CHAPTER 6

MINERAL ELEMENT REQUIREMENT OF *ASPERGILLUS NIGER* X_1 FOR BIOLEACHING OF SILICA AND IRON OXIDE FROM BAUXITE ORE.

Carbon and nitrogenous compounds are essential for the growth and metabolism of micro-organisms. They also require some micro-nutrients (more commonly termed trace elements) for their growth. Many trace elements have profound effects on the production of many commercially important primary and secondary metabolites. Weinberg's review listed the required concentration of trace elements which are important in the control of such metabolites (179).

Trace element nutrients can be classified under four heads.

(i) Trace elements required by all bacteria and fungi : Mn, Zn, Fe.

(ii) Trace elements required by many bacteria and fungi under specific growth conditions : Cu, Co, Mo, Ca.

(iii) Trace elements required by some bacteria and fungi under specific growth condition : Na, Cl, Ni, Se.

(iv) Trace elements which are rarely required : B, Al, Si, Cr, As, V, Sn, Be, F, Sc, Ti, Ga, Ge, Br, Zr, W, Li, I.

The lack of an essential trace element will sometimes prevent growth completely. Pirt expressed the relationship between the growth yield and growth rate (174).

Micro-nutrients have specific activities in case of organisms. Iron is present in cytochromes and ferredoxins. The following three elements Fe, Zn, Mn, are the most important among the trace elements responsible for the regulation of secondary metabolism. They also play a vital role in excretion of primary metabolites. Deficiencies of these elements are required for excretion of citric acid by **A.niger**. Iron deficiency is required for the excretion of riboflavin, and its high concentration affects penicillin production by **Penicillium chrysogenum** (180). Zinc is inhibitory to this latter fermentation, to griseofulvin production by **P.griseofulvum** and to aflatoxin production by **A.flavus** (180). Conversely, **streptomyces** cultures require five times the growth requirement of iron for streptomycin production (180). Copper is present in certain respiratory chain components and enzymes and so is probably required by all aerobes, deficiency stimulates, penicillin and citric acid production (180). Cobalt is present in corrinoid compounds for example vitamin B_{12} which is synthesized by many prokaryotes. Corrinoids are found in many methanogenic bacteria growing on H_2 and CO_2 as carbon and energy sources. Molybdenum is a cofactor in nitrate reductase and nitrogenase and so is required for growth of NO_3^- and N_2 gas as the sole nitrogen source. It is also required by methanogen. Nickel is required by methanogens (180).

The trace element requirement is usually small, in the range of 10^{-9} Mol l^{-1} to 10^{-6} Mol l^{-1}. Many of these elements are extremely toxic at higher concentration, usually $>10^4$ Mol l^{-1}.

Fungi have relatively large requirements for phosphorous, potassium, sulphur and magnesium but much smaller requirements for at least five micronutrients namely iron, zinc, copper, manganese and molybdenum (181-191). Phosphorous deficiency causes metabolic disturbance in **A.niger**. The most easily observed is a lowered rate of glucose utilization (181). **Aspergillus niger** utilizes either ferrous or ferric ion. Aspergillin, the spore pigment of the black **Aspergillus** species contains 0.26% iron. Probably 0.5-1 ppm of zinc is adequate, but at higher concentration it is toxic, especially at higher pH. Copper is required by **A.niger** for normal growth and sporulation at about 0.01-0.1 ppm. The most striking physiological effect of copper deficiency is reduced pigmentation. Manganese is needed in small amounts about 0.005-0.01 ppm. **A.niger** responds to as little as 0.02 ppb of molybdenum. Cobalt is not essential for **A.niger** (180).

6. EXPERIMENTAL AND RESULTS :

The synthetic medium consisted of glucose 5%, KCl 0.05%, KH_2PO_4 1%, $MgSO_4.7H_2O$ 0.05%, NH_4Cl 0.13%. The pH was adjusted to 4. The time of incubation was 7 days.

The required amounts of these chemicals were dissolved separately in 200 ml of demineralised water, the resulting solution was shaken twice with a mixture of 0.1 gm of 8-hydroxyquinoline and 5 ml of chloroform in a separating funnel, first at pH 7.2 and then at pH 5.2.

After each extraction, the solution was washed three times with 5 ml of chloroform to make the medium free from traces of 8-hydroxyquinoline. After purification all solutions were sterilized in autoclave at 15 $lbs/inch^2$ pressure for 15 min. (192).

The above mentioned solutions were asceptically taken in conical flasks in proper proportions to obtain a medium of 80 ml volume and inoculated with **A.niger** X_1 in the same manner as discussed in earlier chapter. (Chapter 2 Page No. 46).

The basal medium initially did not contain the mineral salt to be tested. The mineral salt under investigation was added in graded doses to the medium to determine the optimum leaching of silica and iron. In each subsequent experiment the composition of the basal medium was so altered as to include an optimal quantity of compound selected from the previous study. Iron is supplemented in the medium as $FeSO_4.7H_2O$. It is evident from Table 6.1 that the optimum concentration of $FeSO_4.7H_2O$ for best leaching of silica (59.6%) from bauxite ore by **A.niger** X_1 is 1 µgm. Higher concentration is proved to be inhibitory to some extent. Results on the effect of Zn on leaching of silica from bauxite ore by **A.niger** X_1 is presented in Table 6.2. Zinc is proved to be an essential trace element for such leaching because the percentage of silica leached is low where no zinc is supplemented. The rate of leaching is optimal at a concentration of 10 µgm/ml of $ZnSO_4.7H_2O$. This element has also inhibitory effect at a concentration higher than this.

Manganese is supplemented in the medium as $MnSO_4.H_2O$. The optimum concentration of $MnSO_4.H_2O$ for best leaching of silica is 5 µgm/ml. As the concentration of Mn is increased, the leaching is decreased. The concentration dependent effect of Mn has also been observed in a different situation by other workers (180).

Zinc and manganese are found to stimulate the leaching of silica from bauxite ore by **A.niger** X_1 at some optimum concentration but decrease in leaching at higher concentration. Whereas nickel and molybdenum do not impart any effect on the leaching of silica from bauxite ore by **A.niger** X_1. However, molybdenum at high concentration inhibits the leaching, Copper is the only element to inhibit leaching at any concentration of treatment.

Table 6.1 Effect of Fe (added as $FeSO_4.7H_2O$) on silica and iron leaching from bauxite ore by A.niger X_1

Concentration of $FeSO_4.7H_2O$ (µg/ml)	Cellular growth Dry wt. (g/1)	Silica* leaching (%)	Iron Oxide* leaching (%)
0(Nō Supplment)	6.7	59.6	68.5
1.0	6.7	59.6	68.5
5.0	6.8	59.6	68.5
10.0	7.2	59.6	68.5
15.0	7.5	57.6	62.7
20.0	7.7	50.06	58.2

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

Table 6.1 indicates that there is no effect on leaching of Si and Fe at lower concentration of iron but at higher concentration there is a decrease of leaching of Si and Fe. The cellular growth is increased with the increase of Fe concentration in the fermentation medium.

Table 6.2 Effect of Zn (added as $ZnSO_4.7H_2O$) on leaching of silica and iron from bauxite ore by A.niger X_1

Concentration of $ZnSO_4.7H_2O$ (µg/ml)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron Oxide* leaching (%)
0(No supplement)	6.7	59.6	68.5
1.0	6.7	62.4	70.2
5.0	6.7	65.7	73.5
10.0	6.7	66.9	70.4
15.0	6.7	56.6	66.1
20.0	6.8	50.8	62.9

*Each figure is the mean value of 3 individual experiments

Table 6.2 shows that the leaching of Si and Fe is maximum at 5 µg but there is a decrease of leaching of Si and Fe after that. There is no considerable change in cellular growth also.

Table 6.3 Effect of Mn (added as $MnSO_4.4H_2O$) on leaching of silica and iron from bauxite ore by A.niger X_1.

Concentration of $MnSO_4.4H_2O$ (µg/ml)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron oxide* leaching (%)
0(No supplement)	6.7	65.7	73.5
1.0	6.7	67.0	74.2
5.0	6.8	70.2	77.0
10.0	6.9	66.8	72.4
15.0	7.1	62.3	69.4
20.0	7.4	58.1	65.4

*Each figure is the mean value of 3 individual experiments

Table 6.3 shows that the leaching of Si and Fe is increased at lower concentration of Mn but there is a decrease of leaching of Si and Fe after that. Leaching percentage is highest at 5 µg/ml concentration. Cellular growth is increased with the increasing concentration of Mn.

Table 6.4. Effect of Mo (added as $(NH_4)_6Mo_7O_{24}.4H_2O$) on leaching of silica and iron from bauxite ore by <u>A.niger</u> X_1

Concentration of $(NH_4)_6Mo_7O_{24}.4H_2O$ (µg/ml)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron oxide* leaching (%)
0(No supplement)	6.8	70.2	77.0
1.0	6.8	70.2	77.0
5.0	6.8	70.2	77.0
10.0	6.8	68.4	75.5
15.0	6.8	63.0	72.4
20.0	6.8	60.7	67.7

* Each figure is the mean value of 3 individual experiments

Table 6.4 indicates that the molybdenum has no effect on Si and Fe leaching from bauxite ore by <u>A.niger</u> X_1 at lower concentration but there is a decrease of Si and Fe at higher concentration. There is no effect of Mo on cellular growth.

Table 6.5 Effect of Cu (added as $CuSO_4.5H_2O$) on leaching of silica and iron from bauxite ore by <u>A.niger</u> X_1

Concentration of $CuSO_4.5H_2O$ (µg/ml)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron oxide* leaching (%)
0(No supplement)	6.8	70.2	77.0
1.0	5.1	51.2	60.7
5.0	4.0	31.7	33.2
10.0	3.2	11.2	15.0
15.0	NIL	NIL	NIL
20.0	NIL	NIL	NIL

* Each figure is the mean value of 3 individual experiments

Table 6.5 shows that there is considerable inhibitory effect of copper on Si and Fe leaching from bauxite ore by **A.niger** X_1 with the increasing concentration of copper. Si and Fe leaching and also the growth, all are inhibited in presence of Cu^{2+}.

Table 6.6 Effect of Co (added as $CoCl_2$) on leaching of silica and Fe from bauxite ore by A.niger X_1.

Concentration of $CoCl_2$ (µg/ml)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron oxide* leaching (%)
0(No supplement)	6.7	70.2	77.0
1.0	6.7	70.2	77.0
5.0	6.7	66.3	70.7
10.0	6.7	55.4	62.0
15.0	6.6	51.1	55.7
20.0	6.6	45.0	53.9

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

Table 6.6 shows that Co has no effect at lower concentration on both Si and Fe leaching, but at higher concentration there is decrease of leaching of Si and Fe. There is no effect on cellular growth of **A.niger** X_1.

Table 6.7 Effect of Ni (added as $NiSO_4$) on leaching of silica and iron from bauxite ore by A.niger X_1.

Concentration of $NiSO_4$ (µg/ml)	Cellular growth Dry wt (g/l)	Silica* leaching (%)	Iron oxide* leaching (%)
0(No supplement)	6.8	70.2	77.0
1.0	6.8	70.2	77.0
5.0	6.8	70.2	77.0
10.0	6.8	70.2	77.0
15.0	6.8	70.2	77.0
20.0	6.9	69.6	76.8

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

Molybdenum is supplemented in the medium as $(NH_4)_6Mo_7O_{24}.4H_2O$. Result as incorporated in the table 6.4 shows that Mo has hardly have any effect on the leaching of silica from bauxite ore by **A.niger** X_1. Moreover, the element inhibits the leaching at higher concentration.

Copper was supplemented as $CuSO_4.5H_2O$ in the medium. This element is found to be toxic at all levels of treatment because the percentage of silica leaching is inhibited by the presence of this element (Table 6.5).

The mineral element cobalt is found not to stimulate the leaching process at a concentration of 1 μgm/ml, or 5μgm/ml of $CoCl_2$. Concentration higher than this leads to the decrease of leaching (Table 6.6). It has been demonstrated by other workers that cobalt is capable of stimulating the production by fungi (193). It is apparent from table 6.7 that Nickel when added to the medium as $NiSO_4$, does not have any effect on the leaching of silica. It has also been reported by other workers that Nickel does not interfere with the biosynthesis by **A.niger** even at moderately high concentration (194).

It is evident from the results that the effects of the mineral elements studied in the present work can be divided into three categories.

(i) elements that stimulate the leaching process.

(ii) elements that have no effect on leaching process.

(iii) elements that inhibit leaching process at any concentration level.

Table 6.7 indicates that there is no effect of Ni on leaching of Si and Fe from bauxite ore by **A.niger** X_1. Cellular growth also remained unchanged.

As a result of the present study the following suitable synthetic medium was recommended with a composition of glucose 5%, KCl 0.05%, KH_2PO_4 1%, $MgSO_4.7H_2O$ 0.05%, NH_4Cl 0.13%, $ZnSO_4.7H_2O$ 10 µg/ml, $MnSO_4.4H_2O$ 5 µg/ml, pH 4.0.