CHAPTER 4

DETERMINATION OF OPTIMUM CULTURAL CONDITIONS FOR BIOLEACHING OF SILICA AND IRON OXIDE FROM BAUXITE ORE BY *ASPERGILLUS NIGER* X_1.

The effect of the size and pulp density of the ore has been discussed in the previous chapter. Other physical conditions which are necessary to understand the adoption and silica tolerance level of the microbe with respect to the mineral substrates are (i) temperature of fermentation (ii) volume of medium (iii) pH of the medium (iv) incubation period (v) inoculum volume (vi) age of inoculum. The optimum conditions of these physical parameters are discussed in the present chapter.

Microbial growth rate, as with all chemical reactions, is a function of the temperature. In general, micro-organisms will grow over a temperature range of 25 to 30°C. However, it is interesting to note that there are micro-organisms in the environment that grow at temperature below 0°C and above 93°C. The effect of temperature on the specific growth rate is described by the Arhenius equation

$$\mu = Ae^{-Ea/RT}$$

Where A is the Arhenius constant, Ea is the activation energy (Kcal/mol), R is the Universal gas constant, T is absolute temperature. The typical values of activation energy for microbial growth are 13 to 17 Kcal/mol. Above that maximum temperature the growth rate begins to fall. This results from increased rate of microbial death. The dependence of death rate is a strong function of temperature and is characterised by activation energies of 70 to 90 Kcal/mol. This high values of Ea for microbial death means that the rate of death increases

much faster with temperature, than the rate of growth (low values of Ea) (157).

Temperature also affects the efficiency of the carbon-energy substrate conversion to cell mass. This has been proved in the studies of the growth of a yeast and mixed population of bacteria (158).

It is not surprising that temperature affects a variety of metabolic processes in the cell. Macromolecular composition, especially RNA content, as well as growth rate are strong functions of temperature. This was established by Alroy and Tannenbaum (159). So, in process optimization, it is important to realise that temperature may affect both the growth rate and the product synthesis rate.

As a consequence of the laws of conservation of mass, it is essential to balance the elemental requirements for growth and product formation. Volume of medium meet up the nutritional requirements of organism. No consideration was given to the effect of excess amounts of nutrient on the fermentation process. High concentration of many nutrients are in fact inhibitory to a fermentation process. This inhibition can result from non-specific osmotic effects involving interaction of a chemical nutrient with a particular enzyme or membrane component. In addition nutrients exert metabolic effects at the level of transcription of necessary genes. In particular, there may be carbon, sulfur, phosphate and nitrogen catabolite repression. These repressive phenomena may have

little or no effect on growth but may have substantial effect on product synthesis. The most common means of designing an appropriate repression effect is to utilize a fed-batch on continuous culture method of operation. These procedures allow one to supply the necessary nutrients at the same rate as the demand for the nutrient and the process maintain an optimal chemical environment for growth and product formation.

C source + N source + O_2 + minerals + specific nutrients ——> Cell mass + Product + CO_2 + $2H_2O$

The use of the stoichiometry in above equation is necessary to calculate minimal nutrient requirements, Haggstrom focused on this aspect **(160)**. The volume at which the organism obtain the most suitable nutrition will be the optimal volume of fermentation medium.

The pH, a measure of the hydrogen ion (H^+) activity, is particularly important as a parameter of microbial growth. Most micro-organisms grow over a pH range of 3 to 4 pH units. This represents a 1000 to 10,000 fold range of hydrogen ion concentration. It is possible to make some generations that are useful in defining a chemical environment for the cell. Molds often have pH optima between 5 to 7 but the yeast will tolerate a wide range of pH values eg, 3 to 8.5. There appears to have a relationship between pH and temperature according to Brock et,al. **(157)**.

While control of pH is important to the maintenance of an optimal environment of growth and product formation, it is

also important to examine the means of pH control. The pH changes in response to microbial activities : a H^+ is generated during NH_4^+ uptake, H^+ is consumed during NO_3^- metabolism, H^+ consumed when amino acids are used as a carbon source. Thus, by monitoring pH and controlling pH, it is possible to gain both qualitative and quantitative insight into the fermentation. Titration of pH changes, however, will also alter the chemical environment of the cell. If NH_3 is used to balance H^+ generation from ammonia metabolism, then the NH_4^+ concentration will be maintained constant, however, if it is used to titrate an acid that is being formed, one may quickly develop ammonia toxicity. When titrating acid production, it is more common to use NaOH or $Ca(OH)_2$, however, care should be taken there, because they affect the ionic strength of the medium and also product stability. Thus in industrial fermentation, the maintenance and control of pH is a critical variable.

For silica solubilization, that pH will be the optimum, where the final pH values are minimum. Organic acids produced from glucose by **Aspergillus niger** are mainly responsible for silica solubilization. Organic acids dissolve the silica and iron similarly as mentioned by other investigators(161-163)Mishra et al. (164) also discussed the optimization of pH in case of bacteria.

In microbial growth optimum time period is of importance because the organism takes a specific time for its growth and once the growth is complete, further aging does not impart any positive effect on the desired culture, on the

contrary, it causes the inhibition of growth and cell destruction due to over population. So, the optimization of time period of fermentation for silica solubilization is important.

The size of the inoculum is important for the initiation of growth in suspension culture. Below a critical ratio of inoculum to volume of medium, cell division ceases. This observation led to the assumption that the medium has to be conditioned by factors released from the cells of inoculum. Growth only resumes after a critical concentration of these factors is attained in the medium **(165)**. Ten to fifteen percent of v/v of an actively growing suspension culture has been found to be a satisfactory inoculum for most species according to Veliky and Genest **(166)**, Verma and Van Huystee **(167)**, and Wang and Staba **(168)**. The ratio of free cells to cell aggregates influences the growth rate **(169)**. In some investigations the inoculum was perfiltered to obtain a more homogeneous cell suspension **(170,171)**. Larger inoculum may reduce the conditioning time of the medium but may lower the relative growth rate of cell **(172)**. So, for silica solubilization optimum volume of inoculum is important and that has to be determined.

Every micro-organism has its own growth curve. So, for a specific organism specific number of days are required for optimum growth of the organism. On that day concentration is maximum. It supplies the correct amount of inoculum for fermentation. So, age of inoculum has specific effect on fermentation medium and that has to be determined.

4.1 Experimental and Results :

The medium used for the fermentation of bauxite for leaching of silica and iron, consisted of 0.2% $NaNO_3$, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.05% KCl and Yeast extract 0.01%. The pH was adjusted to 4. It was sterilized at 121°C for 15 min, 4% glucose was sterilized separately and added to the medium aseptically.

The optimum cultural conditions for the bioleaching of silica and iron from bauxite ore by **A.niger** X_1 were worked out by keeping all the facts constant except the one which was varied.

The micro-organism and the determination of concentration of silica and iron were same as discussed in the earlier chapter (Chapter 2 Page 47 of the thesis).

4.1.1 Effect of Temperature on Leaching :

Temperature had a very significant role in the release of silica and iron by **A.niger** X_1. Resutls are shown in Table 4.1.

Table 4.1 Effect of temperature on leaching of silica and iron from bauxite ore by Aspergillus niger X_1.

Temperature (°C)	Cellular growth Dry wt. (g/l)	Silica* leaching(%)	Iron Oxide* leaching(%)
22	3.2	16.0	18.0
24	4.0	28.2	30.4
26	5.4	40.4	48.6
28	6.0	52.0	59.2
30	6.4	55.0	59.6
33	6.4	42.0	44.8
35	6.1	33.1	37.0
37	5.2	26.2	28.2
40	3.8	11.0	14.2

* Each figure is the mean value of 3 individual experiments

Table 4.1 indicates that as the temperature was increased from 22 to 30°C, the release of silica and iron was greatly increased from 16.0 to 55.0 and 18.0 to 59.0 percent respectively. Further increase in temperature significantly inhibited the release of silica and iron. The highest activity at 30°C may be due to the metabolically more active synthesis of the silica solubilizing agent at that temperature.

4.1.2 Effect of volume of medium on leaching :

The optimum volume of medium for leaching of silica and iron was determined by carrying out the surface culture fermentation experiments, adjusting the different volume at 50 ml, 80 ml, 100 ml, 130 ml, 150 ml in 250 ml conical flasks. The results are shown in Table 4.2.

Table 4.2 Effect of different volume of medium on leaching of silica and iron from bauxite ore by Aspergillus niger X_1.

Volume of of medium (ml)	Cellular growth Dy wt. (g/l)	Silica* leaching (%)	Iron Oxide* leaching (%)
40	4.8	42.8	50.0
50	5.7	50.7	56.6
80	6.0	52.0	59.2
100	6.4	49.5	50.3
130	6.7	40.1	47.8

*** Each figure is the mean value of 3 individual experiments.**

Table 4.2indicates that the optimum volume of medium for maximum leaching of silica and iron is 80 ml, maximum growth of the organism, however, is at 130 ml volume. This is probably

because of the fact that at more than 80 ml volume the metabolically produced silica and iron solubilizing agents are getting diluted.

4.1.3 Effect of initial pH of the medium on leaching.

These series of experiments were carried out with the medium containing 4% glucose as substrate. The optimum pH for leaching of silica and iron was determined by carrying out fermentation experiments adjusting the different initial pH values to 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 with 2 (N) HCl or NaOH in 250 ml flasks at 28°C, with 5 ml spore suspension of A.niger X_1. The results are shown in Table 4.3.

Table 4.3 Effect of initial pH on the medium on leaching of silica and iron from bauxite ore by A.niger X_1.

Initial pH of the medium	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron Oxide* leaching (%)
3.0	5.0	30.0	35.2
3.5	5.7	42.7	48.5
4.0	6.0	52.0	59.2
4.5	6.6	47.0	50.7
5.0	7.0	37.4	40.0
5.5	6.3	32.0	36.4
6.0	5.5	28.6	30.0

*** Each figure is the mean value of 3 individual experiments.**

Table 4.3 indicates that the optimal initial pH of the medium for maximum leaching of silica and iron is 4.0. At higher and lower initial pH values the leaching of silica and iron was considerably decreased. The optimum growth of the organism, however, is at initial pH 5.0. The final pH is decreased in the

solution where the initial pH was 5.0 or higher. At lower initial pH values a slight increase was noticable.

4.1.4 Effect of time period of fermentation on leaching

Surface culture fermentation was carried out at time periods 5, 6, 7, 8, 9, 10 days. The results are shown in Table 4.4.

Table 4.4 Effect of time period of fermentation on leaching of silica and iron from bauxite ore by A.niger X_1.

Period of fermentation (Days)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron Oxide* leaching (%)
5	5.1	37.1	40.3
6	5.6	39.5	50.3
7	6.0	52.0	59.2
8	7.0	48.2	49.7
9	7.4	41.6	43.0
10	6.7	33.0	34.6

***Each figure is the mean value of 3 individual experiments**

Table 4.4 indicates that the maximum leaching of silica and iron is obtained in 7 days of fermentation and beyond this there is a decrease of leaching capacity of A.niger X_1.

4.1.5 Effect of inoculum volume on leaching :

The optimum volume of inoculum for leaching of silica and iron was determined by carrying out fermentation experiments adjusting the different volume of inoculum to 3, 4, 5, 6, 7, 8 and 10 percent, respectively, of the volume of the medium containing 12 x 10^7 spores/ml. The results are shown in

Table 4.5.

Table 4.5 Effect of inoculum volume on leaching of silica and iron from bauxite ore by *A.niger* X_1.

Volume of inoculum (%)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron oxide* leaching (%)
3.0	4.0	37.0	41.0
4.0	5.4	45.7	50.4
5.0	6.0	55.0	50.6
6.0	6.4	56.0	62.6
7.0	7.0	49.0	51.2
8.0	6.5	44.0	46.4
10.0	6.2	40.2	41.8

*Each figure is the mean value of 3 individual experiments.

Table 4.5 indicates that the optimal volume of inoculum for maximum leaching of silica and iron oxide is 6%. The optimum growth of organism however is at 7%.

4.1.6 Effect of age of inoculum on leaching

Culture of 3 days, 4 days, 5 days, 6 days, 7 days and 8 days were used for inoculation of fermentation medium. Table 4.6 indicates the results.

Table 4.6 Effect of age of inoculum on leaching of silica and iron from bauxite ore by *A.niger* X_1.

Age of inoculum (Days)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron oxide* leaching (%)
3	4.2	21.4	25.4
4	5.4	32.6	36.5
5	6.1	48.0	51.2
6	6.4	56.0	62.6
7	6.4	47.0	48.0
8	5.8	39.4	40.3

*Each figure is the mean value of 3 individual experiments.

Table 4.6 shows that the optimal release of silica (56% and iron oxide (62.6%) from bauxite ore is affected when the microbe is more old than 6 days.