medium provided was incorporated into the cell as cell nitrogen. Only a small percentage of Ammoniacal and Amino nitrogen was formed during the entire fermentation period.

INTRODUCTION

Bioleaching of silica and iron from chromite ore upgrades the ore and at the same time make the ore suitable for the required specification of chromite for its use in metallurgy and refractory purposes. *Aspergillus niger* utilizes the different nutrients supplied in the fermentation medium during the fermentation and produces the organic acids necessary for bioleaching. Carbon and nitrogen are the main components responsible for growth, biochemical process and organic acid production by *Aspergillus niger*. The other macro and micro elements, facilitated the biochemical processes necessary for growth and metabolism[1]. Therefore growth of the organism and bioleaching process is directly related to the availability of the chemical nutrients in the fermentation medium during the bioleaching process. Many investigators used different carbon and nitrogen sources for the study of growth and leaching process by the organism[2,3]. Different organic acids produced as metabolic products by the organism are involved in bioleaching have also been studied some authors[4]. Glucose and Sodium nitrate were used as the suitable carbon and nitrogen source for bioleaching and our present study was conducted to study the utilization of glucose and sodium nitrate from the fermentation medium by *Aspergillus niger* AB_{200} and its bioleaching capacity with relation to the time period of fermentation. Different organic acids produced as a result of metabolism during the fermentation process by the organism were also noted.

MATERIALS AND METHODS

Chromite ore: Chromite ore used in the study of bioleaching is obtained from the SukindaValley, Orissa, India. The ore was crushed to 200 mesh size and the silica and iron content in the ore was estimated[5].

Microorganism: The parent of *Aspergillus niger* was isolated from the soil of North Bengal and silica tolerant *Aspergillus niger* AB200 was developed from the parent culture which was used for the present study[5].

Growth medium and growth conditions: The cultures were maintained on agar slants having composition :- glucose – 5%, NaNO3 – 0.2%, KH2PO4 – 0.1%, KCl – 0.05%, MgSO4.7H2O – 0.05%, $Na_2O_3Si.9H_2O$ – 0.18%, Agar – 4%. pH was adjusted to 4.8-5.0. The slants were incubated for 7days at 30˚C. Preparation of Inoculum: Full grown slant cultures were scrapped off and suspended in 100ml double distilled water. The suspension contained 1.4×10^6 spores/ml and was used as inoculum[5].

Composition of the synthetic medium and conditions for bioleaching : The following synthetic media was used for bioleaching studies:- glucose – 10%, NaNO3 – 0.2%, KH2PO4 – 0.1%, KCl – 0.05%, MgSO4.7H2O – 0.05%. pH – 4.5.

Surface culture fermentation was carried out using 100ml Erlenmeyer flask which contained 0.3 gm chromite ore and 30 ml fermentation media. 6 ml inoculum was added to each flask and incubated at 33˚C for 7 days.

Estimation of silica and iron in the fermentation media : The organism leached out silica and iron oxide from ore into the fermentation media, which is then estimated colorimetrically by silico molybdate method[6] and thiocyanate method respectively[7].

Estimation of Dry cell weight: The cells were separated from the fermentation media by filtration, washed with distilled water and filtered once again. The mass was dried in an oven at 100˚C for 24hrs.it was then weighed to obtain the dry cell mass[5].

Estimation of sugar in the fermentation medium : The residual sugar in the fermentation medium was estimated by Nelson-Somogyi method[8]

Estimation of Nitrogen: Total nitrogen in the cell and in the broth was estimated by Micro-Kjeldahl method[9]. Ammonium nitrogen was estimated by Conway[10] and the amino nitrogen by Paper chromatography[11].

.Estimation of Organic acids : The total acid content in the fermentation medium is obtained by titration. Individual organic acid in the fermentation were detected by paper chromatography[12]. Gluconic acid in the fermentation medium is estimated as Ca-gluconate by EDTA titration[13]. Citric acid is estimated by Pyridine-Acetic anhydride method[14] and Oxalic acid by titration with $KMnO_4$[15].

Statistical Analysis: All data were expressed as Mean ± SEM, where n=6.

RESULT AND DISCUSSION

Bioleaching of silica and iron from chromite ore by *Aspergillus niger* AB_{200} was carried out by utilizing the glucose present in the fermentation medium and it was found that with the increase in glucose utilization by the fungus, bioleaching also increased(shown in Fig 1.).

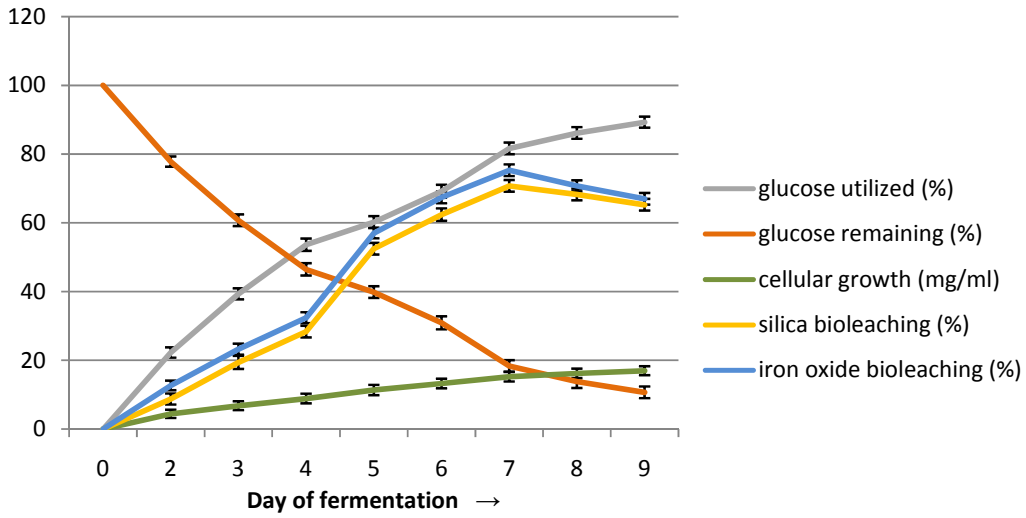

Fig 1. Utilization of glucose, growth of *Aspergillus niger* AB_{200} and its bioleaching capacity with respect to the time period of fermentation.

From Fig 1, it has been found that initially glucose utilization sharply increased and upto the 7th day of fermentation glucose utilization was high, but beyond that i.e., on the 8th and 9th day, glucose uptake was not that much high . Maximum 89.30% of glucose, provided in the fermentation medium was utilized by the organism till the 9th day of fermentation. This can also be correlated with the growth of the organism and its bioleaching capacity, which shows that maximum bioleaching of silica and iron from chromite ore was obtained on the 7th day of fermentation. pH of the fermentation medium also decreased from 4.5 to 2.8 which signifies production of some organic acids in the fermentation medium responsible for the solubilization of silica and iron from the ore. The biochemical mechanisms leading to organic acid production is important because of their relevance to fungal physiology. The biochemistry of carbon metabolism through glycolysis and Krebs cycle have attracted intense attention already. Many posssible mechanisms for organic acid production *by Aspergillus niger* have also been proposed by many authors[16]. Detection and Quantitative estimation of Organic acids from the fermentation medium revealed 3organic acids from the medium, which were Gluconic acid(0.064M), Oxalic acid(0.028M), Citric acid(0.008M). The organic acids produced as a result of the carbohydrate metabolic pathways for glucose metabolism may be responsible for the solubilization of silica and iron.

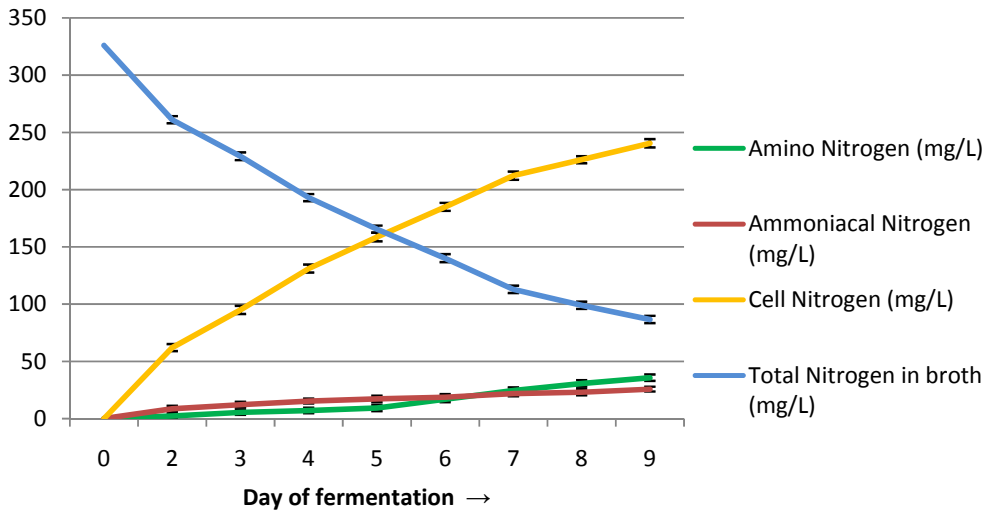

Fig 2. Nitrogen balance during bioleaching by *Aspergillus niger* AB_{200}.

From Fig 2. it has been found that, maximum percentage of nitrogen in the fermentation medium was taken up by the fungal cells for the structural development of the organism and and this nitrogen uptake increased till the 9^{th} day of fermentation. According to some authors presence of Ammonium nitrate used as the nitrogen source required for the mycelial growth is necessary for the citric acid production by the organism. This is because nitrogen is not only important for metabolic rates in the cells but it is also the basic part of cell proteins [17].A small percentage of ammoniacal and amino nitrogen were also produced in the fermentation medium. This shows that nitrogen is well balanced with respect to the amount of nitrogen provided in the medium and cell nitrogen along with ammoniacal and amino nitrogen formed.

REFERENCES

1. Pelczar, J.M., Krieg, N.R., Microbiology, 5^{th} Edition, Tata McGraw-Hill, 1993; 99-101.
2. Mohanty, B.K., and Mishra, A.K., Physical and chemical factors affecting the release of silica from Magnesite ore by a *Bacillus* sp., J. Gen. Appl. Microbiol, 1988; 34: 233-24.
3. Bansal, V., Syed, A., and Sastry, M., Zirconia enrichment in Zircon sand by selective fungus mediated bioleaching of silica, Langmuir, 2007; 23: 4993-4998.
4. Saeed, S., Bhatti,T.M., Bioleaching studies on Rock Phosphate using *Aspergillus niger*, Online Journal of Biological Sciences, 2002; 2(2): 76-78.
5. Ball,D., Banik, A.K., Studies on bioleaching of silica from Indian chromite ore by a silica tolerant *Aspergillus niger* AB_{200}: Characterization of some physical parameters and scanningelectron microscopic studies, Journal of Chemical Society, 2010; 87:857-862.

6. Jeffery G.H., Basset,J., Mendham, J., Denney, R.C., Vogel's Text book of Quantitative Chemical Analysis, 5th Edition , Longman Scientific and Technical, 1989; 703-704.
7. Jeffery G.H., Basset,J., Mendham, J., Denney, R.C., Vogel's Text book of Quantitative Chemical Analysis, 5th Edition , Longman Scientific and Technical, 1989; 690-691.
8. Nelson, A.N., J.Biol. Chem.,1944;153: 375;
9. Somogyi, B.A., *J.Biol. Chem*., 1945; 160: 62.
10. Allen, W.F., Accurate and adaptable micro-kjeldhal method for nitrogen determination. Ind. Eng.Chem., 1931; 3: 239-240.
11. Conway, E.J. and Byrne A., An absorption for the micro-determination of volatile substances, Biochem J., 1933; 27(2): 419-429.
12. Spies J.R, Colorimetric procedures for amino acids, Methods in Enzymol,1957;3:468-471.
13. Isherwood, F.A., and Hanes, C.S., Separation and Estimation of organic acids on paper chromatograms, Biochem. J., 1953; 55(5): 824-830.
14. Jeffery G.H., Basset, J., Mendham, J., Denney, R.C., Vogel's Text book of Quantitative Chemical Analysis, 5th Edition , Longman Scientific and Technical, 1989; 426
15. Marier J.R. and Boulet M., "Direct determination of citric acid in milk with an improved pyridine-acetic anhydride method", J Diary Sci., 1958; 41: 1683-1692.
16. Ahluwalia, V.K., Dhingra, S., Gulati, A., College Practical Chemistry, Universities Press(India) Private Limited, 2005; 66-67.
17. Magnuson, J.K., Lasure, L.L., Organic acid production by filamentous fungi, Advances in Fungal Biotechnology for Industry, Agriculture and Medicine, Kluwer Academic/Plenum Publishers, 2004; 307-340.
18. Ali S., Haq I., Qadeer, M.A., Production of citric acid by *Aspergillus niger* using cane molasses in a stirred fermentor, Electronic Journal of Biotechnology, 2002; 5(3): 258-271.