CHAPTER 5

SELECTION OF SUITABLE CARBON AND NITROGEN SOURCES ON SILICA AND IRON LEACHING FROM BAUXITE ORE BY *ASPERGILLUS NIGER* X_1 AND DETERMINATION OF THEIR OPTIMUM CONCENTRATION.

High percentage of leaching depends to a great extent on the response of organism to the composition of the medium.

Fungi lack the green pigment, chlorophyll and therefore cannot produce their own food material. The majority of the known fungi obtain food either from living organisms or from dead organic substitutes. All fungi require C, O, H, N, P, to fulfil their nutritional requirements. Glucose, nitrogenous and ammonium compounds and nitrates form the best food for many fungi. Food molecules of smaller size especially are easily absorbed by the fungi and the large sized molecules are converted by some extra cellular enzymes secreted by the fungus to smaller ones and are absorbed.

It is to be expected that growth rate, as any chemical reaction rate, will depend on the concentration of chemical nutrients. The most commonm expression used to describe the dependence is the Monod relationship (173).

$$\mu = \mu_m \ [S/(K_2 + S)]$$

where μ and μ_m are the specific growth rate and maximum value respectively (h^{-1}), S is the substrate concentration and K_s is the value of the substrate concentration at $\mu = 0.5\ \mu_m$. The formulation of this equation although based on the analogy with saturation kinetics in monomolecular adsorption and not theoretically described, is quite convenient. The model suggests that the dependence of growth on chemical concentration can be described by two constants, the maximum growth rate and the saturation constant K_s. This has been found to be true for a wide variety of nutrients as shown by PIRT (174).

5.1 Selection of suitable carbon source on silica and iron leaching from bauxite ore *A.niger* X_1 :

Fungus when cultured in the laboratory, carbon containing compounds are supplied as food molecules. It is observed that compounds containing more than three carbon atoms serve as better source. This is because of the fact that after one or two initial reactions the majority of those compounds are oxidised via glycolysis and the TCA cycle. This route involves, as intermediates three carbon compounds which can be carboxylated to give four carbon intermediates in the TCA cycle, and satisfy the condition of the biosynthetic precursors necessary for growth.

For various carbon sources, there will have the optimum value of carbon source concentration in each case. Such carbon source concentration like all other nutrients have an upper concentration limit above which further increase will cause a decrease in growth rate. This is called substrate inhibition effect. The reason is usually osmotic pressure. The increasing concentration causes partial dehydration of the cell and the effect is reduced growth rate. Bacteria are more sensitive to osmotic effects than are the yeasts and molds.

The carbon source concentration may also affect product formation. At low concentration, an increasing substrate concentration is usually favourable to product synthesis. However, many fermentation products of interest are subject to carbon catabolite repression. Thus, in order to maintain high rate of product formation, the carbon source must be kept below some critical value.

5.1.1 Experiment and Results for selection of suitable carbon source on silica and iron leaching from bauxite ore by A.niger X_1 :

The basal medium used for investigating the effect of carbon source consisted of 0.2% $NaNO_3$, 0.1% KH_2PO_4, 0.05% KCl and 0.05% $MgSO_4.7H_2O$ and 0.01% yeast extract. pH was adjusted to 4. Time of incubation was 7 days.

A series of carbon sources were used in the medium to observe their effect on the leaching of Si and Fe from bauxite ore and the results are shown in Table 5.1.

Among the monosaccharides used glucose was the best carbon source for the maximum leaching of silica and iron, which was incidentally also the best carbon source among all. Fructose mannose and mannitol were capable of leaching silica and iron to a small extent, whereas ethyl alcohol as a carbon source failed to yield any result. Some reports are obtained on the effect of carbon source and their concentration on bacterial leaching of silica **(175)**. An observation was reported for the growth of fungi regarding its nutrient requirements **(176)**. Investigations of copper ore leaching indicate that the copper dissolution as well as the fungal activity were dependent upon the medium composition **(177)**, but optimum nutrient concentration was not reported. The requirement of the 5% glucose concentration reported in case of <u>P.simplicissimum</u> (177).

It is apparent from table 5.1 that among the carbon rich sources used, glucose produced best results. When sucrose and starch were used, moderate leaching of iron observed but the release of silica was very low. The release of silica is less in case of lactose. When sodium succinate, sodium acetate were used as carbon source, no growth of the organism was observed. In case of salts, may be the pH of the medium was so lowered down that no growth could be observed.

Table 5.1 Effect of different carbon sources on the leaching of silica and iron by *Aspergillus niger* X_1

Carbon sources (4% conc. taken)	Cellular growth Dry Wt. (g/l)	Silica* leaching (%)	Iron Oxide* leaching (%)
Monosaccharides			
Glucose	6.4	56.0	62.6
Fructose	5.4	37.2	32.7
Manitol	5.1	26.1	24.2
Mannose	4.2	18.1	16.1
Xylose	4.4	10.1	11.2
Disaccharides			
Sucrose	6.6	38.2	36.2
Lactose	5.8	21.9	27.2
Polysaccharides			
Amylose	5.7	23.0	24.0
Cellulose	4.1	10.0	11.1
Glycogen	4.0	2.4	2.0
Starch	6.0	30.6	32.0
Organic Acids			
Citric acid	6.2	21.7	20.0
Glutamic acid	5.5	11.8	12.0
Lactic Acid	5.2	7.7	8.2
Fumaric acid	Nil	0	0

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

Table 5.1 (Continued)

Carbon sources (4% conc. taken)	Cellular growth Dry wt.(g/l)	Silica* leaching (%)	Iron Oxide* leaching (%)
Salts of organic acids			
Calcium gluconate	4.2	16.0	14.2
Sodium succinate	Nil	0	0
Sodium acetate	Nil	0	0
Alcohol			
Ethyl alcohol	Nil	0	0

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

5.1.2 **Determination of optimum concentration of glucose :**

As glucose is the best carbon source for the leaching of silica and iron from bauxite ore by **A.niger** X_1 different concentrations of glucose were tested to determine the optimum concentration for the leaching of silica and iron as shown in Table 5.2.

Table 5.2 Effect of different glucose concentrations on leaching of silica and iron from bauxite ore by A.niger X_1

Glucose (%)	Cellular Growth Dry wt (g/l)	Silica* leaching (%)	Iron Oxide* leaching (%)
2.0	4.2	20.7	24.2
3.0	5.8	39.15	46.8
4.0	6.4	56.0	62.6
5.0	6.7	58.4	65.30
6.0	7.0	46.13	52.2
7.0	7.3	41.20	46.0

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

Table 5.2 shows that glucose at a concentration of 5% gives maximum leaching of silica and iron from bauxite ore where as a lower or a higher dose of sugar decreases the leaching capacity of <u>A.niger</u> X_1.

5.2 Selection of suitable nitrogen source on silica and iron leaching from bauxite ore by A.niger X_1 :

Nitrogen is an essential element for fungal growth, as about 14% of the dry weight of bacteria and fungi is nitrogen. A wide range of inorganic and organic nitrogen compounds are utilised to satisfy the requirement for this element.

Nitrogen is assimilated in the cell as glutamate and glutamine **(178)**. These compounds serve as a donar of nitrogen to many nitrogen containing molecules like tryptophan, histidine, asparagine and also purine nucleotides.

Micro-organisms are able to grow at their maximum growth rate at very low nitrogen conc. The low values reflect the effectiveness of the active transport systems of cells for these materials. In general the concentration of nitrogen compounds in the environment is low and as a consequence cells that can grow fast in low concentration have an important competitive advantage.

Satisfying the low concentration limit for microbial growth does not usually cause a problem to the fermentation technologists. More often, problems of substrate inhibition develop from providing too much nitrogen. If a culture becomes starved of carbon source in the presence of amino acids, the carbon skeleton of the amino acids is consumed and ammonia accumulates. If NO_3^- is used as nitrogen source, it can be partially reduced to NO_2^- which may be toxic to the cell. Thus the effect of a nitrogen source may be derived from its metabolic products as well as from its original form.

During studies on the cultural conditions it has been observed that silica and iron leaching from bauxite ore by **Aspergillus niger** X_1 is maximum in the medium constituting of glucose as a carbon source. Since the composition of the medium greatly influences the leaching of silica and iron from bauxite ore, it is necessary to find out suitable carbon and nitrogen sources for silica and iron leaching. In the present work, a study has been made to use the effect of the different nitrogen sources for the leaching of silica and iron from bauxite ore.

5.2.1 Experiment and Results for selection of suitable nitrogen source on silica and iron leaching from bauxite ore by A.niger X_1 :

The basal medium used for investigating the effect of nitrogen source consisted of glucose 5%, KH_2PO_4 0.1%, KCl 0.05%, $MgSO_4.7H_2O$ 0.05%, and yeast extract 0.01%. pH was adjusted to 4. Time of incubation was 7 days. The different solutions containing different nitrogen sources were sterilised and added to the sterile basal medium aseptically to the concentration equivalent to 0.032 gm nitrogen in 80 ml basal medium. 5% glucose was sterilised and added to the medium aseptically.

The results are shown in the following Table 5.3.

Table 5.3 Effect of different nitrogen sources on leaching of silica and iron from bauxite ore by A.niger X_1

Nitrogen source (0.032 gm N)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron oxide* leaching (%)
Sodium nitrate	6.7	58.4	65.3
Ammonium sulphate	7.2	20.6	22.6
Ammonium acetate	4.7	20.3	27.1
Ammonium chloride	6.7	59.6	68.5
Ammonium nitrate	6.6	46.7	53.5
Ammonium oxalate	5.0	Nil	34.6
Triammonium citrate	4.7	Nil	39.7
Ammonium phosphate	5.2	Nil	26.3
Urea	5.9	46.6	47.4

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

Among the nitrogen containing nutrients supplied, NH_4Cl was found to be the most potent source of nitrogen as the release of silica and iron is maximum. Moderate leaching of silica was observed when sodium nitrate, ammonium nitrate, ammonium acetate or urea were used as nitrogen source. The percentage of leaching is very low when ammonium acetate was used as nitrogen source. Others failed to release silica. As ammonium chloride easily dissociates in the medium, pH of the medium gets lowered down readily. This may be the cause for which ammonium chloride is more effective than other nitrogen sources as nutrient.

5.2.2 Determination of optimum concentration of ammonium chloride :

Ammonium chloride being the best nitrogen source for the leaching of silica and iron from bauxite ore by **A.niger** X_1 was added in different levels to the basal medium for optimizing the concentration.

Table 5.4 Determination of optimum concentration of ammonium chloride on leaching of silica and iron from bauxite ore by A.niger X_1

Conc. of Ammonium Chloride (%)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron Oxide* leaching (%)
0.10	6.6	58.2	67.5
0.12	6.7	59.6	68.5
0.13	7.0	59.8	69.0
0.15	7.1	58.1	68.2
0.17	7.1	57.4	67.1
0.20	7.2	56.2	65.0

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

Table 5.4 shows that release of silica and iron is highest when 0.13% of ammonium chloride is added. With the higher conc. of ammonium chloride silica and iron leaching from bauxite ore decreased but cellular growth increased with increasing concentration of ammonium chloride.