Optimization of Process Conditions for the Biodesulphurization of Iron Ore using Sulpholobus Brierleyi

*T.O. CHIME,**O. D. ONUKWULI AND *S. O. EGBUNA
*Department of Chemical Engineering Enugu State University of Science and Technology
**Department of Chemical Engineering Nnamdi Azikiwe University Awka

ABSTRACT - An investigation has been made in this work to optimize the process conditions for the biodesulphurization and biobeneficiation using sulpholobus Brierleyi bacterium strain. Standard central composite design with 22 full fractional design was used to develop the models. The models were optimized using matlab. The surface responses of the plots shows that microbial population and leaching time interacted effectively to enhance the degree of biodesulphurization and biobeneficiation. The result show that 99.93% of biosulphurization was obtained at optimum values of 88 days and 108 cells/ml while optimum values of 71 days and 108 cells/ml of microbial population were obtained for 49.86% degree of beneficiation.

Keywords: Sulpholobus Brierleyi, Biodesulphurization, Microbial Population, Iron Ore, Optimization, Biobeneficiation.

INTRODUCTION

The estimated workable iron deposits in Nigeria stand in excess of 2.5 billion times most of which belong to hematite, magnetite, hematite-geothite and siderite-geothite. However, the estimated reserve of Agbaja iron ore is over one billion tonnes. The utilization of this ore is hindered by its poor response to established industrial beneficiation techniques. This is due to the fine grained texture of the iron ore [2].

Iron ore resources are fast depleting as a result of pressure originating from population and industrial development requirements. However, there exist large stockpiles of low and lean grade ores to be mined of which Agbaja iron ore is one of such ores[3].

Desulphurization mainly takes place at the metal-slag interface, but the behavior of sulphur in the bulk of metal is also of greater importance[4].

Conventional techniques involved in metal extraction from metallic ores are economically expensive in monetary terms and human labour and are also environmentally unfriendly. Many of the by- products of extraction by thermal and chemical means are toxic to man and his environment and constitute sizeable environmental pollutants in cities and industrial layouts[3].

On the search for more environmental sound technologies for the mineral processing industry, biological processes to extract metals from ores, pretreating metallic ores or removing contaminants from metallic ores or industrial wastes have been developed for different metallic mineral resources[5].

Bioleaching is regarded as one of the most promising and certainly the most revolutionary solution to these challenges compared to pyrometallurgy or chemical metallurgy. Toxic chemicals are not added to this leaching process and it is carried out under mild conditions. The biological treatment of ores to remove impurities often referred to as bioleaching[5] is another aspect of chemical processing. In such a process the microorganisms produce, as a consequence of their metabolism, a chemical by-product, mineral acid, organic acids, polymers and enzymes. These chemical by- products attack the gangue minerals contained in the ore, dissolving them and thus producing their selective removal[5]. [6] have reported that iron oxidizing bacterium thiobacillus ferrooxidans leached manganese from manganese dioxide in the presence of sulphide ores of copper.

These investigations have main draw back because the strains used were not associated with the ore being treated. When artificially inoculated in a particular environment, indigenous microorganisms, as a general rule compete better in terms of adaptation and cause fewer ecological distortions than exogenous microorganisms. However, if an efficient biodesulphurization process has to be implemented for treating a determined raw materials, studies on the micro biota naturally living in such a subtraction and evaluation of its desired properties should be the starting step[3].

In this study, an attempt is made to use bacterium harvested from the raw iron ore to desulphurize the ore. Model equations were developed using central composite design. Optimization of the process variables was also carried out using Matlab

MATERIALS AND METHODS

The iron ore sample was obtained from Agbaja, kogi state, Nigeria. The sample was crushed with hammer mill into finer size and sieved to obtain a particle size of 60microns. 20kg of the sample was weighed out. It was divided into two parts of

14kg and 6kg respectively. The 6kg was subjected to immediate microbial and chemical analyses. The remaining 14kg portion was subjected to desliming and subsequent post desliming microbial and chemical analyses.

Microbial reagents (nutrient agar)

A 25g of dehydrated nutrient agar was dissolved in 1 litre of distilled water and the solution heated in a water bath until completely dissolved and dispersed in 25ml bottles, worked and autoclaved at 120^{o}C for 15minutes. The sterile molten nutrient agar was poured into sterile petri dishes and allowed to solidify. They were then oven dried at 50^{o}C for 15 minutes and thereafter incubated for 24hours to ensure complete sterility before use.

Microbial culture

The raw iron ore was mixed with sterilized water and stirred vigorously with glass stirring rod. A loopfull of the ore suspension was streaked on the oven dried sterile nutrient agar in a petri dish with sterile inoculated loop pre heated to redness. The streaked culture in the petri dish was incubated at 37^{o}C for 24 hours. A moderate yield growth was recorded in the culture plate after 24 hours incubation. On close examination five distinctive colonies were revealed.

Isolation of bacteria

A streak was taken from each colony forming unit and transferred into 20ml agar slant tube for pure culture. Pure cultures obtained from the colony were properly stored in an incubator at 37^{o}C for onward identification and confirmatory tests.

Microscopic examination of isolation.

Each isolate was subjected to stain gram test. The gram stain of a particular colony revealed amorphous-like shape and has a single discoid form with purple colour. The stain character test showed positive result implying gram positive specie. Lobus specie is suspected as a result of the pre disposing features. The confirmatory test showed that manitol, sulphide oxidase and phosphorus reductase tested positive while coagulase tested negative. The litmus milk test was acidic and oxidation of ferrous to ferric was neutral confirming the bacterium as Sulpholobus Brierleyi which was used in this study.

Preparation of innoculum and leaching of deslimed sample

A standard culture of 10^{9}cells/ml was prepared from pure culture in the agar scant containing Sulpholobus Brierleyi. This 10^{9} cells/ml standard culture solution was made by pouring 5ml of sterile water on the surface of the agar slant and stirred. It is then poured into a sterilized test tube. Another 5ml of sterile water was again poured on the surface of agar slant and stirred again and this was poured into the same test tube. This result to 10ml concentration of the micro organism in the sterilized test tube and this forms the standard 10^{9} colony forming unit of Sulpholobus Brierleyi. Subsequent serial dilutions were made from the standard culture to obtain 10^{8},10^{7},10^{6},10^{5},10^{4},10^{3},10^{2} and 10^{1} microbial populations.

Each of the microbial population was inoculated into 25g of deslimed iron ore and put into incubating bottles. These samples were collected from the bottles after 8 days interval and were analyzed for sulphur and iron contents. The samples were incubated for 88days at constant temperature of 25^{o}C

Degree of bio desulphurization(%) =

$$\frac{\text{As received value } S^{o}\,(wt\%) - \text{Final value}(wt\%)}{\text{As received value } S\,(wt\%)} \times \frac{100}{1}$$

Degree of bio beneficiation(%)

$$\frac{\text{Final value Fe}(wt\%) - \text{As received value Fe}(wt\%)}{\text{Final value Fe}(wt\%)} \times \frac{100}{1}$$

Design of experiment for bioleaching

Statistically designed experiment was carried out for the bioleaching treatment based on central composite design 2^{2}. The design matrix for the two variables, two levels and eleven experimental runs and response are developed.

Table 1
Experiment range and levels of independent variables (sulphur and iron)

Independent variables	Lower level(-1)	Base level (0)	Upper level (+1)
X_1 Microbial population	10cells/ml	10^{4} cells/ml	10^{8}Cells/ml
X_2 Time(days)	8 days	48 days	88 days

Development of statistical multivariable models

The standard design of experiment is an efficient procedure for planning experiments so that data obtained can be analyzed to yield valid objective conclusions. Standard central composite design of 2^{2} full factorial design was used. This design was constructed from 2^{m-t} for cube portion, which is augmented with centre points and start points

2^{2} full factorial design
$N = K^{m-t} + 2m + N_o$
Where
K = level of experiment = 2
m= Total number of variables(2) x_1, x_2
t= The degree of fractionality t=0 for m<4
No – centre points added = 3
$\therefore N = 2^{2-0} + 2^{2} + 3 = 11$runs

The model equation for the experiment is proposed as

$$\hat{Y} = b_o + b_1x_1 + b_2x_2 + b_2x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2$$

Table 2
Design Matrix and Response

	X_1	X_2	$\hat{Y}$ (S) % biodesulphurization	$\hat{Y}$ (Fe)% biobeneficiation
1	0	0	84.44	42.94
2	-1	-1	70.00	1.33
3	+1	-1	73.33	1.77
4	0	0	84.44	42.94
5	-1	+1	98.89	45.56
6	+1	+1	99.89	42.20
7	0	0	84.44	42.94
8	-1	0	84.44	42.74
9	+1	0	86.61	43.31
10	0	-1	71.11	1.51
11	0	+1	98.89	45.81

Developed model equation for biodesulphurization

$$\hat{Y} = 85.03 + 1.09X_1 + 13.87X_2 + 0.58X_1X_2 + 0.53X_1^2 - 0.03X_2^2$$

Developed model equation for biobeneficiation

$$\hat{Y} = 43.96 - 0.39X_1 + 21.49X_2 - 0.95X_1X_2 - 0.95X_1^2 - 20.29X_2^2$$

Optimization of model equation using Matlab

Biodesulphurization of agbaja iron ore using sulpholobus brierleyi

```
>> A=[1 1];
>> b=[2];
>> lb=[-1;-1];
>> ub=[1;1];
>> x0=[0;0];
>>
[x,fval,exitflag,output]=fmincon(@leaching1,x0,A,b,[],[],lb,ub)

x =

    1

    1

fval =

 99.9300

exitflag =

    1

output =

      iterations: 2

       funcCount: 11

        stepsize: 1

       algorithm: 'medium-scale: SQP, Quasi-Newton, line-search'

   firstorderopt: 4.4409e-016

    cgiterations: []

         message: [1x144 char]

>> [x1,x2] = meshgrid(-1:.05:1, -1:.05:1);
>> z=85.02+1.09*x1+13.87*x2-0.58*x1.*x2+0.53*x1.^2;
>> surfc(x1,x2,z)
>>
```

Biobeneficiation of agbaja iron ore using sulpholobus brierleyi

```
>> A=[1 1];
>> b=[2];
>> lb=[-1;-1];
>> ub=[1;1];
>> x0=[0;0];
>>
[x,fval,exitflag,output]=fmincon(@leaching1,x0,A,b,[],[],lb,ub)

x =

   0.4756

    0.5407

fval =
```

```
 49.8626

exitflag =

    1

output =

      iterations: 5

       funcCount: 24

        stepsize: 1

       algorithm: 'medium-scale: SQP, Quasi-Newton, line-search'

   firstorderopt: 4.7684e-007

    cgiterations: []

         message: [1x144 char]

>> [x1,x2] = meshgrid(-1:.05:1, -1:.05:1);

>> z=43.96-0.39*x1+21.49*x2-0.95*x1.*x2-0.95*x1.^2-20.29*x2.^2;

>> surfc(x1,x2,z)

>>
```

Results and Discussion

Table 3
Xray Fluorescence Chemical analysis of Agbaja iron ore before desliming

Component	Average Composition wt%
Fe	56.34
SiO_2	5.16
S	0.12
A_2O_3	6.60
CaO	0.23
MgO	0.07
MnO	0.18
TiO_2	0.15
K_2O	0.04
P	0.79
H_2O	2.06

Table 4
X ray fluorescence chemical analysis of Agbaja iron ore after desliming

Component	Average Composition wt%
Fe	56.90
SiO_2	5.02
S	0.05
A_2O_3	5.20
CaO	0.21
MgO	0.03
MnO	0.17
TiO_2	0.25
K_2O	0.007
P	0.69
H_2O	2.81

Table 3 depicts the chemical analysis of as received Agbaja iron ore. The iron content of 56.34% by weight shows that the iron ore if adequately beneficiated and desulphurized can be used in steel production (7). The iron ore is not exploited because of its high sulphur content despite a huge reserve of over 1.0 billion tones.

The chemical analysis of the deslimed iron ore is shown in table 4. The effect of desliming was not significant though it increased the degree of biobeneficiation of total iron content from 56.34 to 56.9% weight (7). It can also be observed that percentage removal of sulphur was 0.07% showing that desliming had effect on sulphur removal.

Table 5
Result of Microbial Characterization of Sulphobolus Brierleyi

Stain	Gram Stain
Shape	Amorphous
Arrangement	Single discoid
Colour	Purple
Stain Character	+
Presumptive test specie suspective	lobus
Confirmatory test	
Coagulase	-
Manitol	+
Litmus milk	acid
Sulphur oxidase	+
Fe^{2+} - Fe^{3+}	Neutral
Phosphorus reductase	+

The result of microbial characterization of Sulphobolus Brierleyi is represented in Table 5. The grain test showed amorphous-like shape under microscope. The bacterium was arranged in single discoid form and the colour was purple. The stain character test showed positive result implying gram positive specie. Lobus specie was suspected as a result of gram stain test.

The confirmatory test showed that manitol, sulphide oxidase and phosphorus reductase tested positive while coagulase tested negative. The litmus milk test was acidic and oxidation of ferrous to ferric was neutral confirming the bacterium as Sulpholobus Brierleyi[7].

Influence of leaching time on the percentage degree of biodesulphurization at 25°C

In nutrient limited environments such as the one in our experiments, bacteria must colonize mineral surfaces where phosphate or sulphides are located in order to scavenge it[8]. They accomplish this through biofilm formation. Biofilms are

complex aggregates of bacterial cells, bacterial exopolymers, mineral debris attached to a surface.

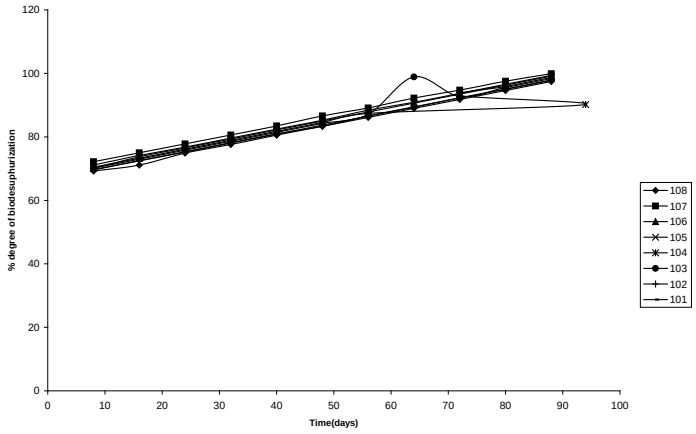

Fig 1. Influence of leaching time on the percentage degree of biodesuphurization of agbaja iron ore

The percentage removal of sulphuric for the 8th day was 71.78%. The degree of biodesulphurization increase progressively with leaching time[7]. Consequently, the highest experimental value of 99.89% removal of sulphur was achieved for 10^8 microbial population at leaching time of 88days. From the analysis above it can be inferred that the degrees of biodesulphurization is strongly affected by the increase in the leaching time.

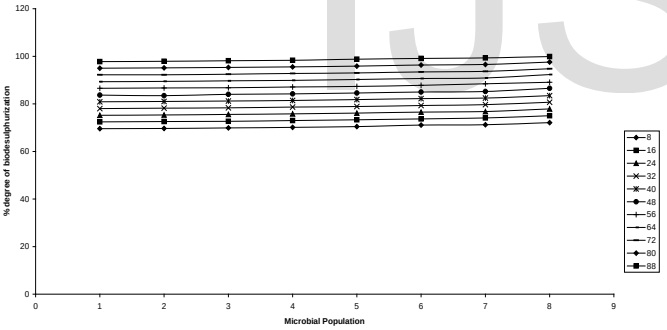

Fig 2: Influence of microbial population on the percentage of Biodesulphurization of Agbaja iron ore.

The percentage difference of removed sulphur between 10^1 and 10^8 cells/ml was 2%. From figure 2, it can be observed that as the microbial population increases the percentage degree of biodesulphurization increases marginally.

Interaction effects and surface responses

The design matrix is shown in table 2. The parameters x_1 (microbial population) and x_2(leaching time) were chosen as independent variables while the percentage degree of biodesulphurization is the output response. The analysis is based on how the percentage degrees of biodesulphurization and biobeneficiation are affected by independent variables in order to study the combined effects of these factors. Experiments were performed at different combinations of the parameters using statistically designed experiments. The determination of polynomial coefficients were followed by statistical analysis (G-test, F-test, T-test) to develop a model that is adequate, significant and homogeneous.[9]

The corresponding interactive surface response plots presented in figure 3 and figure 4 show effective interaction of leaching time and microbial population.

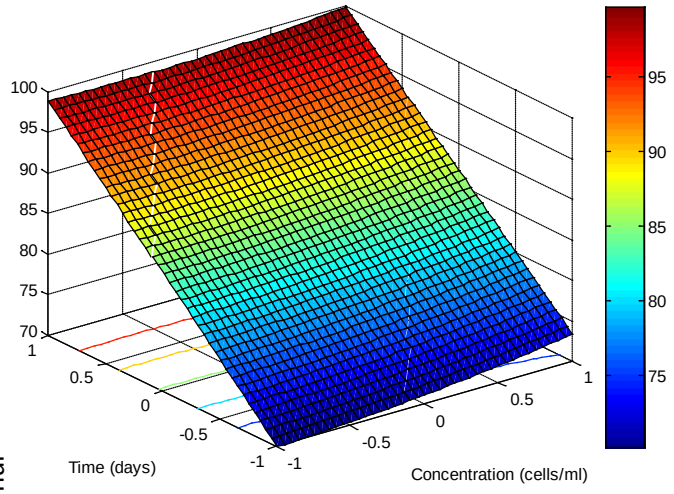

Fig 3: Percentage removal of sulphur using sulpholobus brierleyi (time and concentration as variables)

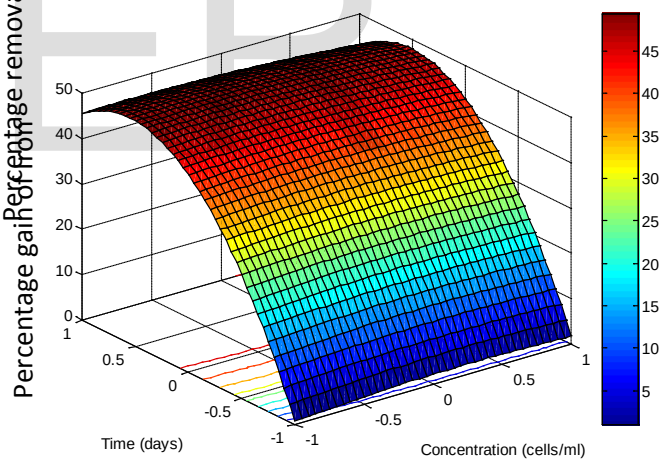

Fig 4: Percentage gain of iron using sulpholobus brierleyi (time and concentration as variables)

CONCLUSION

The sulphur removal was found to be feasible using Sulpholobus Brierleyi bacterium. The models developed were significant,adequate and homogeneous. The optimum percentage degrees of biodesulphurization and biobeneficiation were 99.93% and 49.86% respectively. The surface responses show that leaching time interacted effectively with microbial population.

REFERENCE

ISSN 2229-5518
1. Alafara, A.B; Adekola, F.A.O.and Folashade, A.O. Quantitative leaching of Nigerian ore in hydrochloric acid. Journal of applied science and environment, 2005, 9(3) 1115-20

2. Uwadiele, G.O. O.O.and Whewel, R.S. Effect of temperature on magnetizing reduction of Agbaja iron ore. Metallurgical Transaction B, 1988, B11 31-785.

3. Chime, T.O., Menkiti, M.C.,and Onukwuli, O.D. Biodephosphorization of Iron ore using acidothiobacillus Ferrooxidans New York Science Journals, 2011, 4(1) pp. 1-6

4. Nwoye, C.I. Gaseous state desulphurization of Agbaja iron ore concentrate. Journal of Engineering and applied science, 2007, (3) pp 72-75.

5. Jain, N. and Sharama, D. Biohydrometallurgy for non sulfidic minerals. A review, geomicrobiology journal, 2004, Pp 135-144.

6. Gosh, J. and Imai, K. Leaching mechanism of manganese dioxide by Thiobacillus ferrooxidans. Journal of fermentation technology, 1985,(63) 295 – 298.

7. Chime, T.O. Beneficiation, Dephosphorization and Desulphurization of Agbaja iron ore. Ph.D Thesis ,Nnamdi Azikiwe University, Awka, 2010.

8. Banfield, J Barker, W. Welch, S., and Taunton, A. Biological impact on mineral dissolution: Application of lichen model of understanding mineral weathering in the rhizosphere. Proceeding of national Academy of science USA, 1999, 3404-3411.

9. Obiorah, S.M.O, Menkiti M.C. and Nnuka, E.E. Beneficiation processing of Agbaja Iron ore by chemical leaching technique. New York Science Journal, 2011, 4(5) 22-27