DISCUSSION

The mutagenic study reveals that after ethylene imine treatment a mutant of **A.niger** obtained, which has better leaching capacity. This mutant when treated further with X-rays produce a mutant strain **A.niger** X_1 with still better leaching capacity.

Ethylene imine being an alkylating agent modifies chemically the DNA nucleotide permanently which imparts a permanent change in the DNA structure and hence the mutant strain remains stable through generations.

Investigation on the optimization of physical factors reveals that the maximum leaching is obtained when the particle size of the ore was in between- 170 to 200 mesh. A decrease in leaching is observed when the particle size is either smaller or greater than this. Smaller than this size causes a dilution of sample and a size greater than this leaves a smaller effective surface area resulting in a decreased leaching.

The optimum pulp density for better leaching of silica and iron is 0.3% and the optimum volume of inoculum is 6%. The optimum volume of medium, the optimum initial pH and optimum temperature are 80 ml, 4.0 and 30°C in 250 ml flask respectively. It has been observed that organic acids are produced during the metabolism of **A.niger** X_1 and these acids are at least partly responsible for leaching of silica and iron. So a volume greater than this may cause a dilution of the acids thus produced. The production and effectivity of these acids are perhaps maximum at the above mentioned pH and incubation temperatures.

The leaching is hindered if organism is more aged, this may be because of the fact that the organism proceeds to death phase after certain period.

While performing the experiments on selection of suitable carbon and nitrogen sources for the leaching of silica and iron from bauxite ore by **A.niger** X_1 it was observed that glucose at a concentration of 5% produced best results and 0.13% NH_4Cl was most suitable for effecting highest leaching.

Fungi lack the green pigment, chlorophyll and hence they can not synthesize their own food. All fungi require C, O, H, N, P for their survival and growth. The preferred food materials are smaller molecules, because they can be converted to the desired form through a few steps. Glucose required by many higher form of lives, can easily be oxidised via glycolysis and TCA cycle and can satisfy the condition of biosynthetic precursors necessary for growth of the organism. In the case also **A.niger** X_1 requires glucose as best source of carbon. NH_4Cl is found to be the best suitable nitrogen source. So, it becomes apparent that NH_4Cl can easily be converted to glutamate and glutamine in the cells, because these molecules are responsible for synthesizing nitrogenous molecules like tryptophan, histidine, asparagine and the purine nucleotides, very much required for the propagation of life. Moreover, the leaching process reaches its optimum at a standard lower pH and NH_4Cl on dissociation in the cultural solution can lower the pH of the medium thus facilitating the leaching.

Fungi require many micronutrients, the trace elements, for their growth. Many trace elements have profound effects on the production of many commercially important primary and secondary metabolites.

Zinc and manganese are found to stimulate the leaching of silica and iron at some optimum concentration and leaching is decreased at higher concentrations. Nickel and molybdenum do not have any appreciable effect at moderate concentration, copper, known antimicrobial element, inhibits leaching at all the concentrations studied. Many metaloenzymes contain zinc and manganese in their active sites. Zinc is also and integral part of the enzymes involved in the replication process. Most of the metal ions have antimicrobial effect at higher concentrations because either they block the active sites of essential enzymes or inactivate the enzymes and other essential factors by chelation.

Although bauxite contains sufficient amount of iron studies on the effect of added iron, in the leaching process is still relevant because, probably of the fact, that initially there is no iron in the medium, so, iron to be supplemented as a trace mineral at the initial stage. It appears that initial uptake of iron determines the leaching of silica and iron. Iron in larger quantity comes to the solution from bauxite at a latter stage, i.e. after the optimum growth of the organism and that may not have any appreciable effect on leaching. So, the addition of higher amount of iron in the culture medium at the initial stage are supposed to have inhibitory effect and this is reflected in the result also which shows a decrease in the leaching at higher concentration of view.

Results of the investigation of the effect of complex nutrients on the bioleaching of silica and iron by **A.niger** X_1 reveals that wheat bran extract is effective in effecting good leaching whereas peptone, yeast extract, beef extract also have some effect on leaching. Malt extract, rice bran extract, paddy soak liquor, and corn steep liquor has no appreciable effect. Surprisingly, soyabean meal extract inhibits the leaching with increasing concentration. Complex nutrient are important because they contain many minor and trace nutrients essential for growth and development of the organism, which influences the leaching. So, the complex nutrients like wheat bran extract, peptone, yeast extract, beef extract are capable of supplying the micronutrients essential for better leaching.

The inhibition of leaching by soyabean meal extract clearly demonstrates that, this complex nutrient contains components responsible for blocking the leaching apparatus only because it does not have any effect on growth of the organism.

Investigation reveals that L(-) proline enhances the leaching of silica and iron from bauxite ore by **A.niger** X_1 other amino acids studied inhibits leaching without imparting any appreciable effect on cellular growth.

This indicates that although the essential amino acids are utilized for the growth and development of the organism, only L(-) proline stimulates the leaching. So, L(-) proline is either an integral part of the leaching apparatus or essential for the activation of such apparatus.

Among the very many number of vitamins, folic acid when added at a concentration of 3 µg/ml gives maximum leaching of silica and iron. So, folic acid is also an essential micronutrient and it has to be added for effecting maximum leaching.

Most of the metabolic inhibitors inhibited the leaching of silica and iron as well as cellular growth. So, probably the inhibition is effected both by interfering with the leaching apparatus and by the release of the lower amount of metabolic chemicals responsible for leaching because of the lower growth of the organism.

Results on the effect of antibiotics on leaching entail that tetracycline and penicillin G has no inhibitory effect on leaching of silica and iron but other antibiotics like streptomycin and chloramphenicol inhibits leaching. This inhibition of leaching is effected via the inhibition of cellular growth.

The results demonstrate that the surface active agents Tween 80, Tween 40 and sodium lauryl sulphate does not have any effect on leaching of silica and iron from bauxite ore by **A.niger** X_1 and hence these agents do hardly attack the surface of the fungus **A.niger** X_1.

That silica is removed from the ore is evident from the scanning electron micrograph and petrographic thin section micrographs of the ores taken (raw bauxite and leached bauxite). A probable mechanism of silica solubilization is given in Chapter 10.

Finally in the last chapter we have shown how glucose is utilised with the leaching of silica and iron from bauxite ore by **A.niger** X_1. Cellular growth increases as the no. of days of fermentation increases. Silica and iron leaching is maximum in 7 days.

Regarding the nitrogen utilisation the amount of ammonia-nitrogen decreases gradually upto 6 days as the nitrogen source added in the medium initially as ammonia nitrogen, and it is utilized for the cell synthesis and contribution of amino acids important for the silica and iron leaching. The increase in amino nitrogen in latter phase of fermentation is due to the accumulation of different amino acids coming from the cell after commencement of the autolysis.