Optimization of Process Variables for the Biodephosphorization of Iron ore using Leptospirillum Ferrooxidans

T. O. Chime

Nigeria

Abstract– **An attempt has been made in this study to optimize the processing parameters for the biodephosphorization and bio beneficiation using leptospirillum ferrooxidans. Standard central composite design with 2^2 full fractional was used to develop the models. The models were optimized using matlab. The surface response of the plots shows that microbial population and leaching time interacted effectively to enhance the degree of biodephosphorization and biobeneficiation. The results show that 92.21% of biodephosphorization was obtained at optimum values of 67 days and 10^8 microbial population while optimum values of 10^8 microbial population and 71 days were obtained for 50.98% degree of biobeneficiation.**

Keywords– **Biodephosphorization, Biobeneficiation, Leptospirillum Ferrooxidans and Surface Response**

I. INTRODUCTION

Iron is one of the largest elements in the earth's crust, being the fourth most abundant element at about 5% by weight. The Nigerian mineral appraisal and monetization programme NIMAMOP, 1996, identified about 33 solid mineral commodities including Iron at various stages of exploration and exploitation and which occur in about 450 locations nationwide (Rahaman, 2004).

Iron ore resources are fast depleting as a result of pressure originating from population and industrial development requirement. However there exist large stock piles of low and lean grade ores to be mined of which Agbaja iron is one of such ores. The ore is low grade because of the high phosphorus content (Chime, et al 2011).

Phosphorus, which is frequently found as constituent of iron ore, is deleterious in the manufacture of iron and steel. If steel is produced at high level of phosphorus that steel will be brittle and can easily crack hence the need for its removal. Heat treatment and subsequent leaching is a way for upgrading high-P iron ores (Field et al, 1968, Gooden et al, 1974; Muhammed and Zhang, 1989, Kokal, 1990; Araujo et al; 1994, Cheng et al, 1999, Kokal et al, 2003).

In recent times, an increase in world steel production has increased demand for iron ore with a consequent increase in the price for this commodity, making hydrometallurgical phosphate removal viable (Kokal et al, 2003).

Biohydrometallurgy is an option for the removal of unwanted phosphate from iron ores because it is well established that many microorganisms, especially nutrient – limited environments, are capable of mobilizing the phosphorus contained in minerals (Banfield et al, 1999; Nautiyal, 1999). Some studies have been published on phosphate removal from iron ores using acid – producing microorganisms, including filamentous fungi (Parks et al, 1990, Bius, 1995) and iron – oxidizing bacteria (He and Wei, 2000). Mobilisation of phosphorus from iron ore using Burkholderia Caribensis (Fe GlO_3) a bacterium has been reported (Delvasto et al, 2009).

Recent upsurge in the biohydrometallurgical processes is motivated by the fact that they are efficient and suitable for metal extraction from low grade ores, moreover this is a less energy intensive and an environmentally safer process (Torma and Besecker, 1982).

The microorganisms used in these previous studies were mostly isolated from sources other than the ore to be treated. From practical point of view, the use of microorganisms indigenous to an ore can have some advantages in bioleaching in terms of either adaptation time of the strains to a bioleaching process or in ecological disruption in the surrounding area in an in situ biobeneficiation (Delvasto et al, 2005; Delvasto et al, 2006).

In this study a bacterium, Leptospirillium Ferrooxidans harvested from the Agbaja iron ore was used to remove phosphorus from the iron ore. Standard central composite design of 2 full factorial design was used to develop model equations. The developed model equations were optimized using Matlab.

II. MATERIALS AND METHODS

The iron ore sample was obtained from Agbaja, Kogi state, Nigeria. The iron ore was crushed with hammer mill into finer size and sieved to obtain a particle size of 60 microns. 20 kilograms of the iron ore was weighed out. It was divided into two parts of 14 kilograms and 6 kg respectively. The 6 kilograms was subjected to immediate microbial and chemical analysis. The remaining 14kilograms portion was subjected to desliming and subsequent post desliming chemical and microbial analyses.

A. *Microbial reagents*

A 25g of dehydrated nutrient Agar was dissolved in one litre of distilled water and the solution heated in a water bath until completely dissolved and then dispersed in 25ml bottles,

corked and autoclaved at 120^OC for 15minutes. The sterile molten nutrient Agar was poured into sterile Petri dishes and allowed to solidify. They were then oven dried in the incubator at 50^OC for 15minutes and thereafter incubated for 24 hours to ensure complete sterility before use.

B. Microbial Culture

The raw iron ore was mixed with sterilized water and stirred vigorously with a glass stirring rod. A loopful of the raw ore suspension was streaked on the oven dried sterile nutrient Agar in a Petri dish with sterile inoculating loop preheated to redness. The streaked culture Petri dish was incubated at 37^0C for 24 hours. Incubation for 24 hours yielded moderate growth in the culture plate. On examination with a microscope five colonies of distinctive features were revealed.

C. 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 first colony was properly stored in an incubator at 37^{0C} for onward identification and confirmation tests

D. Preparation of innoculum and leaching of deslimed sample

A standard culture of 10^9 cells/ml was made from pure culture in the Agar slant containing leptospirillum Ferrooxidans. The 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 sterilized test – tube. Another 5ml of sterile water was again poured on the surface of Agar slant and stirred again and this is poured into the same test tube. This results to 10ml concentration of microorganism in the sterilized test tube and this forms the standard 10^9 cell/ml colony forming unit of Leptospirillum Ferrooxidans. 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 10^1microbial populations. Each of the microbial population was inoculated into 2.5g of deslimed iron ore and put into incubating bottles. These samples were incubated for 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, and 88days at 25^oC. Each of the sample was analyzed

Degree of biodephosphorization (%)
= As deslimed value P (wt%) – Final value P (wt%) x 100
      As deslimed value P (wt%)   1

Degree of biobeneficiation (%)
= Final value Fe (wt%) – As deslimed value Fe(wt%) x 100
      Final values Fe(wt%)   1

E. Development of statistical multivariable model

Standard central composite design (CCD) with 2^2 full factorial design were employed. This was constructed from 2^{m-1} design for cube portion, which is augmented with centre points and star points.

$$N = k^{m-t} + 2\,m + No \qquad ...(1)$$

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

$N = 2^{(2-0)} + 2 \times 2 + 3 = 11$runs
The model equation for the experiment is proposed as

$$Y_n = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_2^2 \qquad ...(2)$$

The coefficients were obtained using the following equations:

$$bo = a\sum_{u=1}^{N} yu + P\sum_{j=1}^{M}\sum_{u=1}^{N} x^2 ju\ yu \qquad ...(3)$$

$$b_{1=}e\sum_{u=1}^{N} x_1 u\ yu \qquad ...(4)$$

$$b_2 = e\sum_{\mu=I}^{N} x_2 u\ yu \qquad ...(5)$$

$$b_3 = e\sum_{\mu=I}^{N} x_3 u\ yu \qquad ....(6)$$

$$b_{12} = g\sum_{u=1}^{N} x_1 u x_2 u\ yu \qquad ...(7)$$

$$b_{13} = g\sum_{u=1}^{N} x_1 u\ x_3 u\ yu \qquad ...(8)$$

$$b_{23} = g\sum_{u=1}^{N} x_2 u\ x_3 u\ yu \qquad ...(9)$$

$$b_{11} =$$
$$c\sum_{u=1}^{N} x_{1u}^2\ yu + d\left[\sum_{u=1}^{N} x_{2u}^2 yu + \sum x_{3u}^2\ yu\right] + P\sum_{u=1}^{N} yu \qquad ...(10)$$

$$b_{22} = c\sum_{u=1}^{N} x_{2u}^2\ yu + d\left[\sum_{u=1}^{N} x_{1u}^2 yu + \sum_{u=1}^{N} x_{3u}^2 yu\right] + P\sum_{u=1}^{N} yu \qquad ...(11)$$

$$b_{33} = c\sum_{u=1}^{N} x_{3u}^2 yu + d\left[\sum_{u=1}^{N} x_{1u}^2 yu + \sum_{u=1}^{N} x_{2}^2\ yu\right] + P\sum_{u=1}^{N} yu \qquad ...(12)$$

F. Design of experiment for leaching

Statistically designed experiment for leaching treatment based on Central Composite Design Plan of 2^2

Number of variables = 2
Number of star points = 4
Number of centre points = 3

X_1 = microbial Populations (10' cells/ml - 10^8 cell/ml)
X_2 = Leaching time (8days – 88days)

Table 2.1 experimental range and level of independent variables

Independent Variables	Lower level - 1	Base level O	Upper level + 1
X_1	10^1 cells/ml	10^4 cells/ml	10^8cells/ml
X_2	8days	48days	88days

G. Design matrix and responses

	x_1	x_2	yu(P)% Biodephosphorization	yu(Fe)% Biobeneficiation
1	0	0	79.75	43.50
2	-1	-1	01.26	1.86
3	+1	-1	16.46	2.66
4	0	0	79.75	43.50
5	-1	+1	79.75	46.33
6	+1	+1	82.28	47.48
7	0	0	79.75	43.50
8	-1	0	79.75	43.45
9	+1	0	82.28	44.57
10	0	-1	05.05	1.89
11	0	+1	82.28	46.38

H. Developed model equation for Biodephosphorization

$$yu = 79.75 + 1.47\ x_1 + 38.39\ x_2 - 1.27\ x_1 x_2 - 37.98\ x_2^2 \quad \dots(14)$$

I. Developed model equation for Biobeneficiation

$$yu = 43.56 + 0.52\ x_1 + 22.30\ x_2 + 0.09 x_1 x_2 + 0.45\ x_1^2 - 19.43\ x_2^2 \quad \dots(15)$$

J. Optimization programs using Matlab

For phosphorus for Leptospirillum Ferrooxidans.

```
>> 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 =   1.0000   0.4677 fval =   92.2126 exitflag =    1
output =
    iterations: 3
     funcCount: 15
```

stepsize: 1
algorithm: 'medium-scale: SQP, Quasi-Newton, line-search'
firstorderopt: 7.8512e-007
cgiterations: []
message: [1x144 char]

For Iron for Leptospirillum Ferrooxidans.

```
>> 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 =
   1.0000
   0.5762
fval =
  50.9802
exitflag =
    1
output =
    iterations: 4
     funcCount: 19
     stepsize: 1
    algorithm: 'medium-scale: SQP, Quasi-Newton, line-search'
   firstorderopt: 5.0157e-007
    cgiterations: []
       message: [1x144 char]
>> [x1,x2] = meshgrid(-1:.05:1, -1:.05:1);
>> z=43.56+0.52*x1+22.30*x2+0.09*x1.*x2+0.45*x1.^2-19.43*x2.^2;
>> surfc(x1,x2,z)
>>
```

III. RESULTS AND DISCUSSION

Table 3.1: Chemical analysis of Agbaja Iron ore before desliming

Component	Average Composition (wt%)
Fe	56.00
SiO_2	5.16
S	0.12
Al_2O_3	6.60
CaO	0.23
MgO	0.07
MnO	0.18
TiO_2	0.37
K_2O	0.04
P	0.80
H_2O	2.60

Table 3.2: Chemical analysis of Agbaja iron ore after desliming

Component	Average Composition (wt%)
Fe	56.34
SiO_2	5.02
S	0.09
Al_2O_3	5.20
CaO	0.21
MgO	0.03
MnO	0.17
TiO_2	0.32
K_2O	0.007
P	0.79
H_2O	2.81

Table 3.3: Result of microbial characterization of Leptospirillum Ferrooxidans

Stain	Gram Stain
Shape	Spiral, discoid
Arrangement	Single
Colour	Purple
Stain character	Positive
Presumptive test	Spirillum specie suspective
Confirmatory test Coagulase	-
Manitol	+
Litmus milk	Acidic
Nitrate reductase	-
Fe oxidase	+
Phosphorus reductase	+
Sulphide oxidase	_

Table 3.1 depicts the chemical analysis of as received Agbaja iron ore. The iron content shows that the iron ore, if properly beneficiated and dephosphorized can be used in steel making. The high phosphorus content explains the reason why the iron ore has not been exploited.

The chemical analysis of deslimed iron ore is shown in Table 3.2. The desliming process increased the degree of

beneficiation from 56% to 56.34% and degree of dephosphorization from 0.80% to 0.79%. It can be inferred that desliming removes extraneous materials like sand and dirts but contributes insignificantly to beneficiation and dephosphorization.

Table 3 shows the preliminary and confirmatory tests of the organism. The gram test showed spiral discoid shape under microscope. The bacterium is arranged in single form and the colour is purple. The stain character is positive showing gram positive specie. The spirillum specie is suspected as a result of gram stain test.

The confirmatory test showed that coagulace, nitrate reductase and sulphide oxidase tested positive while the test for manitol, iron oxidase and phosphorus reductase tested negative. The organism is gram positive organism which tested positive to acid test, confirming the bacterium as Leptospirillum Ferrooxidans.

A. Influence of leaching time on degree of biodephosphorization at 25^0c

In Figure 1 the influence of leaching time is shown. In nutrient limited environments, bacterial must colonize mineral surfaces where phosphate is located in order to scavenge (Banfield et al, 1999). They accomplish this through biofilm formation. Biofilms are complex aggregates of bacterial cells, bacterial exopolymers, mineral debris and other metabolites attached to a surface.

Between the 8th day and 16th day the amount of phosphorus removed was 16.46% and 18.99% respectively for microbial population of 10^1 cells/ml. The percentage removal of phosphorus was small due to the fact that Lag phase predominates implying that cells division has not fully commenced. The degree of biodephosphorization for the 32nd day was 74.68% implying full mobilization of Leptospirillum Ferrooxidans. The rate of biodephosphorization increased experimentally from 16thday. This exponential growth rate continues until the 40th day where the maximum rate of
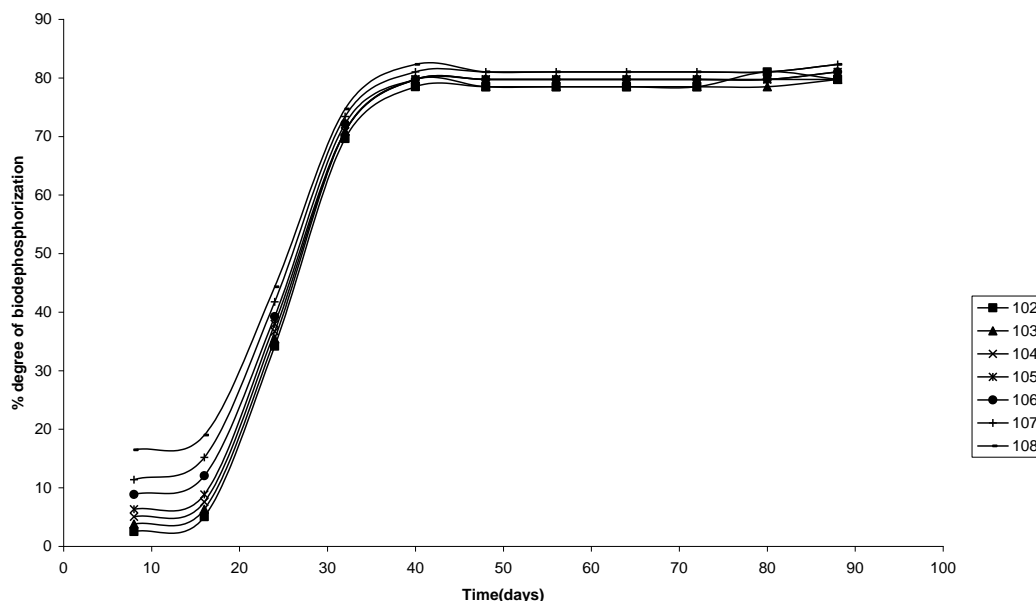

Fig. 1: Influence of leaching time on the percentage degree of biodephosphorization for Leptospirillum Ferrooxidans

biodephosphorization of 81.01% was achieved. A fairly constant degree of phosphorus removal was achieved between 40th day and 88th day. This is due to the dynamic balance between growth and death of leptospirillum Ferrooxidans during metabolism. The work of Delvasto et al (2009) using Burkholderia Caribensis FeGlO$_3$ posted a maximum of 20% degree of dephosphorization in 21 days. This work posted 30% degree of biodephosphorization in 21 days.

B. Effects of microbial population on degree of biodephosphorization at 25°C

Figure 2 represents the influence of microbial population on the degree of biodephosphorization for Leptospirillum Ferrooxidans. From microbial population of 10^{1}cells/ml, there was a steady increase of degree of biodephosphorization for 8 days, 16 days, 24 days and 32 days profile as shown in the figure. The degree of biodephosphorization was fairly constant between 40days and 88 days as the microbial population increases. It can be inferred that increase in the quantity of phosphorus removed.

C. Influence of leaching time on the degree of beneficiation

The influence of leaching time on the percentage degree of bio beneficiation is shown in figure 3. From 8days to 24 days the degree of biobeneficiation slightly increases due to the lag phases of the between 24days and 44days there was a substantial increases of the iron constant showing full mobilization of leptospirillum ferrooxidans. The degree of biobeneficiation was fairly constant between 48 days and 88 days. This is as a result of dynamic balance between growth and death rates of leptospirillum ferrooxidans.

D. Influence of Microbial population on the degree of biobeneficiation

In Figure 4 the influence of microbial population on degree of biobeneficiation is represented. Released metals can be accumulated in biofilms by complexation with active moieties present in exopolymeric substances (Corzo et al, 1994, Conte et al, 2006). This accounts for the initial drop in the degree of biobeneficiation between 8th day and 24th day of treatment as shown in Figure 4. There was an infinitesimal change in biobeneficiation as the microbial population increases. This trend was observed for the rest of the leaching times. But the highest beneficiation was 33% at microbial population of 10^8 as shown in Figure 4.

E. Interactions of process variables and surface responses

The design matrix is depicted in Table 2.2. The process variables x_1 leaching time and x_2 microbial population were taken as independent variables while percentage degrees of biodephosphorization and biobeneficiation were the output responses. The analysis is based on how the percentage degrees of biodephosphorization and biobeneficiation are influenced by independent variables in order to study the surface responses.

Figure 5 shows the surface response plot for removal of phosphorus using time and microbial population variables. The response shows that the leaching time affected the degree of biodephosphorization than the microbial population. The optimum value is located at the dark red portion.

In Figure 6 the surface response plot for beneficiation is depicted. The figure shows that the leaching interacted effectively with microbial concentration. The surface response shows that leaching time responded better than microbial population.
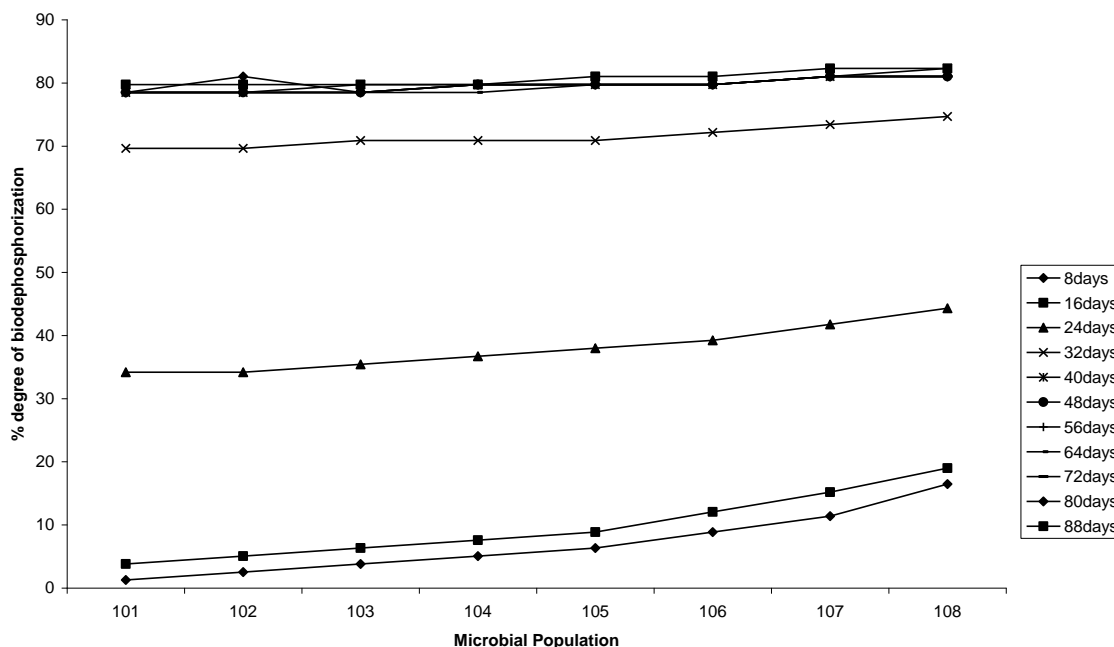

Fig. 2: Influence of Microbial Population degree of biodephosphorization of Agbaja Iron Ore for Leptospirillum Ferrooxidans
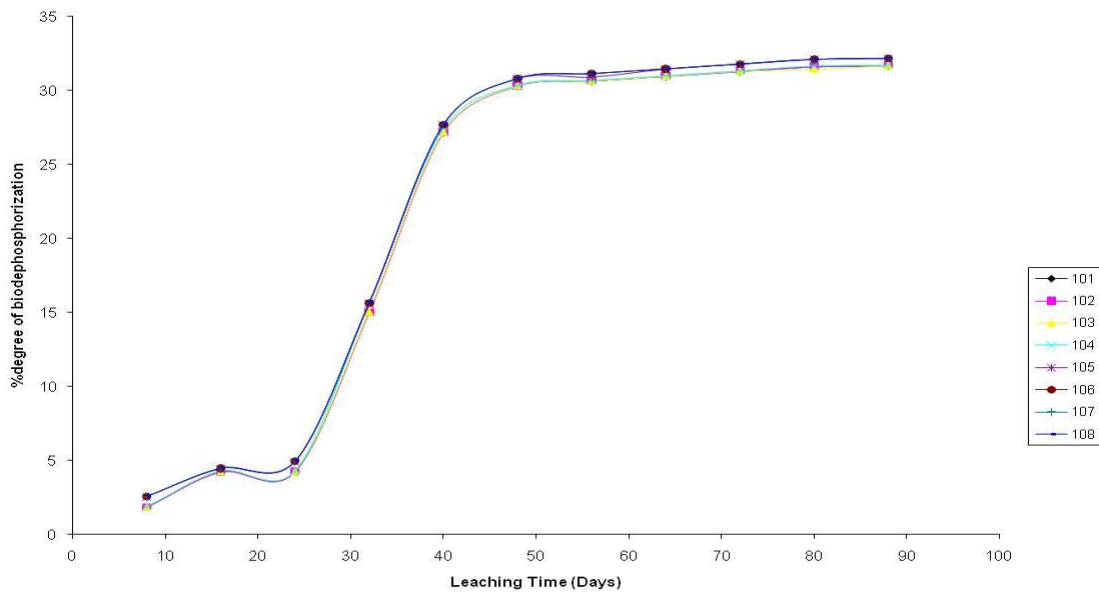
Fig. 3: Influence of leaching time on the %degree of biobeneficiation for microbial population for Leptospirillum Ferrooxidans

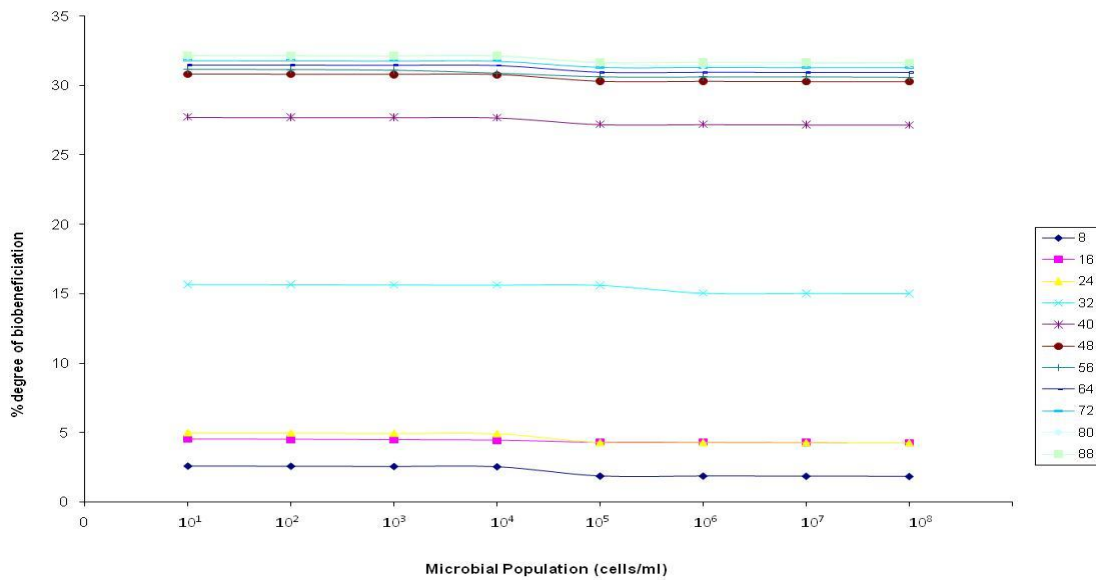Fig. 4: Influence of microbial population on degree of biobeneficiation

Table 3.4: Optimum parameters from developed model equation (Biodephosphorization)

Independent Variables	Ftest	Coded Values	Real Values	Optimum Biodephosphorization %
X_1 leaching time	0.99	0.4677	67 days	92.21%
X_2 Microbial Population		1.000	10^8 cells/ml	

Table 3.5: Optimum parameters from developed model equation (Biobeneficiation)

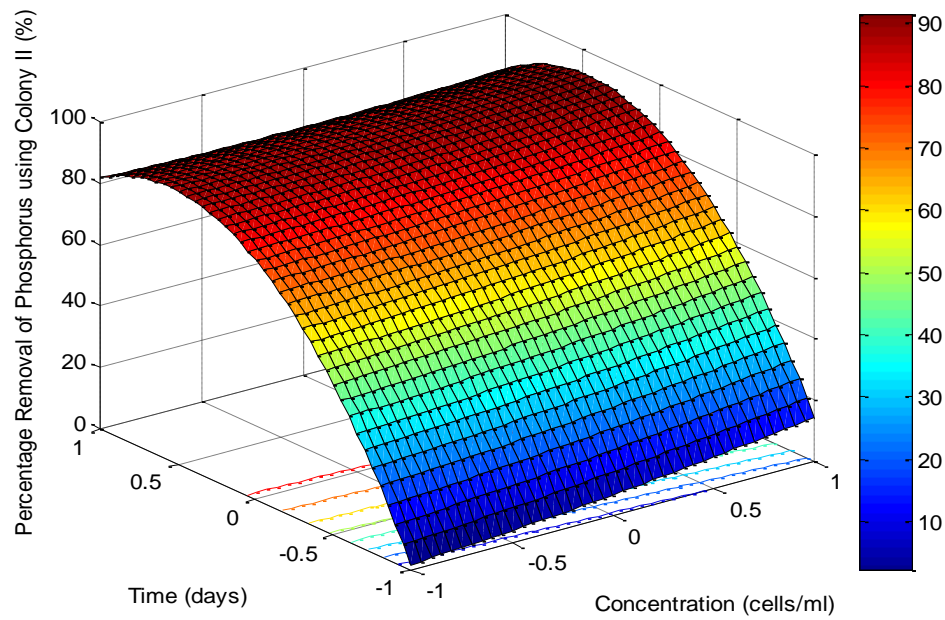| Independent Variables	Ftest	Coded Values	Real Values	Optimum Biobeneficiation %
X_1 leaching time	0.99	0.5762	71 days	50.98%
X_2 Microbial Population		1.000	10^8 cells/ml	

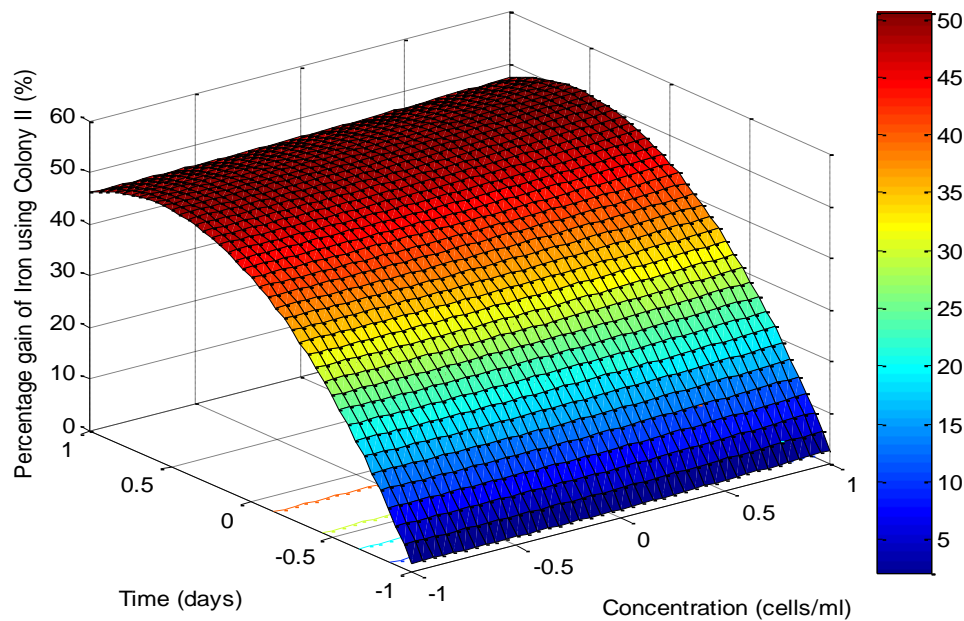Fig. 5: Percentage removal of phosphorus using Leptospirillum Ferrooxidans (time and concentration as variables)

Fig. 6: Percentage gain of Iron using Leptospirillum Ferrooxidans (time and concentration as variables)

Table 3.4 represents the optimum parameters for bio dephosphorization. The result shows that 92.21% degree of biodephosphorization was obtained at optimum value of 67 days and 10 cells/ml. This shows that leptospirillum ferrooxidans was effectively mobilized in the course of biodephosphorization. The F_{test} obtained helped in determination of the adequacy for the model.

Similarly, Table 3.5 depicts the optimum values for biobeneficiation. 50.98% percentage degree of biobeneficiation was obtained at optimum values of 10^8 cells/ml and 71days.

IV. CONCLUSION

Biodephosphorization and biobeneficiation have been carried out on Agbaja Iron ore successfully using leptospirillum. Ferrooxidans. The models developed using central composite design was adequate. The surface response

plots showed that leaching time and microbial population interacted effectively. On optimization of the models the degrees of biodephosphorization and biobeneficiation of 92.21% and 50.98% were respectively obtained.

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