CHAPTER 8

EFFECT OF AMINO ACIDS, VITAMINS, METABOLIC INHIBITORS AND ANTIBIOTICS ON BIOLEACHING OF SILICA AND IRON OXIDE FROM BAUXITE ORES BY *ASPERGILLUS NIGER* X_1.

8.1 Effect of amino acids on bioleaching of silica and iron from bauxite ore by Aspergillus niger X_1 :

Nitrogen is the essential element for microbial growth. Inorganic as well as organic nitrogen compounds can be utilized to satisfy the requirement for this element. All bacteria and fungi can utilize ammonia (as NH_4^+ ion) which reflects its central role in nitrogen metabolism as the form in which nitrogen is incorporated into organic cell components. Among the organic nitrogen sources amino acids can be utilized if the microbe under consideration is capable of breaking down these compounds into smaller fragments. Amino acids are particularly good sources of nitrogen. Nitrogen metabolism by micro-organisms has been recently reviewed by Payne (178). Growth on amino acids relieves the cell of many biosynthetic activities leading to more efficient growth. Again, some essential amino acids can often act as growth inhibitors. This is often linked to transport into the cell since many of these compounds share a permease with resulting antagonism of uptake. Drew and Demain (198) described the repressive effect of amino acids and NH_4^+ ion. The cellular assimilation of nitrogen involves only two key compounds, glutamate and glutamine. Ammonia is incorporated into glutamate by glutamate dehydrogenase or glutamine synthetase. When amino acids are the source of nitrogen to the cell, the amino acid is degraded to glutamate by the classical amino acid degradation pathway, or nitrogen is passed to glutamate in transmination reactions. Glutamine is the primary donor for the incorporation of amino groups into amino acids in transmination reaction. This is the

main source of nitrogen to the biosynthetic pathways, glutamate + $NADP^+$ + $RCoCO_2H$ $\rightleftharpoons$ 2-oxoglutarate + NADPH + $RCHNH_2CO_2H$, glutamine serves as the donor to, for example, tryptophan, histidine, aspargine, and also purine nucleotides to satisfy the requirements for nitrogen within the monomer skeleton. Therefore, all cellular nitrogen has to pass through glutamine, glutamate or both in order to be assimilated, unless amino acids are supplied in which case they are incorporated directly (178).

From the above discussions it in clear that amino acid has vital role in the growth of micro-organism which in turn affects leaching process.

Mohanty et al. has used glycine in their medium composition at the time of microbial silica solubilization from magnesite ore through mutagenic treatment of **B.licheniformis** (197).

In the present work, a study has been carried out to observe the effect of different amino acids on bioleaching of silica and iron from bauxite ore by **A.niger** X_1.

8.1.1 Experimental and Results :

The basal medium used for the experiments consisted of glucose 5%, NH_4Cl 0.13%, KH_2PO_4 0.1%, KCl 0.05%, $MgSO_4.7H_2O$ 0.05%, $ZnSO_4.7H_2O$ 10 µgm/ml, $MnSO_4.4H_2O$ 5 µg/ml, pH was maintained at 4. The solutions of individual amino acids were made in double distilled water and sterilized by filtration and added asceptically to the sterile basal medium

at 0.5 and 1 mg/ml concentrations. The cultural condition and method of silica and iron estimation are same as before. (Page No. 47 of this thesis).

The results are given in Table 8.1.1.

Table 8.1.1 Effect of different amino acids on the leaching of silica and iron from bauxite ore by A.niger X_1

Amino acid added	Concentration amino acid (mg/ml)	Cellular growth Dry wt. (g/l)	Silica* leaching (%)	Iron Oxide* leaching (%)
Control (no supplement)	-	6.8	65.8	70.2
DL Alanine	0.5	6.2	Nil	45.8
	1.0	6.0	Nil	25.3
L Tyrosine	0.5	5.9	42.7	38.03
	1.0	5.8	42.27	36.2
L(+) Lysine	0.5	6.3	Nil	37.93
	1.0	6.5	Nil	29.08
L(-) Phenylalanine	0.5	6.6	48.09	50.12
	1.0	6.2	31.57	38.39
L(-) Methionine	0.5	6.7	27.92	42.18
	1.0	6.4	20.94	35.42
L Tryptophan	0.5	6.8	54.8	70.2
	1.0	7.1	65.9	70.4
Valine	0.5	6.9	65.8	70.2
	1.0	7.0	65.7	70.3
L Histidine	0.5	6.8	65.9	70.3
	1.0	6.6	65.7	70.5
L(-) Serine	0.5	5.9	Nil	39.4
	1.0	5.7	Nil	22.5
L(-) Proline	0.3	6.8	66.3	71.4
	0.5	6.8	70.4	73.5
	1.0	7.0	72.4	77.8
	1.5	7.2	65.0	73.1
L(+) Cysteine	0.5	6.4	Nil	34.1
	1.0	6.0	Nil	24.9

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

It appears from table 8.1.1 that L(-) proline has increasing leaching effect on silica and iron from bauxite ore. DL-Alanine, L(+) Lysine, L(-) Serine, L(+) Cysteine completely inhibit the leaching of silica and L(-) Phenyl alanine, L(-) Methionine have inhibitory effect on leaching of silica and iron from bauxite ore. The effects on cellular growth however are found to be different.

8.2 Effect of vitamins on bioleaching of silica and iron from bauxite ore by A.niger X_1 :

Many micro-organisms have specific nutritional requirement for growth as a consequence of their inability to synthesize them. For the sufficient growth and metabolism of the organism, some vitamins must be needed. The type and amount of vitamins depend much on the type of organism.

Thiamine, is the vitamin, most frequently required by the fungi. The metabolically active form of thiamine is the pyrophosphate, long known as carboxylase because of its co-enzyme function in the decarboxylation of pyruvic acid (181).

Biotin is required by many fungi, the absolute requirement is usually less than 5 µgm/litre (181).

Pyridoxin appears to be required by fewer fungi than the biotin or thiamine (181).

Riboflavin is only rarely required by microorganisms isolated from nature.

Riboflavin formation by **A.niger** is favoured by any of a variety of nutritional factors (181).

The known function of nicotinic acid is, as a constituent of the respiratory co-enzyme diphosphopyridine nucleotide and triphosphopyridine nucleotide (181).

Requirement for inositol is rather rare in fungi. No co-enzyme function involving inositol is known in any organism (181).

Although the folic acid molecule contains the structure of PABA, it seems doubtful that the major function of PABA is in the form of folic acid. Several metabolic roles of PABA have been suggested especially a function in the introduction of carbon intermediates into other compounds (181). Vitamin B_{12} is not required by fungi as known so far (181).

R.C. Thompson described the requirement of different vitamins of bacteria (199).

In the present study, an attempt has been made to find out the role of different vitamins for the bioleaching of silica and iron from bauxite ore by **A.niger** X_1.

8.2.1 Experimental and Results :

The basal medium used for the experiment consisted of glucose 5%, NH_4Cl 0.13%, KH_2PO_4 0.1%, KCl 0.05%, $MgSO_4.7H_2O$

0.05%, $ZnSO_4.7H_2O$ 10 µgm/ml, $MnSO_4.4H_2O$ 5 µgm/ml, L(-) Proline 1.0 mg/ml, pH was maintained at 4.0.

The vitamin solutions were sterilized by filtration and added asceptically to the sterile basal medium at the concentration of 2 and 5 µg/ml. The cultural conditions and the estimation procedures of silica and iron are same as mentioned in the earlier chapter (Chapter 2 Page No. 47 of the thesis).

The results are shown in Table 8.2.1

Table 8.2.1 Effect of vitamins on bioleaching of silica and iron from bauxite ore by *A.niger* X_1

Vitamins added	Concentration (µg/ml)	Cellular growth Dry wt. (gm/l)	Silica* leaching (%)	Iron* oxide leaching(%)
Control	-	7.0	72.4	77.8
Riboflavin	2.0	7.1	73.2	68.2
	5.0	7.2	59.9	64.0
Mesoinositol	2.0	7.0	72.4	77.8
	5.0	7.1	72.3	77.7
Pyridoxine hydrochloride	2.0	7.2	73.5	70.2
	5.0	7.2	66.0	67.0
Thiamine hydrochloride	2.0	7.0	73.0	68.5
	5.0	7.2	67.2	62.0
Vitamin B_{12}	2.0	7.3	73.8	70.2
	5.0	7.5	68.2	65.5
Biotin	2.0	7.0	72.4	77.7
	5.0	7.2	72.5	77.8
Folic acid	2.0	7.0	73.2	78.2
	5.0	7.3	72.5	78.0
Nicotinic acid	2.0	6.8	64.2	67.4
	5.0	6.6	50.5	58.0
Paraaminobenzoic acid (PABA)	2.0	7.0	60.8	69.2
	5.0	6.7	49.8	54.5

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

It is evident from table 8.2.1 that Riboflavin, Pyridoxine hydrochloride, Thiamine hydrochloride, Vitamin B_{12} has increasing effect at lower concentration on silica leaching but decreasing effect on iron leaching. Folic acid has also increasing effect on both silica and iron leaching, but other vitamins such as mesoinositol, biotin has no effect on leaching of silica and iron. Nicotinic acid and paraamino benzoic acid have decreasing effect on silica and iron leaching from bauxite ore by **A.niger** X_1.

The optimum concentration of folic acid was determined. The results are shown in Table 8.2.2.

Table 8.2.2 Determination of optimum concentration of folic acid on bioleaching of silica and iron by A.niger X_1

Vitamins added	Concentration (µg/ml)	Cellular growth Dry wt. (gm/l)	Silica* leaching (%)	Iron* leaching(%)
Control	0	7.0	72.4	77.8
Folic acid	1.0	7.0	72.7	78.0
	2.0	7.0	73.2	78.2
	3.0	7.2	75.0	80.2
	4.0	7.2	73.6	78.3
	5.0	7.3	72.5	78.0

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

Table 8.2.2 shows that 3 µgm/ml folic acid has maximum effect on silica and iron leaching by **A.niger** X_1.

8.3 Effect of metabolic inhibitor on silica and iron leaching from bauxite ore by *Aspergillus niger* X_1 :

The silica leachinbg is influenced indirectly by the growth and metabolism of the organisms or directly by medium constituents and environmental conditions of the medium. Proper growth, pigmentation etc. stimulates the leaching process. On the contrary, the substances, inhibitory to the organism can inhibit the leaching process by hindering the growth of the organism or directly by interferring with the organic acid production process.

Very limited reports ~~have been obtained~~ are available in the literature about the metabolic inhibitors that influence the bioleaching of silica.

Agbim, N.N. and Doxtader, K.G. (200) reported that with glucose as an energy source, the leaching of silica from zinc-sulfide ore was markedly reduced when sodium azide or DNP was added to the medium.

The rate of uptake of metabolic inhibitor increases with increasing incubation period and environmental conditions.

In the present investigation, an effort has been made to study the effect of metabolic inhibitors, on bioleaching of silica and iron by **Aspergillus niger** X_1. The inhibitors have been added on 0 day, 3rd day and 5th day of inoculation.

8.3.1 Experimental & Results :

The basasl medium used for the experiment consisted of glucose 5%, NH_4Cl 0.13%, KH_2PO_4 0.1%, KCl 0.05%, $ZnSO_4.7H_2O$ 10 µgm/ml, $MnSO_4.4H_2O$ 5 µgm/ml. L(-) proline 1 mg/ml, Folic acid 3 µg/ml and pH was maintained at 4.

Sterile inhibitor solutions were added individually to the sterile basal medium at the concentrations of 10^{-1}(M), 10^{-2}(M) and 10^{-3}(M) on 0, 3rd and 5th day of incubation.

Experiments were carried out in 250 ml of conical flask using 80 ml medium for 7 days at 30°C by surface culture method. The inoculum was prepared in the same way as indicated in (Chapter 2 Page No. 46 of the thesis). Percentage of silica and iron leaching was determined as described before (Page No. 47 of the thesis).

Results are shown in Table 8.3.1.

Table 8.3.1. Effect of metabolic inhibitors on silica and iron leaching from bauxite ore by A.niger X_1

Inhibitors added	Time of addition (days)	Concentration (Molar)	Cellular growth Dry wt (g/l)	Silica* leaching(%)	Iron oxide* leaching(%)
Control (no supplement)	-	-	7.2	75.0	80.2
Mercuric chloride	0	10^{-1}	3.8	Nil	10.13
		10^{-2}	4.0	Nil	14.45
		10^{-3}	4.4	Nil	20.0
	3	10^{-1}	4.2	Nil	11.0
		10^{-2}	4.4	Nil	15.0
		10^{-3}	4.8	Nil	22.8
	5	10^{-1}	4.5	Nil	16.7
		10^{-2}	5.0	10.2	20.0
		10^{-3}	5.5	14.0	30.0
Sodium fluoride	0	10^{-1}	3.0	Nil	10.76
		10^{-2}	4.8	15.98	16.9
		10^{-3}	5.7	19.5	22.06
	3	10^{-1}	4.7	Nil	14.8
		10^{-2}	5.2	17.0	20.1
		10^{-3}	5.8	22.8	29.0
	5	10^{-1}	5.0	12.2	21.5
		10^{-2}	5.5	20.4	30.0
		10^{-3}	6.0	28.5	37.4
Sodium iodoacetate	0	10^{-1}	4.7	Nil	10.8
		10^{-2}	5.2	14.65	25.8
		10^{-3}	5.5	19.8	31.9
	3	10^{-1}	5.0	15.0	17.5
		10^{-2}	5.4	26.2	30.0
		10^{-3}	6.0	34.0	44.7
	5	10^{-1}	5.3	20.3	28.9
		10^{-2}	5.6	32.0	39.0
		10^{-3}	6.4	41.0	57.2
6 Mercaptapurine	0	10^{-1}	4.4	Nil	18.4
		10^{-2}	4.9	17.0	23.07
		10^{-3}	5.0	24.0	33.1
	3	10^{-1}	4.8	22.0	27.0
		10^{-2}	5.5	29.8	34.1
		10^{-3}	5.9	36.0	44.6

Table 8.3.1. (Contd.)

Inhibitors added	Time of addition (days)	Concentration (Molar)	Cellular growth Dry wt (g/l)	Silica* leaching(%)	Iron oxide* leaching(%)
6 Mercaptapurine	5	10^{-1}	5.4	29.5	36.0
		10^{-2}	5.8	35.4	47.0
		10^{-3}	6.0	44.7	55.3
2-thiouracil	0	10^{-1}	5.2	Nil	22.9
		10^{-2}	6.0	Nil	32.2
		10^{3}	6.5	Nil	54.5
	3	10^{-1}	5.7	Nil	28.4
		10^{-2}	6.2	17.2	39.0
		10^{-3}	6.8	23.0	60.1
	5	10^{-1}	6.5	28.2	45.0
		10^{-2}	6.6	39.0	57.3
		10^{-3}	6.8	50.7	68.0
Sodium arsenite	0	10^{-1}	2.2	Nil	20.7
		10^{-2}	2.8	Nil	31.7
		10^{-3}	3.6	Nil	40.1
	3	10^{-1}	2.4	Nil	34.0
		10^{-2}	3.6	Nil	42.0
		10^{-3}	4.9	Nil	51.7
	5	10^{-1}	3.0	14.5	42.2
		10^{-2}	4.5	17.8	49.7
		10^{-3}	5.4	27.4	60.4
Sodium azide	0	10^{-1}	2.1	Nil	22.6
		10^{-2}	2.8	Nil	28.7
		10^{-3}	3.5	Nil	36.0
	3	10^{-1}	2.8	Nil	29.1
		10^{-2}	3.7	Nil	43.4
		10^{-3}	4.9	Nil	55.2
	5	10^{-1}	3.2	Nil	44.9
		10^{-2}	4.5	Nil	54.9
		10^{-3}	5.8	Nil	63.9
2-4-dinitrophenol	0	10^{-1}	3.1	12.1	22.0
		10^{-2}	4.3	15.0	27.2
		10^{-3}	4.8	19.9	30.7

Table 8.3.1. (Contd.)

Inhibitors added	Time of addition (days)	Concentration (Molar)	Cellular growth Dry wt (g/l)	Silica* leaching(%)	Iron oxide* leaching(%)
2-4-dinitrophenol	3	10^{-1}	5.0	22.5	35.0
		10^{-2}	5.8	32.8	48.4
		10^{-3}	6.1	48.7	56.8
	5	10^{-1}	6.6	30.3	48.2
		10^{-2}	7.0	43.0	57.0
		10^{-3}	7.2	56.0	68.5

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

Table 8.3.1 indicates that Mercuric Chloride inhibits silica leaching completely at the concentration of 10^{-1} (M), 10^{-2}(M), 10^{-3}(M). But iron leaching is not totally inhibited, it is leached at lower rate. Sodium fluoride when added at the concentration of 10^{-1}(M) no leaching is observed when added at 0, 3rd day of incubation. Low percentage of leaching is observed at 10^{-2}(M), 10^{-3}(M) concentration when added at 0, 3rd and 5th day. Sodium iodoacetate and 6-Mercaptapurine when added at the concentration of 10^{-1}(M), no silica leaching is observed on 0 day addition. But at the other two concentration 10^{-2}(M), 10^{-3}(M) effect is less. No silica leaching is there when sodium azide is added, very low silica leaching is observed when sodium arsenite added at 5th day of incubation. 2-thiouracil has inhibitory effect when added at 0 day but low silica leaching is observed when added at 3rd and 5th day. 2,4-dinitrophenol inhibited sharply at 10^{-1}(M), 10^{-2}(M) concentration. But silica leaching is comparatively good at 10^{-3} (M) concentration.

8.4 Effect of antibiotics on silica and iron leaching from bauxite ore by A.niger X_1 :

Antibacterial antibiotics when added in the nutritional medium of fungus sometimes stimulate some metabolic activities of fungus or in too high concentrations these can inhibit the growth of the fungus also.

In the present investigation, efforts, have been made to study the effect of different antibacterial antibiotics on bioleaching of silica and iron from bauxite ore by **A.niger** X_1.

8.4.1 Experimental and Results :

The medium used for the experiments consisted of glucose 5%, NH_4Cl 0.13%, KH_2PO_4 0.1%, KCl 0.05%, $MgSO_4.7H_2O$ 0.05%, $ZnSO_4.7H_2O$ 10 µgm/ml, $MnSO_4.4H_2O$ 5 µgm/ml, L(-) proline, 1 mg/ml, Folic acid 3 µg/ml, pH was maintained at 4.

Antibiotic solutions were made in double distilled water and sterilized by filtration and added separately to the sterile basal medium in different concentrations and also at different days of fermentation.

The percentage of silica leaching and iron leaching and cellular growth were determined by the methods as described before (Page No. 47 of the thesis).

The results are shown in Table 8.4.1.

Table 8.4.1. Effect of different antibiotics on silica leaching and iron leaching by *A.niger* X_1

Antibiotics added	Time of addition (day)	Concentration (µgm/ml)	Cellular growth Dry wt (gm/l)	Silica* leaching(%)	Iron* oxide leaching (%)
Control	-	-	7.2	75.0	80.2
Chloramphenicol	0	10	5.0	31.35	51.0
		20	3.3	Nil	33.7
		30	2.2	Nil	24.3
		50	1.0	Nil	18.1
	3	10	5.5	40.1	62.5
		20	5.0	23.0	43.7
		30	3.2	Nil	23.6
		50	2.0	Nil	19.69
	5	10	6.0	48.0	69.9
		20	5.5	32.2	58.6
		30	4.6	18.8	40.3
		50	3.7	10.2	31.26
Streptomycin	0	10	6.2	Nil	62.65
		20	5.8	Nil	50.7
		30	5.0	Nil	38.9
		50	4.1	Nil	31.4
	3	10	6.7	27.75	68.0
		20	6.0	17.89	59.3
		30	5.4	Nil	52.4
		50	4.8	Nil	48.3
	5	10	7.0	47.2	80.2
		20	6.2	36.0	80.2
		30	5.5	25.0	79.62
		50	5.0	12.8	78.37
Tetracycline.HCl	0	10	7.2	75.1	80.0
		20	7.2	75.0	80.2
		30	7.2	75.2	80.1
		50	7.1	75.0	80.0
	3	10	7.2	75.2	80.1
		20	7.2	75.0	80.2
		30	7.2	75.1	80.0
		50	7.0	75.0	80.0
	5	10	7.2	75.1	80.2
		20	7.2	75.0	80.0
		30	7.2	75.2	82.1
		50	7.2	75.0	80.2

Table 8.4.1.(Contd.)

Antibiotics added	Time of addition (day)	Concentration (µgm/ml)	Cellular growth Dry wt (gm/l)	Silica* leaching(%)	Iron* oxide leaching (%)
Na-Salt of Penicillin G	0	10	7.2	75.2	80.0
		20	7.2	75.1	80.2
		30	7.1	75.0	80.0
		50	7.0	75.1	79.8
	3	10	7.2	75.1	80.1
		20	7.2	75.0	80.2
		30	7.2	75.0	80.2
		50	7.0	74.7	80.0
	5	10	7.2	75.2	80.0
		20	7.2	75.0	80.2
		30	7.2	75.0	80.0
		50	7.2	75.1	80.2

*** Each value is the mean of 3 inedividual experiments.**

Table 8.4.1 shows that Chloramphenicol when added on the 0 day, leaching is observed at 10 µgm/ml concentration. But at other concentrations no silica leaching is there. Streptomycin inhibits silica leaching when added at 0 day. Streptomycin when added on 3rd day leaching is decreased when added at the concentration of 10 µgm/ml and 20 µgm/ml but leaching inhibited at the concentration of 30, 50 µgm/ml. Streptomycin when added on 5th day leaching decreases with increasing concentration. Tetracycline and penicillin has no appreciable effect on silica and iron leaching.