BACTERIAL HEAP LEACHING STUDIES OF LOW-GRADE URANIUM ORES FROM SIWALIK SANDSTONE ORE DEPOSITS, SULAIMAN RANGE, PAKISTAN

BY

TARIQ MAHMOOD

A THESIS SUBMITTED TO THE UNIVERSITY OF PUNJAB, LAHORE FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY

1994

INSTITUTE OF CHEMISTRY UNIVERSITY OF THE PUNJAB LAHORE, PAKISTAN
Dedicated

to
Irfan, Farhan, Ali, Umer, Farah,
Kalsoom and Jani Shah
PREFACE

The Government of Pakistan is eager to develop biotechnology in the country so as to purposefully utilize in the field of agriculture, energy, health, and industry. It is becoming clear that biotechnology can, indeed, play a role in production and conservation of energy and can thus contribute to the overall energy situation.

One of the primary mandates of the Pakistan Atomic Energy Commission is to generate sufficient electricity by properly harnessing the nuclear energy. It is indeed, through this objective, that power crisis which country is facing today, can be averted. The importance of uranium as a feed stock source for power generation, is ever increasing and is anticipated to be more in future in view of the commissioning of CHASMA Nuclear Power Plant (CHASNUPP). Keeping in view, this augmented requirement of uranium, a project on "uranium biohydrometallurgy" was initiated at NIBGE to develop a low-cost and environmentally safe process for the extraction of uranium from indigenous low-grade ores, which are not economically feasible for uranium extraction by conventional hydrometallurgical methods. Therefore, the present industrial interests in biohydrometallurgy are motivated by the fact that it can produce uranium from low-grade ores, overburdens, and tailings residues for approximately one-third to one-half of the cost of conventional techniques without polluting the environment. Furthermore, selective implementation of living systems can offer opportunities for reduced labor, increased productivity and other technological advantages.

Another aspect of mineral biotechnology is equally important to control environmental pollution problems which are commonly associated with mining and ore-processing operations. Biohydrometallurgical techniques, which are indeed pollution-free, but nevertheless, also offer attractive solutions for bioremediation of environmental damages caused during mining and ore-processing operations. Metals trapped in tailings dams and overburden heaps, which are potential hazards to the environment, can very easily be leached out, by properly organizing biotreatment operations, thus alleviating adverse affects on existing eco-systems.
ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. M. Waheed Akhtar, Professor of Biochemistry, Institute of Chemistry, University of the Punjab, Lahore and Dr. Kauser A. Malik (T.I.), Director, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, for their continued guidance and technical suggestions during the course of these studies. They provided encouragement, suggestions and often, timely insight into the aspects of my research that I had overlooked.

I am heartily grateful to Dr. Olli H. Tuovinen, Professor of Microbiology, The Ohio State University, Columbus, USA, for his valuable technical suggestions, comments and constructive criticism of this script during his scientific visit to Pakistan in December, 1993. Discussions with Dr. Arpad E. Torma, Professor of Metallurgy, Department of Materials and Metallurgical Engineering, New Mexico Institute of Mining and Technology, Socorro, NM, USA and Dr. R.G.L. McCready, Professor of Microbiology, CANMET Department of Energy, Mines and Resource, Ottawa, Ontario, Canada were invaluable during their visits to Pakistan, and I am indebted to them for their guidance and technical suggestions.

I am also indebted to Mr. Abdul Mateen, Chief Scientific Officer, Centre for Nuclear Studies, PINSTECH, Islamabad and Dr. Muhammad Amin, Head Chemistry Division, BC-1 Project, Dera Ghazi Khan for their technical guidance and diligent discussions about the performance of these studies. It is my pleasure duty to offer my thanks to Mr. Zafar M. Khalid, Dr. Javed A. Qureshi, Dr. M. Sajjad Mirza and Mr. Tariq Mahmood (NJAB) for their encouragements, valuable comments and critically reviewing of this script.

I am very thankful to Mr. Muhammad Aslam Alvi, Project Manager, BC-1 Project, and Mr. Haq Nawaz Khan, Manager (Baghalchur Mines), Dera Ghazi Khan, for providing samples of uranium ore, tailings residue and mine water. I am also grateful to Mr. Abdul Hameed, Manager (Plants), and Muhammad Ilyas, Principal Engineer (Chemical), Ore-Processing Plant, D.G. Khan for providing technical assistance and cooperation during this work. I would also like to thanks to Mr. S.M. Ifzal (Late), PSO, New Laboratories, PINSTECH, Islamabad for providing help in ICP analysis of yellow cake and Mr. M.A. Rahman, Principal Geologist, BC-1 Project, D.G. Khan, for technical assistance in carrying out mineral analysis of uranium ore used in these studies.
I would also like to acknowledge with thanks the help provided by numerous colleagues of NIAB and NIBGE, particularly Mr. Tanveer Ahmad and Ms. Naima Hamid for their help and cooperation in computer work. Special thanks are due to Massod Ahmad and Hamid Rashid for their help in photography. I also wish to appreciate Mr. Nasir Hayat Butt and Syed Habib-ur-Rehman Shah for their help and technical assistance.

It is a great pleasure for me to mention my father and mother who inspired me high ideas of life and enabled me to achieve this goal.

In the last, but not least, I must also owe my gratitude to my wife, sons and daughter for their patience, cooperation and moral support during the research work and writing of this thesis.

TARIQ MAHMOOD
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREFACE</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>5</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>14</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>51</td>
</tr>
<tr>
<td>MATERIALS</td>
<td>51</td>
</tr>
<tr>
<td>Sandstone Uranium Ore</td>
<td>51</td>
</tr>
<tr>
<td>Mill Tailings Residue:</td>
<td>51</td>
</tr>
<tr>
<td>Elemental Sulfur and Sulfur Slag</td>
<td>51</td>
</tr>
<tr>
<td>Pyrite Mineral and Sulfidic Ore</td>
<td>51</td>
</tr>
<tr>
<td>Microorganisms</td>
<td>52</td>
</tr>
<tr>
<td>METHODS</td>
<td>52</td>
</tr>
<tr>
<td>Sieve Analysis</td>
<td>52</td>
</tr>
<tr>
<td>Bromoform Separation</td>
<td>52</td>
</tr>
<tr>
<td>Isodynamic Magnetic Separation</td>
<td>53</td>
</tr>
<tr>
<td>Stereomicroscopic Analysis</td>
<td>53</td>
</tr>
<tr>
<td>X-Ray Diffraction Analysis</td>
<td>54</td>
</tr>
<tr>
<td>Chemical Analyses of Sandstone Uranium Ore</td>
<td>54</td>
</tr>
<tr>
<td>Sample Preparation</td>
<td>54</td>
</tr>
<tr>
<td>Uranium Determination</td>
<td>55</td>
</tr>
<tr>
<td>Ferrous and Total Iron Determination</td>
<td>55</td>
</tr>
<tr>
<td>Elemental Analysis of Uranium Ore</td>
<td>56</td>
</tr>
<tr>
<td>Induced Coupled Plasma Spectroscopic Analysis</td>
<td>56</td>
</tr>
<tr>
<td>Acid Determination</td>
<td>57</td>
</tr>
<tr>
<td>Free Acid Determination</td>
<td>57</td>
</tr>
<tr>
<td>Sulfur Determination</td>
<td>57</td>
</tr>
<tr>
<td>Chemical Analyses of Uranium Mine Water Samples</td>
<td>57</td>
</tr>
<tr>
<td>Micobiological Growth Media</td>
<td>58</td>
</tr>
<tr>
<td>Liquid Media</td>
<td>58</td>
</tr>
<tr>
<td>Iron medium (9KFe²⁺)</td>
<td>58</td>
</tr>
<tr>
<td>Sulfur medium (9KS⁰)</td>
<td>58</td>
</tr>
<tr>
<td>Sulfur Slag medium</td>
<td>59</td>
</tr>
<tr>
<td>Pyrite medium</td>
<td>59</td>
</tr>
<tr>
<td>Tetrathionate medium</td>
<td>59</td>
</tr>
<tr>
<td>Glucose medium</td>
<td>59</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Solid Media</td>
<td>60</td>
</tr>
<tr>
<td>Gelrite-FeSO₄ medium</td>
<td>60</td>
</tr>
<tr>
<td>Thiosulfate medium (Agarose-S₂O₃²⁻ medium)</td>
<td>61</td>
</tr>
<tr>
<td>Glucose medium (Agarose-Glucose medium)</td>
<td>62</td>
</tr>
<tr>
<td>Isolation and Characterization of Acidophilic Iron-and Sulfur-Oxidizing <em>Thiobacillus</em> Bacteria</td>
<td>62</td>
</tr>
<tr>
<td>Sampling Procedure</td>
<td>62</td>
</tr>
<tr>
<td>Isolation and enumeration of <em>Thiobacillus ferooxidans</em></td>
<td>62</td>
</tr>
<tr>
<td>Isolation and enumeration of <em>Thiobacillus thiooxidans</em></td>
<td>63</td>
</tr>
<tr>
<td>Direct Bacterial Counts</td>
<td>64</td>
</tr>
<tr>
<td>Maintenance of Culture</td>
<td>65</td>
</tr>
<tr>
<td>Preparation of Inocula</td>
<td>65</td>
</tr>
<tr>
<td>Oxidation of Ferrous Iron by <em>T. ferooxidans</em></td>
<td>66</td>
</tr>
<tr>
<td>Oxidation of Pyrite by <em>T. ferooxidans</em></td>
<td>66</td>
</tr>
<tr>
<td>Oxidation of Elemental Sulfur and Sulfur Slag by <em>T. ferooxidans</em> and <em>T. thiooxidans</em></td>
<td>67</td>
</tr>
<tr>
<td>Bioleaching Studies of Sandstone Uranium Ore</td>
<td>67</td>
</tr>
<tr>
<td>Shake Flask Leaching Studies</td>
<td>67</td>
</tr>
<tr>
<td>Tank Leaching Studies</td>
<td>68</td>
</tr>
<tr>
<td>Air-Lift Percolator Column Leaching Studies</td>
<td>68</td>
</tr>
<tr>
<td>PVC Column Leaching Studies</td>
<td>69</td>
</tr>
<tr>
<td>Microbial Heap Leaching Studies</td>
<td>70</td>
</tr>
<tr>
<td>Heap Construction</td>
<td>70</td>
</tr>
<tr>
<td>Heap Operation</td>
<td>72</td>
</tr>
<tr>
<td>Adsorption of Uranium by Anion-Exchange Resins</td>
<td>72</td>
</tr>
<tr>
<td>Packing of Resin Column</td>
<td>72</td>
</tr>
<tr>
<td>Loading of Uranium</td>
<td>73</td>
</tr>
<tr>
<td>Elution of Uranium from Loaded/ Adsorbed Resin</td>
<td>73</td>
</tr>
<tr>
<td>Production of Uranium Concentrate (Yellow Cake)</td>
<td>73</td>
</tr>
<tr>
<td>RESULTS</td>
<td>75</td>
</tr>
<tr>
<td>Mineralogy of Low-Grade Sandstone Uranium Ore</td>
<td>75</td>
</tr>
<tr>
<td>Sandstone Uranium Ore</td>
<td>75</td>
</tr>
<tr>
<td>Sieve Analysis</td>
<td>75</td>
</tr>
<tr>
<td>Light and Heavy Minerals Separation</td>
<td>77</td>
</tr>
<tr>
<td>Magnetic Separation</td>
<td>78</td>
</tr>
<tr>
<td>Isodynamic Magnetic Separation</td>
<td>79</td>
</tr>
<tr>
<td>Stereomicroscopic Analysis</td>
<td>80</td>
</tr>
<tr>
<td>X-Ray Diffraction Analysis</td>
<td>81</td>
</tr>
<tr>
<td>Chemical Analyses of Sandstone Uranium Ore</td>
<td>83</td>
</tr>
<tr>
<td>Chemical Analyses of Sulfur and Sulfur Slag</td>
<td>85</td>
</tr>
<tr>
<td>Chemical and Microbiological Analyses of Tailings Liquid Samples</td>
<td>86</td>
</tr>
<tr>
<td>Physico-Chemical and Microbiological Analyses of Mill Tailings Residues</td>
<td>90</td>
</tr>
<tr>
<td>Chemical and Microbiological Analyses of Mine Water Samples</td>
<td>92</td>
</tr>
</tbody>
</table>
Isolation and Characterization of Acidophilic Iron-and Sulfur-Oxidizing Thiobacillus Bacteria ................................................................. 94
  Isolation of Iron- and Sulfur Oxidizer (T. ferrooxidans) .... 94
  Isolation of Sulfur-Oxidizer (T. thiooxidans) ................. 98
  Cell Morphology and Characterization of Isolated Strains .. 101

Growth Studies of Thiobacillus Bacteria ................................. 102
  Oxidation of Ferrous Iron (Fe^{2+}) by T. ferrooxidans .... 102
  Oxidation of Pyrite (FeS$_2$) by T. ferrooxidans ......... 103
  Oxidation of Sulfur by T. ferrooxidans and T. thiooxidans 105
  Oxidation of Slag by T. ferrooxidans and T. thiooxidans .... 107

Optimization of Leaching Parameters for Bioleaching of Sandstone Uranium Ore ............................................................ 112
  Shake Flask Studies ...................................................... 112
    Effect of External Energy Substrates ..................... 118
    Effect of Inoculum Size ...................................... 119
    Uranium Leaching Tests with Sulfuric Acid ........... 120
    Effect of Different Proportions of Sulfur and Slag .... 123
    Effect of Pulp Density .................................. 126
    Effect of Particle Size .................................... 128

  Shake Flask Leaching Studies of Tailings Residues ......... 129

Tank Leaching Studies .................................................... 131
  Air-Lift Percolator Column Leaching Studies .............. 132
  PVC Column Leaching Studies .................................... 134
    Column Effluents Analyses ................................. 138
    Core Samples Analyses .................................... 141

  PVC Column Leaching Studies of Tailings Residues ........ 146

Microbial Heap Leaching Studies ........................................ 148
  Heap Effluents Analyses ....................................... 152
  Heap Core Samples Analyses .................................. 154
  Heap Sludge Samples Analyses ................................. 156

  Down-Stream Processing of Uranium ............................ 158
    Adsorption of Uranium by Strongly Basic Anion-Exchange Resins .................................................. 158
    Elution Studies of Uranium .................................. 160
    Production of Uranium Concentrate (Yellow Cake) .... 163

  DISCUSSION ................................................................. 168

  REFERENCES ..................................................................... 181
SUMMARY

The principal objective of the present investigations was to optimize leaching parameters for uranium extraction from Baghalchur low-grade sandstone uranium ores (0.023% \( \text{U}_3\text{O}_8 \)) by bacterial heap leaching process. Baghalchur sandstone uranium ore is alkaline in nature and was found to contain 3.5% calcite (\( \text{CaCO}_3 \)), and 0.1% pyrite (\( \text{FeS}_2 \)) as the only sulfide mineral. Hematite (\( \text{Fe}_2\text{O}_3 \)) and magnetite (\( \text{Fe}_3\text{O}_4 \)) were the main iron oxide minerals and constituted the major portion of the heavy minerals. Quartz (\( \text{SiO}_2 \)) was the main silicate mineral present in the sandstone uranium ore and represented 59% of the frame grains. Actinolite, biotite, chlorite, epidote, hornblende, muscovite, pyroxene, and tourmaline were the mica minerals present in sandstone uranium ore.

Tyuyamunite [\( \text{Ca(VO}_4\text{)}_2(\text{UO}_4\text{)}_2\cdot 5\cdot 8 \text{H}_2\text{O} \)] was identified as the main uranium mineral present in the sandstone ore. Clay minerals fraction of the ore contained major portion of uranium content (0.021% \( \text{U}_3\text{O}_8 \)) which was 91.30% of the total uranium content present in the ore sample (0.023% \( \text{U}_3\text{O}_8 \)). Uranium content was much higher in the fine size ore fraction as compared to coarse one. The ore contained 46.9% (wt/wt) of ore fraction with a particle size of \( \geq 595 \mu\text{m} \) (\( \geq 30 \) ASTM mesh size) which was a major fraction and almost the weight of ore fraction was found to decrease concomittantly with particle size.

The indigenous strains of \( \text{Thiobacillus ferrooxidans} \) (TFe-1 and TFe-2) and \( \text{Thiobacillus thiooxidans} \) (TTh-1) were isolated from ecosystem of uranium ore-processing unit. These strains were Gram-negative, motile, and rod-shaped bacteria. \( \text{T. ferrooxidans} \) (TFe-2) oxidized \( \text{Fe}^{2+} \), pyrite, sulfur and reduced sulfur compounds like thiosulfate and tetrathionate. The bacterial oxidation of pyrite, sulfur and reduced sulfur compounds produced sulfuric acid which followed a drop in initial pH-value of the medium. \( \text{T. thiooxidans} \) (TTh-1) oxidized only sulfur and reduced sulfur compounds to sulfuric acid through its metabolic activity. Sulfuric acid thus produced acts as lixiviant for uranium solubilization from sandstone ore.

The release of iron from pyrite was much more pronounced (about 3-folds) by inoculated system as compared to uninoculated one. It was observed that 14.66 g/L \( \text{Fe} \) (total iron) was released from the bacterial oxidation of pyrite by indigenous strain of \( \text{T. ferrooxidans} \) (TFe-2) in 20-days of incubation. The release of iron (\( \text{Fe} \)) from pyrite mineral was relatively higher (16 g/L \( \text{Fe} \)) in standard strain of \( \text{T. ferrooxidans} \) (ATCC 13361) as compared to indigenous strain of \( \text{T. ferrooxidans} \) (TFe-2).

\( \text{Thiobacillus ferrooxidans} \) (TFe-2) produced 10.80 and 20.80 g/L \( \text{H}_2\text{SO}_4 \) from the bacterial oxidation of elemental sulfur and sulfur slag respectively, in 20-days of incubation. Similarly, \( \text{T. thiooxidans} \) (TTh-1) produced 13.52 g/L and 22.0 g/L \( \text{H}_2\text{SO}_4 \) from the oxidation of sulfur and sulfur slag, respectively, under similar experimental
conditions. However, a mixed culture of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) produced 18.6 g/L and 27.0 g/L H$_2$SO$_4$ from the oxidation of sulfur and sulfur slag, respectively. *T. thiooxidans* (TTh-1) showed a comparable results of sulfuric acid production and change in initial pH-value of the medium with the axenic strain of *T. thiooxidans* (ATCC 8085). *T. thiooxidans* (TTh-1) produced 39.80 g/L H$_2$SO$_4$ with a drop in initial pH-value of 2.50 to 0.68 in 30-days incubation; whereas 42.60 g/L H$_2$SO$_4$ was produced from sulfur oxidation by *T. thiooxidans* (ATCC 8085).

In shake flask bioleaching studies, the isolated strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) solubilized 91% and 88% U$_3$O$_8$, respectively, from low-grade sandstone uranium ore (0.023% U$_3$O$_8$) in 30-days of incubation. *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) exhibited comparable leaching data of uranium solubilization from ore with those of axenic strains of *T. ferrooxidans* (ATCC 13661) and *T. thiooxidans* (ATCC 8085). In chemical control samples, only 1.67% U$_3$O$_8$ solubilized from sandstone ore. A 10% (wt/vol) ore pulp density was used for these studies. During bioleaching of sandstone uranium ore, a significant amount of iron (Fe$_3$) was also solubilized by chemical reaction between H$_2$SO$_4$ and iron minerals like hematite (Fe$_2$O$_3$), magnetite (Fe$_3$O$_4$) and pyrite (FeS$_2$) etc., present in the ore matrix.

A higher uranium recovery of 92% U$_3$O$_8$ was obtained from sandstone ore amended with sulfur slag as compared to elemental sulfur (86.7% U$_3$O$_8$) and pyrite (84.3% U$_3$O$_8$). It was due to higher acid production during bacterial oxidation of slag and the presence of iron (Fe$^{2+}$ and Fe$^{3+}$) in sulfur slag, which catalyzed the chemical reaction for uranium solubilization from ore.

In batch tests, it was observed that the elemental sulfur to uranium ore ratios [S$_8$: ore] of 4:100 to 6:100 (wt/ wt) was found to be the best proportions for optimal recovery of uranium (63%-64% U$_3$O$_8$) from sandstone ore by a mixed culture of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1). Similarly, a uranium recovery of 68% U$_3$O$_8$ was obtained from sulfur slag to ore ratio of 5:100 to 6:100 (wt/ wt). It was found during acid leaching studies of sandstone ore that 130 kg H$_2$SO$_4$/ ton of ore was required for maximum uranium recovery of 80% U$_3$O$_8$. Uranium concentration of the acid leach liquor was 434.62 mg/L U$_3$O$_8$ with a pH-value of 1.52. Uranium extraction from sandstone ore was found inversely proportional to the ore pulp density. A maximum uranium recovery of 83.5% U$_3$O$_8$ was obtained from ore-pulp density of 1.0% (wt/ vol) during 15-days of incubation. Uranium recovery of 89.12% U$_3$O$_8$ was obtained from ore fraction of particle size of ≤53 μm (ASTM Mesh No. ≤ 300). Microbial leaching of tailings residues showed that a significant amount of uranium could be leached out successfully with supply of energy substrate and nutrient(s).

Bioreactor leaching studies showed a uranium recovery of 87.3% U$_3$O$_8$ from low-grade sandstone uranium ore by a mixed culture of *T. ferrooxidans* (TFe-2) and
T. thiooxidans (TTh-1) during 20-days of incubation. Physical appearance of bacterial leachate was light green, which indicated the presence of soluble iron salts resembling to acid leach liquor of Uranium Ore-Processing Plant, CPC. D.G. Khan.

PVC column leaching studies revealed that when the sandstone ore was amended with elemental sulfur and mine water at pH-value of 3.5, a uranium recovery of 66% U₃O₈ was achieved during 150-days leaching experiments with indigenous microbial populations of acidophilic thiobacilli. However, when mine water was used as such (pH 7.40), the uranium solubilization was found to be upto 48% U₃O₈ under similar conditions. The addition of ammonium sulfate [(NH₄)₂SO₄; 3.0 g/L] in mine water of an adjusted pH-value 3.50, was found to increase the microbial populations concomitantly enhancing the uranium leaching to 90% U₃O₈ from column filled with ore amended sulfur slag. Similarly, maximum uranium recovery of 84.08% U₃O₈ was obtained from PVC column leaching studies on mill tailings residue during 100-days of leaching time.

Uranium recovery of 4.9% U₃O₈ was obtained (calculated on the basis of heap effluents) from low-grade sandstone ore by microbial heap process during 150-days. But on the basis of chemical data of leached residues (core samples taken at depths of 00-100 cm), an average uranium recovery of 31.64% U₃O₈ was leached out during heap operation. During microbial heap leaching process on sandstone uranium ore, an off-white fluffy solid material (sludge) was observed emerging along with heap effluents. A significant amount of uranium (0.0517% to 0.6283% U₃O₈) was found to be present in these samples. It seemed that a major portion of the uranium leached so far during heap leaching process had precipitated/entrapped into these sludge samples. The formation of off-white sludge and precipitation of uranium in these sludge samples might be due to the presence of high calcium content (150.0 mg/L) in subsoil water being used for inocula preparation for microbial heap leaching process. However, no such problem was encountered during microbial column leaching studies of sandstone uranium ore earlier since mine water was employed as inoculum. The mine water sample contained less calcium content (27.6 mg/L Ca²⁺) as compared with NIBGE water.

Amberlite IRA-400 was capable of adsorbing 97.6% U₃O₈ present in the microbial leach liquor as compared to Dowex-I and Duolite® A-147 anion-exchange resins, which absorbed 82% and 87% U₃O₈, respectively. A 2.0 M NH₄HCO₃ solution of pH-value 7.94 showed the maximum uranium elution efficiency (E) and stripped out 99.8% U₃O₈ from the uranium loaded resin column. Strip solutions obtained by 2.0 M NH₄HCO₃ solution was found to contain 15.5-25.6 g/L U₃O₈.

Uranium concentrate (yellow cake) produced from bacterial leach liquors by separation, stripping, precipitation and drying was found to contain 89.6% U₃O₈ on dry matter basis with minor impurities of others metal ions. Boron (B) was present in a concentration of 4.0 μg/g of the sample. The purity of the yellow cake sample
was found to meet the specifications of the Canadian UO₂ powder to some extent.

These studies have indicated that uranium can be leached out, microbially from low-grade sedimentary type ores and uranium mill tailings residue. Only through the applications of biohydrometallurgical processes, the valuable U₃O₈ contents of mill tailings residues can be recovered on commercial scale. Certain amendments like supply of external energy source, nutrient, and following proper leaching techniques i.e. heap leaching process, under natural conditions at very low capital investment are however, required. This process is simple, economically viable and free of environmental hazards. The results of present investigations are very encouraging, and yielding a high uranium recovery from low-grade ores and waste residues. As a consequence of these studies, a heap of 5,000 tons of low-grade sandstone uranium ore has been planned to extract uranium on commercial scale at the mine site, Baghalchur, D.G. Khan.
INTRODUCTION

With the development of civilization and technological advances the consumption of metals and their products has been increased tremendously. This has created problems for metal industry since many of the known high-grade mineral deposits are diminishing at an alarming rate. Consequently, the increased metal demands has to be met more often from low-grade or complex ores. Ebner (1978) estimated that many high-grade metal reserves would be depleting within the next 10-50 years. Bacterial leaching technology presents a potential solution for the problems which are being faced in many countries, where continuing depletion of high-grade ore deposits has created a need to develop cost-effective and environmentally acceptable methods for recovery of metals from low-grade resources.

Bacterial leaching is currently being applied commercially in many developed countries in three major areas: namely, the leaching of (i) copper ores, (ii) uranium ores, and (iii) the pretreatment of gold-containing pyrite and arsenopyrite ore concentrates (Tuovinen et al. 1991). The application of biotechnological principles for obtaining uranium and copper offers a number of advantages over conventional hydrometallurgical techniques. The major benefits perceived are low-cost, ease of maintenance and safety of environment (Bhatti et al. 1991a; Torma 1991a). Selective implementation of living systems can offer opportunities for reduced labour, increased productivity and technological advances (Torma 1986). During the 1970's this technology was coined as "biohydrometallurgy" (Torma and Banhegyi 1984).

Copper can be produced from mining wastes and low-grade ores by bioleaching methods for about one-third to one-half of the costs of copper production from high-grade sulfide concentrates by conventional smelting processes (Torma 1991a). It was estimated in 1980's that about 20-25% of the total copper production in the USA and 5% of the world's total copper production was obtained through bioleaching processes (Brierley 1982; Torma 1986). Another aspect of mineral biotechnology which is equally important is to control environmental
pollution problems which are commonly associated with mining and ore-processing operations. Biohydrometallurgical techniques may offer solutions for bioremediation of environmental damages caused during mining and ore-processing operations. Metals trapped in tailings dams and overburden heaps are potential hazards to the environment, and may be leached out via proper bioleaching processes, thus alleviating adverse affects on existing eco-systems (Bhatti et al. 1993a).

The mineral biotechnology proved to be important for the Canadian uranium industry in the 1960's by resulting in substantial savings in the production costs. At that time, the production price of yellow cake (uranium concentrate) was C$ 3.50/lb, which was below the production costs for chemical processing of ores (Fisher 1966; Gow and Ritcey 1969; MacGregor 1969). Yttrium, one of the rare earth elements, was also recovered as a by-product from the uranium leach solution, which further improved the overall process economics (Torma 1991b). The exploitation of bacterial leaching enables the recovery of uranium from low-grade ores (0.01-0.050% \( \text{U}_3\text{O}_8 \)), which is otherwise uneconomical to recover by conventional hydrometallurgical methods. The process can be applied to recover even poorer and, of course, high-grade materials, such as uranium rich pillars supporting the roof of mine (Kelly 1976). Bioleaching process was used to recover uranium as by-product from gold waste residues at the Buffelsfontein in South Africa (Livesey-Goldblatt 1986).

The biohydrometallurgical processes are mediated by natural populations of acidophilic iron- and sulfur-oxidizing bacteria, such as \textit{Thiobacillus ferroxidans}, \textit{Thiobacillus thiooxidans} and \textit{Leptospirillum ferrooxidans} (Ehrlich 1991; Kelly 1988; Tuovinen et al. 1991), moderate thermophiles such as \textit{Sulfobacillus} spp. (Karavaiko et al. 1988; Norris 1985), and extreme thermophiles such as \textit{Sulfolobus} spp. and \textit{Acidianus} spp. (Segerer et al. 1986). These are obligate or facultative chemolithotrophs and have been isolated from acid mine drainages and leach liquors (Smith and Hoare 1977). These bacteria are capable of oxidizing ferrous iron to ferric iron as well as reduced sulfur compounds to sulfuric acid or metal sulfates (Ehrlich 1991; Norris 1990; Tuovinen et al. 1991). The main physiological
characteristics of these bacteria have been described in detail by Norris (1990).

The principal acid-generating bacterium affiliated with mineral leaching is *T. ferrooxidans*, which is often associated with acid mine effluents and leach liquors (Norris and Ingledew 1992; Tuovinen et al. 1981). *T. ferrooxidans* is capable of utilizing the sulfide moiety of minerals including pyrite [FeS₂] (Basaran and Tuovinen 1987), pyrrhotite [Fe₇₆S₃₄] (Ahonen et al. 1986; Bhatti et al. 1993b), chalcopyrite [CuFeS₂] (Mehta and Murr 1982), chalcocite [Cu₂S], bornite [Cu₄FeS₄] (Torma 1984), arsenopyrite (Carlson et al. 1992; Tuovinen et al. 1994) and sphalerite [ZnS] (Groudev 1980).

\[
\begin{align*}
\text{FeS}_2 + 3\frac{1}{2} \text{O}_2 + \text{H}_2\text{O} & \rightarrow \text{FeSO}_4 + \text{H}_2\text{SO}_4 & [1] \\
2 \text{FeSO}_4 + \frac{1}{2} \text{O}_2 + \text{H}_2\text{SO}_4 & \rightarrow \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{O} & [2] \\
\text{FeS}_2 + \text{Fe}_2(\text{SO}_4)_3 & \rightarrow 3 \text{FeSO}_4 + 2 \text{S}^0 & [3] \\
2 \text{S}^0 + 3 \text{O}_2 + 2 \text{H}_2\text{O} & \rightarrow 2 \text{H}_2\text{SO}_4 & [4]
\end{align*}
\]

Reactions [1], [2], and [4] are catalyzed by acidophilic *Thiobacillus* type or related bacteria (Tuovinen 1990). The involvement of a number of heterotrophs, fungi, yeasts, algae and protozoa have also been found in symbiotic association with sulfidic minerals oxidations (Ehrlich 1991; Groudev 1987; Madgwick and Ralph 1980).

Chemical and biochemical reactions, such as oxidation, reduction, chelation, biosorption, biocorrosion, probably simultaneously take place in the biohydrometallurgical processes under the metabolic influence of acidophilic, chemolithoautotrophic and heterotrophic microorganisms in symbiotic growth (Wichlacz and Unz 1981). Often, the leach medium is dilute sulfuric acid containing microorganisms, nutrients, ferric iron, organic acids, polysaccharides, proteins, and chelating agents which may be the products of bacterial metabolism (Karavaiko
In bioléaching processes, microorganisms catalyze the dissolution of metals from minerals (Lundgren et al. 1986) and are carried out at atmospheric pressure and temperatures which vary from 5 to 85°C (Karavaiko 1985). Bacterial leaching processes are much faster than chemical (abiotic) leaching processes at ambient temperature and atmospheric pressure (Torma 1991b).

The microbial leaching studies of uranium ores have extensively utilized pure or enrichment cultures of *T. ferrooxidans*. The use of *T. ferrooxidans* and *T. thiooxidans* for uranium ores leaching has also been investigated by a number of workers (Bhatti et al. 1993a; Bosecker and Wirth 1980; Bruynesteyn 1985. Duncan and Bruynesteyn 1970; Guay et al. 1976; Guay and Silver 1980; Harrison et al. 1966; Khalid et al. 1990; Silver 1985; Soljanto and Tuovinen 1980; Tomizuka and Takahara 1972; Tomizuka et al. 1976). Investigations with mixed cultures have demonstrated the beneficial use of various combinations of acidophilic thiobacilli and *Leptospirillum ferrooxidans* or other mixed cultures in the solubilization of pyritic materials (Puhakka et al. 1985). It has already been established that complex heterogeneous microbial populations exist in acid mine waters (Grondev 1980; Wichlacz and Unz 1981) including uranium mine leach liquors (Tuovinen et al. 1981). In heap and dump leaching operations, no extra inoculation is required since the bacteria responsible for leaching processes are already ubiquitous, occurring wherever acidic conditions are maintained in the presence of sulfidic minerals (Ito 1976).

The bacterial oxidation of U(IV)-compounds is of particular interest in biohydrometallurgical processes (Duncan and Bruynesteyn 1971; MacGregor 1969; McCready et al. 1969). In acid leaching, tetravalent uranium [U⁴⁺] is oxidized by ferric iron to the hexavalent form [U⁶⁺], which is readily soluble in dilute sulfuric acid. In this reaction, Fe³⁺ is reduced to Fe²⁺ which in turn is oxidized to Fe⁴⁺ by iron-oxidizing thiobacilli (Derry et al. 1977; Guay et al. 1976; 1977; Soljanto and Tuovinen 1980). In case of metal sulfides, the production of sulfuric acid and soluble ferric iron is one of the mechanisms by which *T. ferrooxidans* indirectly accelerates the leaching of uranium ores (Lundgren and Silver 1980):
\[
\begin{align*}
\text{U}^\text{V} + \text{H}_2\text{SO}_4 + \frac{1}{2} \text{O}_2 & \rightarrow \text{UO}_2\text{SO}_4 + \text{H}_2\text{O} & [5] \\
\text{U}^\text{V} + \text{Fe}_2(\text{SO}_4)_3 & \rightarrow \text{UO}_2\text{SO}_4 + 2 \text{FeSO}_4 & [6] \\
2 \text{FeSO}_4 + \text{H}_2\text{SO}_4 + \frac{1}{2} \text{O}_2 & \rightarrow \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{O} & [7] \\
2 \text{FeS}_2 + 7\frac{1}{2} \text{O}_2 + \text{H}_2\text{O} & \rightarrow 2 \text{Fe}_2(\text{SO}_4)_3 + 4 \text{H}_2\text{SO}_4 & [8] \\
\text{UO}_2 + 2 \text{Fe}^{3+} & \rightarrow \text{UO}_2^{2-} + 2 \text{Fe}^{2+} & [9]
\end{align*}
\]

Reactions [7] and [8] are catalyzed by \textit{T. ferrooxidans} at low pH values (≤2.0).

The rate of bioleaching of uranium is directly related to the rate at which the iron-oxidizing bacterium (\textit{T. ferrooxidans}) oxidizes ferrous iron, pyrite, and reduced sulfur compounds for its growth requirements (DiSpirito and Tuovinen 1982a, 1982b; Ferroni et al. 1986). Many laboratory studies have demonstrated that these bacteria can accelerate, with varying success, leaching rates and thereby enhance uranium recovery under controlled conditions (Lundgren and Silver 1980). Particularly, in the Elliot Lake area, uranium ores were investigated for the development of bacterial extraction processes both in surface heaps and underground stopes (Gow et al. 1971). Bacterially mediated leaching processes for uranium can be represented by a generalized flow-sheet as shown in Figure 1.

Ore leaching under static condition by gravity flow through the pile is generally termed as a heap leaching process. Heap leaching process is useful for the treatment of low-grade ores or for small, isolated ore-bodies which do not justify the transportation of ore to a conventional mill or installation of capital investment for equipment and depending upon the characteristics of the ore as mined, can be directly used for heap formation and the most of crushing system can be avoided. Agitation and liquid separation equipments are not required as leach solutions are reasonably clean. Solution purification and precipitation facilities are required nearby to avoid transportation of large volume of solution collected from the heap (Zaman et al. 1980).
Figure 1: A generalized proposed flow-sheet for bacterial leaching of uranium ore.

Microbial biotechnology can potentially be applied to recover zinc (Baldi et al. 1991; Derry and Whittenmore 1983; Groudev 1980), gallium (Jiang and Torma 1989), cobalt (Baldi et al. 1991), nickel (Bosecker 1986), zirconium and rare earth elements (Glombitza et al. 1987) as well as precious metals such as gold (Lawrence 1990; Lindstrom et al. 1992; Livesey-Goldblatt 1986) and silver (Ehrlich 1986) from
their respective sulfide-bearing matrices. This technology has also been tested for the recovery of heavy metals from municipal sludge (Blais et al. 1992; Tyagi et al. 1991; Tyagi and Couillard 1989) and fly ash (Lodi et al. 1989). In addition, the pyritic and organic sulfur content of coal can be removed by the bacterial action of \textit{T. ferrooxidans} (Dugan 1986) and \textit{Sulfobatus acidocaldarius} (Kargi and Robinson 1985).

In spite of the relative slowness of the recovery of metals by bacterial leaching processes as compared to hydrometallurgical procedures, there is likely to be a continuing and increasing usage of dump, heap and solution mining operations in the future. The state of the art with respect to the basic science and the application in bacterial leaching and related areas has been progressively reviewed in the recent years (Brierley 1982; McCready and Gould 1990; Ralph 1985; Tuovinen 1990; Torma 1991a; Torma 1987; Torma and Bosecker 1982). The proceedings of the international conferences on biohydrometallurgy in recent years provide evidence of increasing interest in this technology (Ehrlich and Holmes 1986; Hutchins et al. 1986; Karavaiko and Groudev 1985; Lawrence et al. 1986; Norris and Kelly 1988; Rossi 1990; Rossi and Torma 1983; Torma et al. 1993; Trudinger et al. 1980).

The well-established bacterial leaching techniques have been applied to sulfidic ores or to ores containing iron sulfide minerals. The microbial degradation of silicate minerals required an external energy source (Ralph 1985). Little information is available in the literature on biohydrometallurgical applications for the recovery of uranium from sandstone-type ore deposits. Some work on the sedimentary type ore deposits from Fortsru, Austria (Bosecker and Wirth 1980), and Baghalchur, Pakistan (Bhatti et al. 1991a; Khalid et al. 1990) have been reported. Like most of the ore deposits mined commercially, the uranium content of the ore being mined at Baghalchur, varies from a maximum of 0.10\% \text{U}_3\text{O}_8 to a minimum of 0.02\% \text{U}_3\text{O}_8 as the cut-off grade. Conventional leaching (acid/carbonate leaching) of uranium ore containing \leq 0.03\% \text{U}_3\text{O}_8 is not economically feasible (Bhatti et al. 1993a).
The importance of uranium as a feedstock in the production of energy in nuclear powers has been recognized during the past four decades and its importance will be growing in the future, too. The world’s nuclear power reactors produce more than 19.0% of all the electricity consumed (CNA 1989). Hence an efficient and safe extraction of uranium is of great interest worldwide. The significance of processing of some low-grade resources of uranium like sea-water, coal-ash, cinders and shale has become increasingly important.

In Pakistan, earnest efforts are being made to overcome the acute shortage of electricity by producing it through nuclear energy. The importance of uranium as a feedstock source for power generation is ever increasing and is anticipated to be more in future in lieu of the commissioning of CHASMA Nuclear Power Plant (CHASNUPP). In keeping with this perceived additional requirement of uranium, the present work is aimed at investigating the microbial leaching of Baghalchur low-grade sandstone uranium ore and at ascertaining the role of microorganisms in this process. In order to systematically test the optimum leaching parameters for bacterial heap leaching of low-grade sandstone uranium ore, the following objectives are identified. It is hoped that this study will open up a new era for the extraction of uranium from low-grade ores, overburdens and industrial wastes in Pakistan.

- Mineral chemistry of low-grade sandstone uranium ore.
- Isolation and characterization of iron- and sulfur-oxidizing bacteria from various ecosystems of uranium ores, such as mining, processing and tailings.
- Oxidation studies of various external energy sources like elemental sulfur, sulfur slag, ferrous iron and pyrite by isolated strains of *Thiobacillus*.
- Effect of various leaching parameters such as acid consumption, time, pulp density, particle size and different proportions of external energy substrates.
on bioleaching of sandstone uranium ore.

- Optimization of bacterial leaching processes in the laboratory through various leaching techniques including shake-flask, tank, air-lift percolator and column.

- Microbial heap leaching studies of sandstone uranium ore.

- Separation of uranium from bacterial leach liquors through ion-exchange resins.

- Production of uranium concentrate (yellow cake).

- Chemical analyses of uranium and associated elements in low-grade ores, bacterial leach liquors, strip solutions and uranium concentrates by various analytical techniques.
LITERATURE REVIEW

Biogeochemical activity began with the appearance of life on earth as a product of interacting evolutions of biosphere, atmosphere, hydrosphere and lithosphere (Cloud 1980). Since geological times, microorganisms are known to play an important role in the formation and solubilization of mineral deposits (Ehrlich 1981; Krumblin 1983). Early evidence for the ability of some microbial species to influence the course of inorganic chemical reactions was provided by the work of Winogradsky (1888), which indicated the growth of filamentous bacteria in ferruginous and was accompanied by the accumulation of oxidized and insoluble forms of iron. The presence of sulfuric acid in coal-mine drainages and of iron and copper in the effluents of copper-mines had long been considered to be the solely results of chemical reactions (Cloud 1980).

In recent years, biological methods enjoy an increased awareness for mineral prospection (Brooks 1983) and sedimentology (Nedwell and Brown 1982). Nevertheless, the extraction of metals from ores by bacterial leaching process can be traced back to the Phoenicians and Romans who recovered copper released by bacterial dissolution from its minerals (Nicolaidis 1987). The recovery of copper by treatment of drainage waters from abandoned mines and dumps of overburden materials containing low-grade copper sulfide minerals had been practiced during 960-1279 in China (Pu 1982). Similarly, the leaching of copper ores was being practiced in Northern Hungary in 1497 (Podanyi 1980). Although during 16th century in the Harz Mountains, Germany (Habashi 1980); around 1670 at the Rio Tinto, Spain (Taylor and Whelan 1943) and in the 18th century in Wicklow, England (Wadsworth 1982) copper was produced through leaching operations but the presence of bacteria in the leach liquors of the Rio Tinto mines were not confirmed until 1963 (Razzell and Trussell 1963a).
Heap and dump leaching operations were introduced in the USA in the early 1920’s for the recovery of copper from sulfidic ores by the Phelps-Dodge Corporation at Bisbee, Arizona and Tyron, New Mexico, without knowing about the contribution of microorganisms in these processes (Trussell et al. 1964). In the former USSR, the extraction of copper from mine effluents of copper-pyrite deposits was practiced in the Urals during the mid of 1940’s (Karavaiko 1985). Rudolfs and Helbronner (1922), however, later reported the oxidation of zinc sulfides by soil microorganisms but no real understanding of the microbial role in geochemical phenomena existed until Colmer and Hinkle (1947), isolated a microorganism from acid mine drainage of bituminous coal mines and identified as *Thiobacillus ferrooxidans* being the microorganism principally responsible for leaching of metal sulfides. The biotechnology of microbial mining was started in 1963, when results of laboratory studies confirmed the involvement of bacteria in solubilization of copper from sulfidic ores (Razzell and Trussell 1963b). The elementary process involved was the percolation of acidified water through a mineral ore-body during which oxidative reactions, catalyzed by bacteria occurred which led to metal extraction.

Uranium recovery by bacterially-assisted leaching was started during the 1960’s from acid leaching of mining wastes and worked out stopes of Stanrock uranium mines in Canada (Duncan and Bruynesteyne 1971; Fisher 1966; Fletcher 1970). The procedure was simply to hose down the roof, walls and floors of mine working stopes at cyclical intervals of about three months producing acidified water of pH 2.3-2.8 which contained economically workable concentrations of dissolved uranium, and a monthly recovery of 7.50 tons U₃O₈ was obtained in this way (Sasson 1975) at production cost of C$ 1.58/kg U₃O₈ (MacGregor 1966). Over 84,000 lbs of U₃O₈ was recovered from ore left behind after mining activities, waste piles and ore-bearing pillars by bacterially-assisted leaching methods at the Denison Mines, Canada (Marchbank 1987). The first industrial heaps for uranium extraction built during 1952-53 at Urgeirica in Portugal, were fully described by Cameron (1963) and subsequently, this method has been studied in Argentina, France, USA, Spain, South Africa and former Yugoslavia and USSR (Lowson 1975). In the early 1960’s, mine
operators at several uranium mines in the Elliot Lake Area, Northern Ontario, Canada, noticed that the mine drainage had become very acidic and contained appreciable amounts of soluble iron and uranium (Harrison et al. 1966). Harrison and his coworkers also isolated *T. ferrooxidans* from the Denison Mine water thereby demonstrating that bacteria were necessary for uranium solubilization from its ore.

McCready (1988) reported that the Denison Mines produced 33,000 kgs/month $U_3O_8$ in 1987 by bacterial leaching process from forty-eight stopes containing $1.1 \times 10^6$ tons of broken. During 1964 and 1965, a total recovery of 58,000 kgs $U_3O_8$ was obtained from the Rio Algom's Milliken Mine by spraying the worked out stopes of uranium mine with acidic mine drainage (Fisher 1966). The mine water pumped out to the surface contained $0.135 \text{ kg/m}^3 U_3O_8$. At the Nordic Uranium Mine in the Elliot Lake area, where 14 experimental worked out stopes were sprayed with bacterial solutions enriched with suitable nutrients, so the production was as high as $9.0 \text{ kg } U_3O_8/\text{hr}$ from pregnant solutions containing up to $2.5 \text{ kg/m}^3 U_3O_8$ (Guay et al. 1977). In 1978, the Agnew Lake Operation produced 1,81,437 kgs $U_3O_8$ from surface heaps, whereas 1,13,398 kgs $U_3O_8$ was produced from underground stopes (Anonymous 1979). The Agnew Lake Mine was considered the first mine in the world to apply *in-situ* bioleaching technique to a virgin orebody (Rossi 1990).

**Uranium Ores and Minerals**

Although uranium was discovered in 1789 by Martin Klaproth in pitchblende $[(U^{+4},U^{+6})O_2]$ ores at a German mine but its presence has been known since 1727. As an element it was isolated in 1842 and its nature as radioactive element was first noted in 1896 by William Bacquerel. The radium, a daughter product of uranium decay was first discovered in 1898 by Madame Curie and G. Bemont from pitchblende ores at Joachimsthal, Czechoslovakia. These mines were the first to produce uranium, mined for its radium content being used in medical radiotherapy (Elevatroski 1978).
In 1874, carnotite \( [K_2(VO_4)_2(UO_2)_2·1·5H_2O] \) a bright-yellow uranium mineral was first discovered at Jim Thrope (formerly Mauch Chunck), Pennsylvania, USA and later in 1881, it was discovered as rich veins in the Roc Creek area of Colorado, USA. The uranium ores containing carnotite mineral were first shipped to France in 1898 for radium production. From 1911 to 1923, there was a commercial production of carnotite for its radium and vanadium contents in the Paradox District, Colorado, USA. A small amount of uranium was recovered but most of it was disposed of as tailings (Elevatroski 1978). The Shinkolobwe high-grade uranium ore deposit containing up to 60% \( U_3O_8 \) located in the Katanga Province, Republic of Zaire (formerly Belgian Congo) was discovered in 1913. From 1923 to 1930’s, this deposit largely supplied the source material for radium and the US uranium mines were remained closed. In 1933, uranium production began from a new discovery at the Great Bear Lake in Canada’s Northwest Territory and the uranium market was shared with Shinkolobwe until the early 1940’s. Uranium, mined at the Great Bear Lake deposit was the source material imported by the US for making the first atomic bomb during the World War II (Hewlett and Anderson 1962). The earliest use of uranium was in ceramic paints prior to its use for radium production (Elevatroski 1978). Uranium-vanadium deposits in the Colorado Plateau region were mined for their vanadium content from 1936 to 1944. In the late 1940’s and 1950’s, uranium was sought for atomic weapon fissionable material (Merritt 1971).

Uranium in the earth’s crust was introduced hydrothermally or magmatically and the source of uranium present at specific deposits was postulated to be alkalic granites, arkoses or volcanic ash-tuffs. In many areas, the alkalic granites showed high uranium content which was leached out from granites by syngenetically and disseminated in the sediments and epigenetically it was concentrated by ground-water (Tugarinov 1975). Uranium solubility is favoured by high temperature and pressure conditions that are found in hydrothermal solutions released from cooling magmas (Langmuir 1978; Taylor 1979). Ground waters may transport uranium derived from weathering of granites or ash-tuffs through aquifers, pore spacings, cracks, and fissures in the rock formations. This movement of water may lead either to
dissemination of uranium or to concentrations under favourable conditions. Physical characteristics of the host sediment act to channel the solutions and assist in the concentration of uranium (Taylor 1979).

Uranium is widely disseminated in nature with estimates of its average abundance in the earth's crust varying from 2-4 ppm, which is near to that of molybdenum, tungsten, arsenic, and beryllium, but higher than metals such as cadmium, bismuth, mercury, and silver (Merritt 1971). Igneous rocks with a high silica content such as granite, tend to show slightly higher uranium concentrations approaching to 6 ppm, while rocks with less silica and more magnesium, aluminum, and iron content may contain less than 1 ppm of uranium (Taylor 1979). Apart from ore deposits, uranium is most abundant in high silica igneous rocks and shales, especially black shales. For example, in USA some Colorado Front Range and other granites are enriched in alkali contents, depleted in calcium and contain over 100 ppm uranium. Typical uranium concentrations in various host-materials are reported in Table 1 (Merritt 1971).

There are eleven known isotopes of uranium, of which three with atomic weights 234, 235 and 238 occur in nature. They are all radioactive with half-life (in years) of 2.35 X 10⁴, 7 X 10⁸ and 4.5 X 10⁹, respectively. The relative abundance of isotopes varies depending on the age and geological history of uranium occurrence: a typical distribution of uranium is ²³⁵U:99.28%; ²³⁴U:0.71%; ²³۸U:0.005% (Anonymous 1969). Uranium in nature exists in different oxidation states, such as trivalent [U³⁺], tetravalent [U⁴⁺], pentavalent [U⁵⁺] and hexavalent [U⁶⁺] (Eligwe and Torma 1986). The mineralogy and geochemistry of uranium oxidation states are quite different from each other. Uranium is geologically laid down under highly reduced conditions in the tetravalent [U⁴⁺] state (Anonymous 1969; Smith 1990; Taylor 1980). The precipitation of uranium from solution may brought about in nature by lowering of temperature, pressure, reduction, ion-exchange, neutralization, and chemical replacement (Elevatroski 1978). The mechanism of major importance is the formation of tetravalent uranium [U⁴⁺] minerals from hydrothermal and ground
water solutions, involving reduction of soluble uranyl ion $[\text{UO}_2^{2+}]$, which may take place through the action of carbonaceous matter, hydrogen sulfide, and other reductants (Taylor 1979).

Table 1
Uranium Content of Various Host-Materials (Merritt 1971)

<table>
<thead>
<tr>
<th>Host Material</th>
<th>Typical Analysis, $\text{U}_3\text{O}_8$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[%]</td>
</tr>
<tr>
<td>High-grade Veins</td>
<td>30-70</td>
</tr>
<tr>
<td>Vein ores</td>
<td>0.2-1.0</td>
</tr>
<tr>
<td>Sandstone ores</td>
<td>0.05-0.40</td>
</tr>
<tr>
<td>Pegmatitic ores</td>
<td>0.05-0.10</td>
</tr>
<tr>
<td>Lignite ores (USA)</td>
<td>0.20-0.30</td>
</tr>
<tr>
<td>Uraniferous hydrocarbons</td>
<td>0.001-0.10</td>
</tr>
<tr>
<td>Uraniferous phosphates</td>
<td>0.005-0.03</td>
</tr>
<tr>
<td>Alum shales (Sweden)</td>
<td>0.02-0.03</td>
</tr>
<tr>
<td>Gold ores (South Africa)</td>
<td>0.015-0.06</td>
</tr>
<tr>
<td>Marine black shales</td>
<td>0.001-0.02</td>
</tr>
<tr>
<td>Chattanooga shales (USA)</td>
<td>0.006</td>
</tr>
<tr>
<td>Uraniferous granites</td>
<td></td>
</tr>
<tr>
<td>Normal granite</td>
<td>5</td>
</tr>
<tr>
<td>Earth’s crust</td>
<td>2-4</td>
</tr>
<tr>
<td>Uranium mine waters</td>
<td>5-15</td>
</tr>
<tr>
<td>Copper dump leaching solutions</td>
<td>1-12</td>
</tr>
<tr>
<td>Sea water</td>
<td>0.002-0.003</td>
</tr>
</tbody>
</table>
The microbial reduction of soluble U(VI) to insoluble U(IV) may play an important role in the geochemical cycle of uranium and also serve as a mechanism for the bioremediation of uranium contaminated waters (Lovely et al. 1991). The participation of microbes in the geochemistry of uranium is directly related to its chemical properties. The precipitation of U(VI) as a result of uranium (IV) reduction in anaerobic marine sediments is the most significant modern global sink for dissolved uranium (Anderson et al. 1989; Klinkhammer and Palmer 1991). Immobilization of uranium through U(VI) reduction has led to the formation of many economically important uranium deposits (Langmuir 1978; Taylor 1979). The reductive precipitation of uranium may also account for the ability of bottom sediments of algal ponds to remove dissolved uranium from uranium mine waters (Brierley and Brierley 1980). Bioreactors containing U(VI)-reducing microorganisms can rapidly remove dissolved uranium from aqueous solutions (Gorby and Lovely 1991).

The Fe(III)-reducing microorganisms, Geobacter metallireducens (previously known as strain GS-15) and Shewanella putrefaciens (previously Alteromonas) have shown to use U(VI) as a terminal electron acceptor (Lovely et al. 1991; Lovely et al. 1989). The geochemical evidence indicated that Fe(III)-reducing microorganisms have reduced U(VI) within Fe(III)-reducing zones of marine sediments (Colley and Thomson 1985; Langmuir 1978) and other environments (Hoffmann 1990). The recent informations suggested that in some marine sediments, U(VI) has also been reduced within the sulfate-reducing zones (Klinkhammer and Palmer 1991). The enzymic reduction of U(VI) by a sulfate-reducing anaerobic bacterium, Desulfovibrio desulfuricans was observed to be much faster than chemical reduction of U(VI) by sulfide (Lovely and Phillips 1992). D. desulfuricans produced hydrogen sulfide [H$_2$S] gas by feeding on organic matter and sulfates (Alder 1974; Alder 1963). The metal sulfides associated with uranium minerals in many of the major sandstone-type ore deposits in the USA, provided evidence of having been formed by the reducing action of bacteriogenic hydrogen sulfide. The carbonaceous matter not only acts as a reductant but with certain clay, may function as an ion-exchanger to adsorb
uranium from solution (Miller et al. 1954). This mechanism has an importance in uraniferous hydrocarbons and in concentrating uranium in high-clay ores. The accumulation of uranium in the Carboniferous and Cretaceous deposits has attributed to biogenic reactions (Levinson 1974).

Uranium in nature exists as complex oxides, hydrated oxides, carbonates, phosphates, sulfates, vanadates, molybdates, and silicates (Eligwe and Torma 1986). Over 100 minerals of uranium have been described in literature, but minerals of economic importance are uraninite \([\text{UO}_2]\) and pitchblende \([(\text{U}^{4+},\text{U}^{6+})\text{O}_2]\), which represent different crystalline forms of uraninite in vein type ore deposits (Elevatroski 1978; Eligwe and Torma 1986; Gow et al. 1971). Uranium minerals are often associated with metal sulfides such as pyrite \([\text{FeS}_2\text{ cubic}]\), marcasite \([\text{FeS}_2\text{ orthorhombic}]\), pyrrhotite \([\text{Fe}_1-x\text{S}]\), sphalerite \([\text{ZnS}]\) and chalcopyrite \([\text{CuFeS}_2]\) (Smith 1990). According to the data published jointly by the OECD Nuclear Energy Agency and International Atomic Energy Agency (IAEA) in 1983, the world’s reasonably assured resources of uranium which are recoverable at US$ 130/kg U, to be 2.293 X 10^6 tonnes of uranium (Anonymous 1983). The major uranium resources of the world can be assigned on the basis of their geological setting to the following six categories of ore types (Table 2) (Bhatti et al. 1993a). Uranium deposits which occur in the sedimentary rocks have been considered the most important economically (Elevatroski 1978). However, very large and high-grade uranium deposits were reported in metamorphic rocks as in the Northern Australia and Saskatchewan and low-grade deposits in granitic rocks (Elevatroski 1978). The largest known uranium ores deposits are located in Zaire, Canada and Czechoslovakia (Guy and Silver 1980).

Uranium also occurs in sea-water as an anion and has a long residence time of 2-4 X 10^6 years (Klinkhammer and Palmer 1991). The uranium metal behaves as a well-mixed element with an average concentration of 3.30 \(\mu\text{g} \text{ U/L} [1.4 \times 10^{-5} \text{ M U}].\) Cloud (1968) estimated that sea-waters contain 5.0 X 10^9 tonnes of dissolved uranium content. Uranium in sea water is precipitated or adsorbed into marine sediments.
Table 2
Major Types of Uranium Ore Deposits of the World

<table>
<thead>
<tr>
<th>Deposit Type</th>
<th>Mineralization of Uranium</th>
<th>Uranium Minerals Present</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High-Grade Uranium Deposits:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandstone</td>
<td>Grain binding material</td>
<td>Uraninite, Coffinite and Pitchblende in unoxidized deposits; Carnotite, Tyuyamunite and Uranophane in oxidized ores; upto 5% calcite and minor pyrite.</td>
</tr>
<tr>
<td>Quartz-Pebble Conglomerate</td>
<td>Cementing material and pebbles</td>
<td>Pitchblende, Uraninite, Brannerite, Uranothorite; upto 5% pyrite; gold present in the South African deposits.</td>
</tr>
<tr>
<td>Proterozoic Unconformity and Related</td>
<td>Disseminated form</td>
<td>Pitchblende, Uraninite and massive arsenides and sulfides of nickel and other metals, like gold. High content of carbonates.</td>
</tr>
<tr>
<td>Disseminated</td>
<td>Wide range of mineralization</td>
<td>Syenite, Pegmatite, Uraninite and Uranothorite.</td>
</tr>
<tr>
<td>Vein-type</td>
<td>Uranium minerals fill the cavities of host rocks</td>
<td>Pitchblende and uraninite. Some deposits contain sulfides and rhenides.</td>
</tr>
<tr>
<td>Uraniferous Shales</td>
<td>Thin coating of carnitite in voids and limestone</td>
<td>Carnotite</td>
</tr>
</tbody>
</table>

**Low-Grade Uranium Deposits:**
Lignites
Phosphates
Calcretes
Porous limestone
(Deuser 1971; Bonatti et al. 1971). The uraniferous black shales and phosphorites are the products of this process (Merritt 1971). The present occurrence and forms of uranium deposits in sedimentary and metamorphic rocks are not necessarily those of the original deposits and in some regions, several cycles of dissolution and precipitation and/or metamorphism have occurred (Baturin 1973; Jenson 1958; Hostetler and Garrels 1962).

In Pakistan, sedimentary-type uranium ore deposits occur in the foothills of the Sulaiman Range in Dera Ghazi Khan District, and the best exposure of uranium mineralization containing 0.05-0.30% U\textsubscript{3}O\textsubscript{8} content is at Baghalchur (Moghal 1974). Sandstone-type uranium ore deposits mostly contain up to 5.0% calcite [CaCO\textsubscript{3}] and minor amounts of pyrite [FeS\textsubscript{2}] and uranium minerals occur in the grain-binding material (Gow 1985).

**Extractive Chemistry of Uranium from Ores**

The current technology of uranium extraction from its ores involves leaching with either dilute sulfuric acid [H\textsubscript{2}SO\textsubscript{4}] or sodium carbonate-sodium bicarbonate [Na\textsubscript{2}CO\textsubscript{3}-NaHCO\textsubscript{3}] solution (Eligwe and Torma 1986; Merritt 1971). The choice for lixiviant depends upon the ore composition and the effects of several factors such as reagent costs, solubility of contaminants, recovery methods, environmental considerations, and mill-tailings management (Merritt 1971). Sulfuric acid is the most widely used leaching agent for uranium extraction from its ores (Clegg and Foley 1958; DeVries 1985). The sulfuric acid leaching process results in the formation of soluble uranyl sulfate [H\textsubscript{4}(UO\textsubscript{2})(SO\textsubscript{4})\textsubscript{3}], while carbonate [Na\textsubscript{2}CO\textsubscript{3}-NaHCO\textsubscript{3}] leaching gives soluble tetrasiocidum uranyl tricarbonate [Na\textsubscript{4}UO\textsubscript{2}(CO\textsubscript{3})\textsubscript{4}]. Uranium ores are generally considered low-grade, if they contain an average uranium content of less than 0.05% U\textsubscript{3}O\textsubscript{8} (Brierley 1978). In these leaching processes, hexavalent uranium minerals undergo non-oxidative dissolution (Lundgren and Silver 1980):
\[
\text{UO}_3 + 3 \text{H}_2\text{SO}_4 \rightarrow \text{H}_2\text{UO}_2\text{(SO}_4)_3 + \text{H}_2\text{O} \quad [10] \\
\text{UO}_3 + \text{Na}_2\text{CO}_3 + 2 \text{NaHCO}_3 \rightarrow \text{Na}_4\text{H}_{\text{2}}\text{UO}_2\text{(CO}_3)_3 + \text{H}_2\text{O} \quad [11]
\]

The tetravalent form of uranium \([\text{U}^{4+}]\) is insoluble in dilute sulfuric acid and/or carbonate solution and must be oxidized for dissolution to occur (Eligwe and Torma 1986). The most frequently used oxidants in sulfuric acid leach system are sodium chlorate \([\text{NaClO}_3]\) and manganese dioxide \([\text{MnO}_2]\) (Merritt 1971). These oxidants maintain iron in solution in ferric state (Eligwe and Torma 1984; Mattus and Torma 1980), which oxidizes tetravalent uranium by a surface electrochemical reaction (Nicol et al. 1975). During leaching process, the redox potential is maintained for the system above 400 mV by the oxidation of appropriate amount of oxidants are manganese dioxide \([\text{MnO}_2]\) and sodium chlorate \([\text{NaClO}_3]\) (Merritt 1971). Molecular oxygen is known to be a relatively inefficient oxidant for tetravalent uranium in sulfuric acid leaching (Haque and Ritecy 1982). Ferric iron \([\text{Fe}^{3+}]\) is one of the most effective oxidants for solubilizing uranium and a concentration of about 1-2 g/L of \text{Fe}^{3+} is usually adequate for an effective dissolution of uranium (Mattus and Torma 1980).

\[
\text{2Fe}^{2+} + \text{MnO}_2 + 4 \text{H}^+ \rightarrow 2 \text{Fe}^{3+} + \text{Mn}^{2+} + 2 \text{H}_2\text{O} \quad [12] \\
\text{2Fe}^{2+} + \text{ClO}_3^- + 6 \text{H}^+ \rightarrow 2 \text{Fe}^{3+} + \text{Cl}^- + 3 \text{H}_2\text{O} \quad [13]
\]

The ferric iron \([\text{Fe}^{3+}]\) then oxidizes \(\text{U}^{4+}\) to \(\text{U}^{6+}\):

\[
\text{UO}_2 + \text{Fe}^{3+} \rightarrow \text{UO}_2^{2+} + \text{Fe}^{2+} \quad [14]
\]

The applicability of a large number of other oxidants, such as nitric acid and Caro's acid \([2\text{KHSO}_3, \text{K}_2\text{SO}_4]\) for uranium extraction from ores have also reported (Chutinara et al. 1984; Haque and Ritecy 1982; Ring et al. 1985). In recent years, the use of hydrogen peroxide \([\text{H}_2\text{O}_2]\) as "clean" alternative oxidant in uranium hydrometallurgy has advocated (Derry and Whittemore 1979; Hiskey 1980) and even practiced in solution mining operations (Eligwe et al. 1982).
Uranium (IV) oxidation by *T. ferrooxidans* was believed to be an indirect mechanism, in which soluble ferric iron [Fe$^{3+}$] acts as a redox carrier (Tuovinen et al. 1983) as illustrated in Figure 2. The catalytic role of *T. ferrooxidans* for regenerating ferric iron in uranium leaching process was demonstrated by several authors (Guay and Silver 1979; Manchee 1977; Tuovinen et al. 1983). The oxidation of tetravalent uranium [U$^{4+}$] to hexavalent [U$^{6+}$] state by ferric ions [Fe$^{3+}$] occurs more rapidly in the presence of *T. ferrooxidans* (Lundgren and Silver 1980).

![Figure 2: Indirect oxidation of uranium (IV) by *T. ferrooxidans* with iron as an electron carrier.](image-url)
The major role of microorganisms in uranium leaching was demonstrated by various workers (Brierley 1980; Brierley 1978; Bruynesteyn et al. 1980; Campbell et al. 1985; Manchee 1977; Tomizuka and Yagisawa 1978; Tuovinen et al. 1991). The oxidation of tetravalent uranium oxide \(\text{UO}_2\) by ferric sulfate \([\text{Fe}_3(\text{SO}_4)_2]\) requires continuous regeneration of ferric sulfate, which can be achieved through biological oxidation of either soluble ferrous sulfate \([\text{FeSO}_4]\), or iron-containing sulfide minerals present in the uranium ores (Harrison et al. 1966; Torma 1991a). Livesey-Goldblatt et al. (1977) designed and developed a pilot plant unit for continuous oxidation of recycled acidified ferrous sulfate leach solution obtained from uranium ore-processing plants. This process was known as "BACFOX" process; which was based on rapid oxidation of an acidified solution of ferrous sulfate to ferric sulfate, when solution was saturated with air and passed over a biofilm of \(T.\) \textit{ferrooxidans} adhering to an appropriate solid surface.

The rate of bioleaching of uranium is directly related to the rate at which the bacterium oxidizes \(\text{Fe}^{2+}\) to provide energy for its growth requirement (DiSpiito and Tuovinen 1982a, 1982b; Ferroni et al. 1986; Ivarson 1980). The Urgeirica ore containing 0.1\% \(\text{U}_3\text{O}_8\) and 5.0\% pyrite leached rapidly by \(T.\) \textit{ferrooxidans} upon addition of 1.0\% \(\text{FeSO}_4\) (Zajic 1969). The \textit{rate of oxidation} of \(\text{Fe}^{2+}\) to \(\text{Fe}^{3+}\) was observed 7.5 g/m\(^2\)/hr. \(T.\) \textit{ferrooxidans} produces ferric sulfate \([\text{Fe}_2(\text{SO}_4)_3]\) solution from oxidation of pyrite in leaching of uranium ores has of interest to uranium hydrometallurgists (Torma 1991b).

The direct oxidation of uranous compounds in the absence of iron was investigated by microcalorimetric (Soljanto and Tuovinen 1980) and manometric techniques (DiSpirito and Tuovinen 1981, 1982a). The first attempt to demonstrate the direct oxidation of uranium was based on microcalorimetric measurement of heat liberated during oxidation of \(\text{UO}_2\) by \(T.\) \textit{ferrooxidans}. Heat evolution was not observed with \(\text{UO}_2\) alone nor it was detected upon addition of boiled cells of \(T.\) \textit{ferrooxidans}. The reaction mixture contained 1.72 \(\mu\)mole total soluble Fe ml\(^{-1}\), and the rest being bound and incorporated into bacterial cellular material. Thus, the
results were found to have presented a combination of direct and indirect oxidation of UO$_2$ by T. ferrooxidans and Fe$^{3+}$. Similarly, Ivarson's (1980) demonstration of uranous sulfate oxidation was inconclusive because the contaminating iron was recycled as an electron carrier between U$^{4+}$ and bacteria according to the reaction proposed as follow:

$$2 \text{U}^{4+} + \text{O}_2 + 2 \text{H}_2\text{O} \rightarrow 2 \text{UO}_2^{2+} + 4 \text{H}^+$$  \[15\]

Uranium from leach liquors solubilized from ores by the action of acid or bacteria as uranyl sulfate [UO$_2$SO$_4$] can be separated by ion-exchange resins and solvent extraction techniques (Gow et al. 1973; Hartley 1972; Mindler and Termini 1956; Naden et al 1985; Ross and George 1971). The application of ion-exchange resin [1X] for the recovery of uranium from acid leach liquors was first time applied in 1952 at the West Rand Corporation Mines near Krugersdorp, South Africa. Typical commercial resins used in uranium hydrometallurgy are listed in Table 3 (Merritt 1971).

The recovery of uranium from sulfuric acid leach solutions is adsorbed by ion-exchange resin according to the following equations:

$$\text{UO}_2^{2+} + 2 \text{SO}_4^{2-} \rightarrow \text{UO}_2(\text{SO}_4)_2^{2-}$$  \[16\]

$$\text{UO}_2(\text{SO}_4)_2^{2-} + \text{SO}_4^{2-} \rightarrow \text{UO}_2(\text{SO}_4)_3^{4-}$$  \[17\]

$$4 \text{R}^+\text{HSO}_4^- + \text{UO}_2(\text{SO}_4)_3^{4-} \rightarrow (\text{R}^+)\text{UO}_2(\text{SO}_4)_3^{4-}$$  \[18\]

Where "R" stands for the ion-exchange resin. The UO$_2$(SO$_4$)$_3^{4-}$ complex is readily adsorbed by the ion-exchange resin in the form of sulfate or bisulfate, leaving most of the metal impurities behind in the spent solution. The complex [(R$^+$)UO$_2$(SO$_4$)$_3$]$^+$ is readily eluted from ion-exchange resin with a suitable solution, such as sulfate, carbonate, nitrate, chloride salt, which resulted strongly concentrated eluate (strip solution). Uranium is precipitated by any one of these bases
(NH₃, MgO, NaOH), in the form of sodium diuranate [Na₂U₂O₇] or ammonium diuranate [(NH₄)₂U₂O₇], commercially known as "yellow cake" which has a grade equivalent to 88-91% U₃O₈ after filtration and drying (Hartley 1972; Litz and Coleman 1980; Merritt 1971).

### Table 3

Typical Commercial Anion-Exchange Resins Used in Uranium Hydrometallurgy

<table>
<thead>
<tr>
<th>Resin</th>
<th>Uranium Loading Capacity [meq/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amberlite IRA-400</td>
<td>3.9</td>
</tr>
<tr>
<td>Amberlite IRA-405</td>
<td>-</td>
</tr>
<tr>
<td>Amberlite IRA-427</td>
<td>-</td>
</tr>
<tr>
<td>Amberlite XE-270</td>
<td>-</td>
</tr>
<tr>
<td>Dowex 1</td>
<td>3.5</td>
</tr>
<tr>
<td>Dowex 11</td>
<td>4.0</td>
</tr>
<tr>
<td>Dowex 21K</td>
<td>4.5</td>
</tr>
<tr>
<td>Duolite A-101D</td>
<td>4.2</td>
</tr>
<tr>
<td>Duolite A-101D-U</td>
<td>-</td>
</tr>
<tr>
<td>Ionac A-580</td>
<td>-</td>
</tr>
<tr>
<td>Ionac A-590</td>
<td>-</td>
</tr>
<tr>
<td>Nalcite</td>
<td>-</td>
</tr>
<tr>
<td>Permutit SK</td>
<td>4.3</td>
</tr>
<tr>
<td>Permutit SKB</td>
<td>-</td>
</tr>
</tbody>
</table>

Amberlite IRA-400 [Rohm and Haas], a strong base ion-exchange resin has widely used in the Canadian uranium ore-processing plants. The uranium loading capacity of Amberlite IRA-400 was observed pH dependent and the maximum uranium loading capacity was found to be 4.0-5.0 lbs U₃O₈/ft³ at pH-values of 1.8-2.0
(Gow et al. 1971). This resin showed a substantial preference for $[\text{UO}_2(\text{SO}_4)_2]^-$ ion over the $[\text{Fe}_2(\text{SO}_4)_2]^-$ ion and consequently found suitable for the treatment of acid leach liquors obtained from bacteria-based processes having relatively high $\text{Fe}^{III}/\text{U}^{VI}$ ratios (Rohm and Haas research report). The recovery of uranium from sea-water with hydrous titanium oxide (HTO) considered the most suitable adsorbent (Best and Driscoll 1980).

Similarly, uranium can be extracted from sulfuric acid leach solutions by either cationic or anionic liquid ion-exchange solvents, because both ionic forms of uranium such as $\text{UO}_2^{2+}$, $[\text{UO}_2(\text{SO}_4)_2]^-$, and $[\text{UO}_2(\text{SO}_4)_2]^-$ are present (Naden et al. 1985). These anionic liquid solvents are most commonly alkyl phosphoric acids and amines viz., secondary [tributyl phosphate (TBP)], tertiary [tricaprylamine known as Alamine-336] and quaternary amines. However, in solvent extraction system, kerosene is generally used as a diluent (Merritt 1971) and isodecanol for aqueous-organic phase separation (Zaman et al. 1980).

Recently, various microorganisms including bacteria (Brierley 1990; McLean and Beveridge 1990), fungi and yeast (Gadd 1990) and algae (Darnall 1990; Greene and Dornall 1990) have been reported for separation of heavy metals from effluents industrial wastes, estuaries, aqueous process streams, sea-water and mine drainages (Beveridge and Koval 1981; Beveridge et al. 1982; Tsezos and Volesky 1981; Volesky 1986). Several samples of inactive biomass were examined for their absorptive uptake capacity for uranium, thorium and radium (Strandberg et al. 1981; Volesky and Tsezos 1982). The retention of cations from aqueous solution by microbial biomass is called "biosorption" (Tsezos 1985; Volesky 1986). Bioaccumulation of metals by several microorganisms, especially for uranium and thorium have been reported (Kayucak and Volesky 1988; Hutchins et al. 1986; Townsley et al. 1986). Biosorbent-based granules have been developed for waste water treatment that can absorb heavy and precious metals (Brierley et al. 1986).
Acidophilic Sulfur- And Iron-Oxidizing Bacteria

The contributions of acidophilic sulfur- and iron-oxidizing bacteria in mineral biotechnology have been amply demonstrated (Ehrlich 1991; Lundgren and Silver 1980; Norris 1990; Norris and Ingledew 1992; Rossi 1990; Tuovinen et al. 1991). These bacteria are chemolithotrophic, which may belong to three taxonomic groups as mesophiles, moderate thermophiles and extreme thermophiles on the basis of their temperature response (Tuovinen et al. 1991). They are further classified into autotrophs, mixotrophs and heterotrophs on the basis of their assimilation of carbon source (Ehrlich 1991). These bacteria oxidize ferrous iron to ferric iron and reduced sulfur compounds to sulfuric acid (Brierley 1978; Kelly 1988; Ralph 1979; Tuovinen and Kelly 1972). The most common sources of these microorganisms are mining operations, waste dumps or tailings dams or in the drainage waters from mined ore bodies or bituminous coal mines (Ralph 1985). The methods for isolation, purification, identification, and characterization of these microorganisms have been fully described (Harrison 1984; Johnson and McGinness 1991; Johnson et al 1990; Khalid et al. 1993; Manning 1975; Mishra and Roy 1979; Marsh and Norris 1983; Silverman and Lundgren 1959; Tuovinen and Kelly 1973; Visca et al. 1989). Some of the known acidophilic bacteria found in ore leaching environments are cited in Table 4. Among these bacteria, *T. ferrooxidans*, *T. thiooxidans* and *L. ferrooxidans* have received much attention because of their potential role in environmental, ecological, and mineral biotechnology and therefore, are explained in some details as under:

*Thiobacillus ferrooxidans*

*T. ferrooxidans* is known to be a principal ore leaching bacterium which has been frequently studied in connection with biohydrometallurgical treatment of sulfide-bearing ores and concentrates (Ehrlich 1991; Lundgren and Dean 1979; Rossi 1990; Torma 1991a; Tuovinen et al. 1991). The occurrence of *T. ferrooxidans* was first reported in acid mine drainage from bituminous coal mines by Colmer and Hinkley (1947). It is motile and sometimes possess a polar flagellum and/or pili (DiSpiroto
### Table 4

**Major Groups of Acidophilic Bacteria found in Ore Leaching Environments**

<table>
<thead>
<tr>
<th>Bacteria (mol%)</th>
<th>DNA G+C</th>
<th>Growth on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Mesophiles:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thiobacillus thiooxidans</em></td>
<td>50-52</td>
<td>+</td>
</tr>
<tr>
<td><em>Thiobacillus ferrooxidans</em></td>
<td>58</td>
<td>+</td>
</tr>
<tr>
<td><em>Thiobacillus acidophilus</em></td>
<td>65</td>
<td>+</td>
</tr>
<tr>
<td><em>Thiobacillus intermedius</em></td>
<td>66-68</td>
<td>+</td>
</tr>
<tr>
<td><em>Thiobacillus novellus</em></td>
<td>66-68</td>
<td>+</td>
</tr>
<tr>
<td><em>Thiobacillus cupriphilus</em></td>
<td>66-69</td>
<td>+</td>
</tr>
<tr>
<td><em>Thiobacillus albertis</em></td>
<td>61.5</td>
<td>+</td>
</tr>
<tr>
<td><em>Thiobacillus prosperus</em></td>
<td>64</td>
<td>+&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Thiobacillus plumbophilus</em></td>
<td>66</td>
<td>+</td>
</tr>
<tr>
<td><em>Thiobacillus versutus</em></td>
<td>66.1</td>
<td>+</td>
</tr>
<tr>
<td><em>Leptospirillum ferrooxidans</em></td>
<td>50-55</td>
<td>-</td>
</tr>
<tr>
<td><strong>Moderate Thermophiles (Eubacteria):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sulfobacillus thermosulfidoxidans</em></td>
<td>45.5-49.3</td>
<td>+</td>
</tr>
<tr>
<td>Strain TH1</td>
<td>49.5±1.0</td>
<td>+</td>
</tr>
<tr>
<td>Strain TH3</td>
<td>68.5±1.0</td>
<td>+</td>
</tr>
<tr>
<td>Strain BC1</td>
<td>50.0±1.0</td>
<td>+</td>
</tr>
<tr>
<td>Strain ALV</td>
<td>56.6±1.0</td>
<td>+</td>
</tr>
<tr>
<td>Strain LM2</td>
<td>59.8±1.0</td>
<td>+</td>
</tr>
<tr>
<td><em>Bacillus acidocaldarius</em></td>
<td>60.0</td>
<td>+</td>
</tr>
<tr>
<td><em>Bacillus coagulans</em></td>
<td>56.0</td>
<td>+</td>
</tr>
<tr>
<td><em>Thermoplasma acidophilum</em></td>
<td>46.0</td>
<td>+</td>
</tr>
<tr>
<td><strong>Extreme Thermophiles (Archaeobacteria):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sulfolobus acidocaldarius</em></td>
<td>60-68</td>
<td>+</td>
</tr>
<tr>
<td><em>Sulfolobus solfataricus</em></td>
<td>38.0</td>
<td>-</td>
</tr>
<tr>
<td><em>Sulfolobus shibatae</em></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>Sulfovorococcus mirabilis</em></td>
<td>43.8</td>
<td>+</td>
</tr>
<tr>
<td><em>Sulfovorococcus yellowstonii</em></td>
<td>44.6</td>
<td>+</td>
</tr>
<tr>
<td><em>Acidianus brierleyi</em></td>
<td>31.0&lt;sup&gt;**&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td><em>Acidianus infernus</em></td>
<td>31.0</td>
<td>+</td>
</tr>
<tr>
<td><em>Metallosphaera sedula</em></td>
<td>45.0</td>
<td>-</td>
</tr>
<tr>
<td><em>Desulfurolobus ambiguvalens</em></td>
<td>60.0</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>#</sup> = very poor growth on sulfur and ferrous iron
et al. 1982). non-spore forming, gram-negative, rod-shaped \(0.5-1.5 \times 0.5 \, \mu\text{m}\) bacterium occurring generally as single-rods and occasionally in pairs (Tuovinen et al. 1991; Vishniac 1974). When cell growth reaches about the double of single cell size, it divides by binary fission. In the dying cell, the mechanisms which regulate the permeability of cell wall and cytoplasmic membranes do not function and the cell is plasmolyzed and broken under the influence of acidic medium (Karavaiko and Avakyan 1970).

*Thiobacillus ferrooxidans* is an obligately chemolithoautotroph, deriving its energy necessary for growth and carbon dioxide [\(\text{CO}_2\)] assimilation from the oxidation of ferrous iron (Ingledew 1982; Kelly and Harrison 1989), reduced inorganic sulfur compounds including sulfur, sulfides, and various S-oxyanions such as thiosulfate and tetrathionate (Tuovinen et al. 1991), molecular hydrogen (Drobner et al. 1990), and formic acid (Pronk et al. 1991). The microbial oxidation of sulfur and reduced sulfur compounds produce a significant amount of sulfuric acid (Tuovinen and Kelly 1972). In addition to ferrous iron oxidation, *T. ferrooxidans* has also shown the ability to oxidize Cu\(^+\) (Imai et al. 1973; Nielson and Beck 1972), UO\(_2\) (DiSpirito and Tuovinen 1982a; 1982b) and Mo\(^{5+}\) (Sugio et al. 1992) enzymatically. Recently, *T. ferrooxidans* also showed the ability to reduce metal ions (Lizama and Suzuki 1989; Pronk et al. 1991; Sugio et al. 1989; Sugio et al. 1990).

The process of ferrous iron oxidation by *T. ferrooxidans* has been reviewed by Ingledew (1982). The ferrous iron [Fe\(^{2+}\)] oxidation system of *T. ferrooxidans* is associated with lipopolysaccharide-containing outer membrane of cell wall envelope (Holt et al. 1984; Pervorzev et al. 1981). Even the cell wall envelopes of dead cells can oxidize Fe\(^{2+}\) to Fe\(^{3+}\) (Bodo and Lundgren 1974). The iron-oxidizing enzymes are Fe\(^{2+}\)-cytochrome C oxidoreductase, cytochrome a, and coenzymes Q (Cobley and Haddock 1975). Rusticyanin, a copper containing protein was reported to serve as the initial electron acceptor upon oxidation of ferrous iron (Blake and Shute 1987; Cox and Boxer 1978; Ingledew 1986). The Fe\(^{2+}/\text{Fe}^{3+}\) is an electropositive redox couple (Lazaroff 1977). An Fe(II)-oxidizing enzyme was purified from *T. ferrooxidans*
(Fukumori et al. 1988), which rapidly reduced ferricytochrome c-552 with Fe$^{3+}$ ions at pH 3.5. The oxidation of ferrous iron by *T. ferrooxidans* cells passively immobilized in polyurethane foam supporting particles was studied (Armentia and Webb 1992).

*T. ferrooxidans* being an aerobic characteristics, utilizes oxygen as the terminal electron acceptor for the oxidation of inorganic substrates, this bacterium displays the ability to oxidize elemental sulfur under anaerobic condition in the presence of ferric iron which serves as electron acceptor at pH 2.0 (Pronk et al. 1992). The anaerobic oxidation of elemental sulfur by *T. ferrooxidans* may be also coupled with manganese-dioxide [MnO$_2$] and the reduction of molybdic-ion [Mo$^{6+}$] (Sugio et al. 1988a, 1988b). These anaerobic activities of *T. ferrooxidans* do not appear to be coupled with energy-transducing system for cell growth (Sugio et al. 1988c).

*T. ferrooxidans* is a native of extreme acidic environments, which optimally grows at pH 2.3 during ferrous iron oxidation (Torma 1986), yet the internal pH of this microorganism is close to neutrality (Apel et al. 1980; Matin et al. 1982) and intracellular enzymes are known to have pH maxima between 5.0-9.0 (Lundgren et al. 1974). The ability to maintain the internal pH near neutrality could be accomplished by pumping protons [H$^+$] outward via reduction of oxygen to water and by possession of a cell-surface barrier extremely impermeable to protons (Ingledew et al. 1977). A constant internal pH value of 6.5 in *T. ferrooxidans* over external pH values ranging from 1.0 to 8.0 (Cox et al. 1979) and this pH gradient (pH 4.5) could be used by microorganism to derive oxidative-phosphorylation. The proton entry associated with the respiratory chain is responsible for providing H$^+$ to be consumed in the oxidase reaction (Cox and Brand 1984). *T. ferrooxidans* is a mesophile and the optimum temperature for most of its strains lies in the range of 30-40°C. Harrison (1982) divided 23 strains of *T. ferrooxidans* into groups on the basis of their DNA homology, one of which included all the strains able to grow at 40°C. The psychrotrophic iron-oxidizing strains of *T. ferrooxidans* have been enriched from acid mine drainages are capable of growing at temperatures as low as 4°C (Ahonen and
Carbon dioxide [CO$_2$] is assimilated by *T. ferrooxidans*, as in many other chemoautotrophs, primarily via the Calvin-Benson Pathway (Tuovinen et al. 1991). The enzyme catalyzing carbon dioxide fixation: ribulose 1,5-bisphosphate carboxylase/oxygenase was purified from *T. ferrooxidans* (Holuique et al. 1987). The fixation of CO$_2$ and its reduction to the level of carbohydrate is a very energy-expensive biochemical process (Tuovinen and Kelly 1973). For nitrogen requirement, the preferred source is ammonium ion [(NH$_4$)$_2$] and the ability to fix dinitrogen [N$_2$] by some strains of *T. ferrooxidans* has been reported (Machintosh 1978; Stevens et al. 1986). The osmoregulatory mechanism of *T. ferrooxidans* was demonstrated by Keift and Spence (1988). They found that proline enhanced the rate of iron oxidation in the presence of NaCl, KCl, Na$_2$SO$_4$, and K$_2$SO$_4$ salt-stressed cultures, whereas betaine acted as an osmoprotectant in sulfate-salt-stressed cultures and in cultures severely stressed by NaCl [0.3-0.4 M]. But the ferrous iron oxidation was inhibited by glutamic acid in all cases.

*Thiobacillus thiooxidans*

*Thiobacillus thiooxidans* (Waksman and Joffe 1921) is an extremely acidophilic chemoautotroph and plays an important role in the bioggydrometallurgical processes. It is motile, gram-negative and rod-shaped [0.5 X 1-2 μm] and cells occur as single rods and rarely in pairs (Vishniac 1974). It often occurs in association with *T. ferrooxidans* in coal mine drainage and in sulfidic ores. *T. thiooxidans* oxidizes reduced sulfur compounds like elemental sulfur, sulfide and thiosulfate to provide energy for its growth (Unz and Lundgren 1961). The pathway of sulfite to sulfate oxidation was investigated (Nakamura et al. 1992) in the strain of *T. thiooxidans* JCM 7814 and found that most of the activities of sulfite oxidation were in the membrane fractions of the cells. Acid production resulting from the oxidation of sulfur by this bacterium tends to enhance the growth of both *T. ferrooxidans* and *T. thiooxidans*. The microbial oxidation of pyrite and leaching of sulfide ores was more efficient in the presence of a mixed culture of *T. thiooxidans*, *T. ferrooxidans* and *Leptospirillum*
ferrooxidans than with alone T. ferrooxidans (Sand et al. 1992).

Thiobacillus thiooxidans has been used industrially in mineral biotechnology to extract metals from ores and in microbial desulfurization of coal in combination with T. ferrooxidans and L. ferrooxidans (Norris 1983). T. thiooxidans is morphologically and in some physiological aspects, similar to T. ferrooxidans (Vishniac 1974), however, this bacterium cannot oxidize ferrous iron, nor it degrade pyrite or chalcopyrite in pure culture, but it influences the leaching of some minerals, particularly zinc sulfide (Lizama and Suzuki 1988) and cadmium sulfide (Kelly et al. 1979), where the leaching depends on the oxidation of elemental sulfur. In mixed culture with L. ferrooxidans, extensive biodegradation of chalcopyrite was reported (Norris 1983). T. thiooxidans strains have shown different morphologies and behavioral traits in sulfur media. Cells of some strains attach themselves to sulfur particles and if motile, they propel the sulfur particle at greater speed, though the particle size exceeds by many times the mass of the cell. Some strains of T. thiooxidans do not attach themselves to sulfur particles, but they oxidize sulfur equally well (Harrison 1984). Schaeffer and Umbreit (1963) identified phosphotidylinositol as a wetting agent for sulfur in a T. thiooxidans culture.

Leptospirillum ferrooxidans

L. ferrooxidans (Markosyan 1972) exhibiting autotrophic growth on ferrous iron oxidation and has shown to be a microorganism, significant for leaching of pyrite mineral (Balashova et al. 1974; Norris and Kelly 1978). This bacterium, like T. ferrooxidans is a gram-negative, mesophile, and more acidophilic than T. ferrooxidans. It is morphologically quite distinct from T. ferrooxidans, because the cell shape varies from rods to spirals. It shows many physiological similarities to T. ferrooxidans and is sensitive to inhibition by ferric iron (Eccleston et al. 1985). The bacterium has a polar flagellum and generally more motile than T. ferrooxidans (Tuovinen et al. 1991). L. ferrooxidans is an acidophilic iron-oxidizing bacterium, incapable of oxidizing sulfur compounds (Balashova et al. 1974; Hart et al. 1991; Norris 1983), and non-ferrous

In mixed cultures with *T. ferrooxidans*, the oxidation of pyrite proceeds to sulfate and some strains of *L. ferrooxidans* have shown to gradually dominate over *T. ferrooxidans* (Merrettig et al. 1989). Coupled with this pyrite oxidation is more extensive when compared to *T. ferrooxidans* alone (Norris and Kelly 1982; Norris 1983). *L. ferrooxidans* displayed a higher affinity for ferrous iron than *T. ferrooxidans* (Norris et al. 1988) and is able to grow at low pH-values which are either marginal or prohibitive to *T. ferrooxidans* (Norris 1983). The influence of pH on the microbial make-up of mixed cultures growing on pyrite showed the dominance of *L. ferrooxidans* over *T. ferrooxidans* at pH value of 1.5; and reversed by increasing pH values from 1.5 to 2.3 (Helle and Onken 1988). The growth of slower growing strains of *L. ferrooxidans* is accelerated by the addition of Zn²⁺ (Norris 1989). The G+C content for *L. ferrooxidans* (Markosyan's isolate) was determined to be 51.7 mol%, while other *Leptospirillum*-like isolates showed a range of 50-55 mol% (Harrison and Norris 1985).

Other Acidophilic Iron- and sulfur-oxidizing Bacteria

Other iron- and sulfur-oxidizing bacteria which are clearly distinct from *T. ferrooxidans*, *T. thiooxidans* and *L. ferrooxidans* have also been isolated (Ehrlich 1991; Norris and Ingledew 1992; Tuovinen et al. 1991), but so far have received relatively little study. *T. albertis* (Bryant et al. 1983), a sulfur-oxidizing acidophile with a higher DNA GC content [61.5 mole%] and slightly less tolerance of acidity than *T. thiooxidans* [52-53 mole% GC], was found active in sulfur oxidation at 30°C. *T. kabobis* (Reynolds et al. 1981), a sulfur-oxidizing bacterium was isolated from an acidic soil. But this strain was not deposited in a type culture collection and thus was subsequently lost. *T. thioparus* (Vishniac 1974) oxidizes elemental sulfur to sulfuric
acid but does not grow below pH 4.5. *T. prosperus* (Huber and Stetter 1989), the first halotolerant mineral-oxidizing bacterium, was isolated from the geothermally heated sea-floor at the beach of Porto di Levante, Volcano, Italy. The cells can grow in a temperature range between 23-41°C with an optimum around 37-41°C at a salt concentration of up to 6% NaCl. *T. prosperus* being extremely resistant to cobalt, nickel and zinc and high salt tolerance it could be suitable for industrial leaching in saline environments where *T. ferrooxidans* is unable to grow.

*Thiobacillus plumbophilus* (Drobner et al. 1992) was isolated from an uranium mine in Germany and was named so because of its ability to grow on expense of lead compounds like galena [PbS] as sole source of energy, which may have formed as decay products from uranium. The cells grow at pH values of 4.0-6.5 and temperatures of about 9-41°C [optimum around 27°C]. The bacterium obtains its energy for growth from oxidation of galena [PbS], hydrogen sulfide [H₂S], and molecular hydrogen [H₂]. Anglesite [PbSO₄] is formed from oxidation of galena. No growth was observed on elemental sulfur, thiosulfate, tetrathionate, ferrous sulfite, synthetic and natural metal sulfides and uraninite. No oxidation of Fe²⁺ to Fe³⁺ was detected neither when FeSO₄ was the sole energy substrate nor in combination with PbS. Growth was neither stimulated nor inhibited by the addition of organic substrates.

*Thiobacillus acidophilus* (Guay and Silver 1975), a sulfur-oxidizing bacterium shares a capacity for heterotrophic growth with species of *Acidiphilium* (Harrison 1984) which derives its energy for growth from the oxidation of elemental sulfur and tetrathionate (DiSpirito and Tuovinen 1981; Norris et al. 1986), thiosulfate and trithionate (Mason and Kelly 1988). The heterotrophic growth of *T. acidophilus* was supported by a number of monosaccharides, TCA-cycle intermediates and some amino acids (Guay and Silver 1975; Pronk et al. 1990). *T. acidophilus* can not oxidize ferrous iron and its autotrophic growth on elemental sulfur was not inhibited in the presence of FeSO₄, but it prevents growth on glucose (Arkesteyn and DeBont 1980). The optimum temperature for growth is between 25-30°C and pH 3.0 with a range
of 1.5-5.6 (Guay and Silver 1975), but its internal pH was found to be 5.6 (Matin et al. 1982). The intermediates formed during aerobic and anaerobic oxidation of reduced sulfur compounds by T. acidophilus was reported (Meuleenberg et al. 1992). T. cuprinus (Huber and Stetter 1990), a facultative chemolithoautotroph, which derives its energy for growth from the oxidation of elemental sulfur, sphalerite, chalcopyrite, galena, and H₂S. No growth was observed on ferrous sulfate, bornite, chalcocite, covellite, pyrite, cinabar, thiosulfate or tetrathionate. The bacterium exhibited organotrophic growth on yeast extract, peptone, cassamino acids, meat extract and pyruvate [0.2%]. T. perometabolis (Myers and Millar 1975), a mixotrophic bacterium which was isolated from an acid mine drainage.

Acidiphilium cryptum (Harrison 1982) was isolated from coal refuse and also as contaminant in cultures of T. ferrooxidans. This bacterium was reported to be the most common heterotrophic contaminant in T. ferrooxidans cultures (Harrison 1984). The G+C contents of DNA were 68-70 mol%. Other three species of Acidiphilium, named as A. angustum, A. facilis and A. rubrum (Wichlacz et al. 1986) were isolated from acid coal mine drainage showed some physiological similarities with A. cryptum but their G+C contents of DNA were relatively lower [63-67 mol%]. Flavobacterium acidurans (Miller 1973) was an aerobic, non-motile, non-spore forming and gram-negative rod [1-3 X 0.3-0.6 μm]. The optimum growth temperature was between 20-30°C. The G+C contents of DNA were 66.3 mol%. Walsh and Mitchell (1972) isolated an acid-tolerant filamentous bacterium of the genus Metallogenium, a microorganism capable of catalyzing ferrous iron oxidation in the pH range of 3.5-5.0. Harrison (1982) isolated an iron-oxidizing bacterium from an old coal strip mine and labelled it T. ferrooxidans "m-1". This bacterium was phenotypically similar to T. ferrooxidans, but lacking the ability to oxidize sulfur. Lane et al. (1985) demonstrated that "m-1" strain was more closely related to Chromatium vinosum, a photosynthetic sulfur-oxidizing bacterium. The G+C contents of DNA were 65 mol%. Stibio bacter senarmontii (Lyalikova 1974), an antimony-oxidizing bacterium also reported.
The ability of bacteria to grow beyond the mesophilic temperature range is well known (Karavaiko et al. 1988; Norris 1990; Norris and Ingledew 1992; Rawlings et al. 1984) and the discovery of thermophilic and psychrophilic chemolithotrophic bacteria was not a major surprise (LeRoux et al. 1977). Thermophilic environments have received much attention due to their greater biotechnological potential (increased leaching rates), and it has recognized that thermal environments develop in oxidizing ore-bodies in dump and heap leaching operations. Thermophilic forms appear to be exclusively prokaryotic and can be divided on the basis of temperature range into: I) moderate thermophiles, with temperature optima around 50°C, and (II) extreme thermophiles, with temperature optima around 70-80°C. Both moderate thermophiles (Brierley et al. 1978) and extreme thermophiles (Norris 1990) have been reported in literature.

Other Microorganisms

A variety of other microorganisms such as fungi, yeasts, algae and protozoa are present in symbiotic growth in the naturally occurring leach solutions (Ehrlich 1991; Groudev 1987). Fungi also solubilize metals by the extraction of organic acids which are additionally useful in increasing the solubility and reducing the toxicity of heavy metal ions at neutral pH-values. The extraction of Cu, Zn, Al, Ni, Fe, K, Li, Si, Ti, Mn, Ag, Hg, U, and Cr from ores have been leached out with fungi (Burgstaller and Schinner 1993). The involvement of Pseudomonas fluorescense, P. putida, Achromobacter, Bacillus licheniformis, B. cereus, B. luteus, B. polymyxa, B. megaterium have been reported in leaching processes (Ehrlich 1991). However, the extent of involvement of these microorganisms in the extraction of metals from minerals is not known. Ralph (1979) presented a reasonable explanation of various microbial populations associated with sulfide oxidation and has proposed a likely microbial succession in sulfdic environments which is influenced by physical and chemical characteristics of mineral component.
Genetic Manipulation of Ore Leaching Bacteria

The genetic manipulation of plasmid biology of *T. ferrooxidans* has recognized to be a potential source of strain improvement for mineral biotechnology (Kulpa et al. 1986; Harrison et al. 1986; Shiratori et al. 1989; Visentin et al. 1986). A number of investigators studied antibiotic resistance of leaching bacteria and their plasmid composition (Holmes et al. 1984; Holt et al. 1984; Polidoro et al. 1993). This route holds at present little promise for heap, dump, and in-situ leaching applications because microbial populations in these processes are typically indigenous and controlled by virtue of their environmental requirements and natural selection. The beneficial use of genetically modified *Thiobacillus* bacteria in biohydrometallurgy has not been well documented. The plasmid DNA was isolated from various strains of *T. ferrooxidans* (Mao et al. 1980; Harrison 1986). The microorganisms involved in the solubilization of metals in acid media develop a natural resistance to high metal ion concentrations by mechanisms not yet completely understood (Chakrabarty 1978). This natural resistance can be achieved by adaptation or by selection (Groudev et al. 1981). Natural selection favours those cells that can best survive and reproduce at low pH and high metal ions concentrations. *T. ferrooxidans* is known to develop high resistance against concentration of metals during leaching process (Torma 1977). The main problems associated with plasmid characterization of *T. ferrooxidans* are the difficulties in growing and maintaining identical bacterium in the laboratories. Studies are in progress in many laboratories and it would be premature at the present time, to draw far-reaching conclusions. However, genetic engineering certainly presents interesting possibilities for biohydrometallurgical applications.

Microbial Ore Leaching Techniques

The profitability of biohydrometallurgical processes depends on chemical and mineralogical characteristics of ore to be leached, so that processes tested on individual types of ores can not be transferred to other ore types. Hence, before preparations are made for microbial leaching of ores on commercial scale, the leaching parameters of the ore concerned must be thoroughly studied. Several
Leaching techniques have been developed to recover metal values from low-grade ores and industrial waste residues on laboratory, pilot and commercial scales. The best results for metal extraction can be obtained with techniques having the highest rate of oxygen [O₂] and carbon dioxide [CO₂] mass transfer into the leach solutions. Numerous laboratory investigations carried out in shake flasks, columns, and vats have resulted in a wealth of information on process performance.

Early laboratory studies used stationary vessels of crushed ore and liquid media containing bacteria (Razzell and Trussell 1963b). This technique is one of the simplest methods of microbial culture, because of the modest cost of equipment and experimental simplicity. The experimental procedure was quite simple: culture medium, substrate and inoculum were introduced into flasks, which were then plugged with adsorbent cotton, and placed in a cabinet or on a bench for duration of the test. Because of the influence of geometric and physical factors, this technique was used solely for investigations of the physiology of microorganisms, amenability of minerals to bioleaching and influence of physiochemical parameters in the process. This technique can not provide information on process kinetics, since they are disturbed or limited by lack of homogeneity in suspension and relative slowness of gas diffusion.

Shake flask leaching technique provides rapid information for a large number of parameters affecting bacterial activity. This technique is used to assess the feasibility of leaching processes and to develop preliminary economical evaluation for microbial leaching of various sulfide bearing concentrates (Torma et al. 1972). Gyratory shaking using Erlenmeyer flasks produces rapid and good aeration and an accelerated rate of leaching (Duncan et al. 1964). For example, using percolators for bacterial leaching of chalcopyrite, 2.70% copper was extracted from one sample and 6.10% from another in 70 days, while using shake flask leaching technique, 72.0% of copper was extracted in 12 days from the same type of chalcopyrite ore sample. However, besides gyratory shaking, there are many other types of mixers available for use in laboratory experiments, such as air-spargers, magnetic stirrers and
reciprocating shakers. The effect of these leaching techniques on microbiological copper extraction was compared and it was observed that magnetic stirring and reciprocating shakers gave results comparable with those for gyratory shaking. Vessels agitated either by stirring provided superior aeration (Duncan et al. 1966).

Large scale experiments were conducted in tanks and fermentors under controlled leaching parameters and the data derived were used for up-scaling of processes (Guay et al. 1977; Konishi and Asai 1993). The continuous-flow stirred tank reactor (CSTR) was used to simulate the oxidation of pyrite by *T. ferrooxidans* (Konishi and Asai 1993). Respirometer vessels are also used, which provide sufficient aeration, but are limited by small sample size (Beck 1960). Microbiological leaching studies of sulfide ores were carried out in air-lift percolators containing 200-600 grams of ore with a total volume of nutrient leach solution of 100-600 mL (Bosecker and Wirth 1980; Perez et al. 1983; Rossi et al. 1983).

Laboratory column leaching technique, either with or without circulation of leaching medium, simulates the commercial procedure for heap and dump leaching processes (Bhatti et al. 1991a; Brinjalwalla and Wadsworth 1973; Choi and Hopkin 1988; Derry et al. 1977; Manchee 1979). Large scale percolators (17 meters height and 0.86 meter dia) made of thermo-resistant glass were used for microbial leaching of low-grade sulfidic ores to recover Cu and Zn (Brierley and Luinsta 1993). An ore sample is placed in a column and the liquid is circulated in it as in air-lift percolators. But the ore-filled columns with or without solution percolation not provided sufficient oxygen transfer (Malouf and Prater 1961). In microbial column leaching technique, 85% $U_3O_8$ was recovered from uranium ore of Elliot Lake mines in 140 days (Guay et al 1976; Manchee 1977). Similarly, 90% $U_3O_8$ was recovered from sandstone-type uranium ore from Baghalchur mines in 150 days (Bhatti et al. 1993a). The ore was amended with sulfur slag as external energy substrate for *T. ferrooxidans* and *T. thiooxidans*. A pilot-scale unit was developed for continuous operation with five columns arranged in series for leaching of uranium ore of 0.12% $U_3O_8$ content (Derry et al. 1977). In this process, one column containing ion-exchange resin was
installed in between for continuous uranium recovery from uranium leach liquor and two columns for ferric iron generation by iron-oxidizing bacteria (*T. ferrooxidans* and *L. ferrooxidans*). In this way, a recovery of 95% U₃O₈ was obtained in 10-days at a cost projected to be cheaper than that of conventional acid leaching slurry. A laboratory scale 30 L capacity of air-lift bioreactor was used in bacterial leaching of sulfidic mine tailings to recover Zn, Cu, Fe, Co and Ni (Barrette and Couillard 1993).

Commercial scale biohydrometallurgical methods are classified as heap, dump, in-situ and vat leaching processes (Torma and Bosecker 1982). These leaching methods are most common modes of solution mining and are generally rely on indigenous microbial populations belonging to *Thiobacillus* and *Sulfobolus* species, which are present in sulfidic environments. The descriptions of dump and heap leaching operations have been discussed in details (Anderson and Allman 1968; Bhappu 1982; Bruynesteyn 1985, 1983; Lundgren and Silver 1980; Marchant 1986; Piercy 1982). Heap leaching is considered a beneficiation method for oxide ores, although it has also been used for oxide-sulfide copper ores and gold recovery (Johnson and Bhappu 1970; Hiskey 1984). Industrial heap and dump leaching processes are being carried out generally close to mining sites in order to minimize transportation and operating costs (Malouf 1971). The low-grade ore is brought by truck or by conveyor belt to an impermeable site and deposited to form truncated cones (Murr 1980; Murr and Brierley 1978). The oxide-sulfide ores are leached under controlled conditions in relatively small heaps of $\leq 10^4$ tonnes on impermeable pads or prepared surfaces.

The commercial application of heap leaching for uranium recovery from low-grade ores or mine wastes [containing 0.05-0.10% U₃O₈] was applied by Western Nuclear Corporation in the Gas Hills of Wyoming, USA (Mashbir 1964). Heaps of 25,000 tons of ore were built and 250,000 gallons of leaching solution at average flow rate of 0.40 gal/hr/ft² was added per paddie and a recovery of 90% U₃O₈ was obtained. The heaps are built up on impermeable pads to prevent loss of solution into the underlying soil. Aeration systems are also installed to increase the flow of
air in the ore piles. The volume of material in heap for copper leaching varies from 100,000 to 500,000 tons. Metals from these ores are generally soluble in sulfuric acid and the leach cycle is completed in months (Wadsworth 1979; Wadsworth 1975). Heap leaching process is more rapid and more complete than dump leaching process. The surface/volume ratio may be greatly increased for better aeration and distribution of leaching solutions by arranging the ore in relatively thin layers or in finger heaps (Robinson 1972). The engineering aspects of heap regarding the solution chemistry, heap aeration, development of the shrinking core model and the discriminant between diffusion and surface chemical reaction control during operation was discussed in detail (Harrington et al. 1993).

Dump leaching process is generally practised on sub-marginal ore material assembled into large dumps containing \(4 \times 10^9\) tons of material highly heterogenous in composition (Brierley 1982). Most of the dumps are formed using the natural formation of the terrain (Biswa and Davenport 1980) and often steep sided valleys are filled or the waste ore is dumped on the hillside providing easier drainage (Torma 1984). Larger dumps may be as high as 200 meters, about 80 meters wide at the top and 250 meters at the bottom and contain 50,000 to 300,000 tonnes of ore. The leaching solution is sprinkled on the top of heap, which percolates through ore body and is collected at the bottom of heap.

In-situ leaching, the ore material is suitably fractured to ensure permeability irrigated in place with appropriate leaching solutions (Brown 1980). Vat leaching process requires installation of special tanks designed for this purpose. The ore material to be leached is placed in a large tank equipped with a false bottom. The leaching solution is added at the top of tank and allowed to percolate through the ore material. Series of tanks are used in counter current system, in which the new solids are brought into contact with the partially reacted leach solution and the fresh leach solution is added to the partially leached one. Tanks having a ore capacity of 12,000 tons or more are in common practice (Torma and Bosecker 1982). Industrial scale vat leaching is practised for the extraction of uranium, copper and gold from
low-grade ores or waste materials (Rossi 1990).

Factors Influencing Bacterial Leaching Processes

A number of factors influence the rates of sulfide mineral biodegradation by the metabolic activity of *T. ferrooxidans* and of these the most common include: the morphology and electronic structure of mineral surface (Tributsch and Bennett 1981a, 1981b), particle size and surface area, pH and redox potential, and temperature (Garrels and Chrish 1965; Pourbaix 1966), the presence or absence of catalysts, partial pressure of oxygen and relative humidity (Lundgren and Malouf 1983). These factors have been discussed in detail (Torma 1977; Lowson 1982). The climatic conditions like temperature and rainfall also play an important role in mineral biodegradation. Highly siliceous or carbonaceous gangue minerals consume acid, thereby changing conditions beyond the pH range suitable for many leaching microorganisms. The optimum leach and growth conditions of microorganisms can be maintained constant in laboratories (Torma and Banhegyi 1984; Torma 1987). A maximum rate of metal extraction can be achieved when the environment is maintained at optimum leaching conditions. The attachment of bacteria at the mineral surface play an important role in leaching process (Berry and Murr 1975). Scanning electron micrographs have revealed that numerous bacteria attach themselves at the surface of sulfide minerals in solutions and the results indicated that bacteria dissolve a sulfide surface of crystal by means of cell contact (Berry and Murr 1978).

Effect of Nutrients

Mesophilic chemoautotrophic bacteria (*T. ferrooxidans*, *T. thiooxidans* and *L. ferrooxidans*) involved in mineral biotechnology require only inorganic nutrients for growth with Fe$^{2+}$ and sulfur compounds (Ewart and Hughes 1991; McCready et al. 1986; Rossi 1990). Some of the major and minor nutrients requirements i.e., P, K, Mg and trace metals may be met with mineral constituents dissolved from ores during biological leaching processes. In contrast, the dissolution of minerals as
nutrient source represents a minor contribution to satisfy the cellular nitrogen requirement. Ammonium (\(\text{NH}_4^+\)) ion appears to be the preferred source of nitrogen for acidophilic chemoautotrophic \textit{Thiobacillus} bacteria. The ammonium amendment (6.0 mM) enhanced \(\text{Fe}^{2+}\) oxidation; whereas the addition of nitrate (6.0 and 12.0 mM) showed a negative effect during biological leaching of a black-schist ore (Niemela et al. 1994).

A number of growth media for nutrients requirements of \textit{T. ferrooxidans} have been described in literature (Silverman and Lundgren 1959; Tuovinen and Kelly 1973). This bacterium synthesizes its cell materials from inorganic sources, which are carbon dioxide \([\text{CO}_2]\) as carbon source, ammonium sulfate \([\text{(NH}_4\text{)}_2\text{SO}_4]\), and dipotassium hydrogen phosphate \([\text{K}_2\text{HPO}_4]\) as nitrogen and phosphate sources for cell growth and potassium chloride \([\text{KCl}]\), magnesium sulfate \([\text{MgSO}_4\cdot7\text{H}_2\text{O}]\) and calcium nitrate \([\text{Ca(NO}_3\text{)}_2]\) as growth factors (Torma 1991a).

Studies on the effect of nutrient concentrations on bacterial leaching of a zinc sulfide concentrate indicated that ammonium concentration controlled the yield, while phosphate concentration influenced the rate of zinc extraction (Torma et al. 1970). Phosphate- or sulfate-limited growth conditions cause serious metabolic imbalance and restricted growth because both nutrients are required by growing cells (Tuovinen 1990). Phosphate produce insoluble complexes with \text{Fe(III)} (Hoffmann et al. 1985), that may effectively scavenge available phosphate from leach solutions. Besides being an essential nutrient, the sulfate ion is also required in ferrous-iron oxidation by \textit{T. ferrooxidans} (Ingledew 1982). These minor nutrients were required by the organisms in such small quantities, that any requirement beyond the ammonium and phosphate salts or in metal sulfide concentrate could not be demonstrated. Ammonium ion \([\text{(NH}_4\text{)}^+]\) is often obtained from the ammonia based explosives used for fracturing ore body and phosphorus is dissolved from the host rocks (Torma 1987). \textit{T. ferrooxidans} was reported to fix elemental nitrogen \([\text{N}_2]\) and its nitrogenase activity was determined by acetylene reduction technique in the presence of ferrous iron as electron donor (Mackintosh 1978). The microbial
mutualism in ore leaching was studied by using copper sulfide ore and Beijerinckia lactica as a nitrogen fixer (diazotroph) and *T. ferroxidans* (Tsuchiya et al. 1974; Trivedy and Tsuchiya 1975). *B. lactica* released NH$_4^+$ into leach solution, which was used by *T. ferroxidans* as nutrient source of nitrogen, while *T. ferroxidans* fixed carbon dioxide and secreted surfactant-like organic substances which served as carbon source for *B. lactica*.

The effect of carbon dioxide on bacterial activity was observed that the oxidation of pyrite by *T. ferroxidans* gradually decreased upon removing carbon dioxide from air, which was used for aeration (Bryner and Beck 1954). An increase in the carbon dioxide content of air, stimulated the growth of *T. ferroxidans* using ferrous iron as substrate. The optimum CO$_2$ concentration was found to be 0.2% in terms of highest rate of zinc extraction from sphalerite (Torma et al. 1972). The oxidation of ferrous iron by *T. ferroxidans* requires the presence of sulfate ion probably as a complexing agent (Dugan and Lundgren 1965). The mass transfer of dissolved oxygen was the rate limiting step in heap leaching process and even in-situ leaching process had a serious problem (Harris 1969).

**Effect of pH and Redox Potential [Eh]**

The biological oxidation of ferrous ion and metal sulfides involves the movements of hydrogen ions [H$^+$] and electrons [e$^-$. Therefore, the pH has a definite effect on the metabolic activity of *T. ferroxidans* in leaching of metal sulfides in the pH range of 1.0 to 5.0 (Silverman and Ehrlich 1964). *T. ferroxidans* is active in the pH range of 1.5 to 3.5 and its growth is generally inhibited below pH 1.0 and above pH 5.0 (Torma 1987). These values are derived in terms of shortest lag time, fastest rate of substrate oxidation and highest yield of metal extraction. By keeping the pH values of medium at 1.9-2.3 and Eh-value less than 500 mV, the massive precipitation of iron hydroxide and hydroxy-sulfates can be avoided (Bigham et al. 1990; Grishin et al. 1988). The chemical stability of iron and its compounds is very sensitive to conditions of pH and Eh and will determine the types of microbial
populations that develop to a large degree (Bruynesteyn and Vizsolyi 1982). Iron oxidation is usually rapid and sensitive to both pH and oxygen concentration at pH-values above 3.50 (Ackman and Kleinmann 1984). After the oxidation of Fe$^{2+}$ to Fe$^{3+}$ state, it tends to hydrolyze to form ferric hydroxide that further interact with various sulfates to form ferric hydroxy-sulfates and oxyhydroxide complexes (Bhatti et al. 1993b; Brady et al. 1986; Nordstorm 1982). The buffering capacity of these compounds also influences the pH of the environment (Lundgren and Dean 1979).

Effect of Temperature

Temperature is an important environmental factor that influences bacterial activities in biological leaching operations (Brierley and Brierley 1986; Kelly and Tuovinen 1988; McCready 1988). The chemistry and biology of leaching process is influenced by temperature of leaching solutions. In harsh winter climatic conditions, such as those experienced in Canada, where the temperature of leaching solution was as low as 1.0 or 2.0°C and summer temperature as high as 35°C could be encountered (Sheffer and Evans 1968). Due to exothermic nature of pyrite oxidation, temperature as high as 80°C have been recorded (Beck 1967). At elevated temperature, the leaching rate is increased due to the metabolic activity of mesophilic acidophiles. Moderate thermophilic temperatures of 56°C in the waste rock dump from a uranium operation in the Northern Australia (Harries and Ritchie 1981) and 59°C in copper bearing waste were reported (Murr and Berry 1979). Similar temperatures were recorded in low-grade copper ore in the New Mexico (Murr and Brierley 1978). Even higher temperature in excess of 80°C was observed in low-grade copper dumps in Balgharia (Groudev et al. 1978) and in the United States (Beck 1967). Higher temperature greater than 40°C would certainly limit the distribution of mesophilic thiobacilli. In evaluating microbial populations in waste rock dumps in the Northern Australia (Goodman et al. 1981), acidophilic iron oxidizers were found to be the most abundant microorganisms associated with oxidation zones at temperatures of 45°C or higher. Iron oxidation occurs at temperature as low as 5 to 6°C (Ferroni et al. 1986; Kovalenko et al. 1982). An increase in the temperature from 30-35°C
slightly enhanced the Fe$^{2+}$ oxidation during biological leaching of a black-schist ore (Niemela et al. 1994), but the effect was statistically not significant. Temperatures in underground mines vary from 5-15°C, whereas surface temperatures in heap leaching processes vary from below freezing to up to 50°C or even higher (Harries and Ritchie 1981; Kelly and Tuovinen 1988; Murr and Brierley 1978).

**Effect of Particle size and Surface Area**

Several investigators have reported the importance of surface chemistry of metal sulfides in the microbiological leaching processes (Beck 1967; Silverman and Ehrlich 1964; Trudinger 1967; Tuovinen and Kelly 1972). The rate of bacterial leaching depends upon the surface of substrate to be leached. A decrease in grain size means an increase in particle-specific surface and in the total surface, so that higher yields of metal can be obtained with no change in total mass of particles (Razzell and Trussell 1963b; Duncan et al. 1966). A grain size of about 42 μm is required as the optimum (Torma 1977). Temple and Delchamps (1953) found that ball milled samples of pyrite and marcasite were more susceptible to action by *T. ferrooxidans* than the coarse ones. These studies emphasized the importance of solid surface area upon the bacterial performance. Razzell and Trussell (1963b) reported that more copper was rapidly extracted from fine particles of chalcopyrite than coarser ones and the best results obtained with 42 μm size fraction. This finding was also confirmed by Duncan et al. (1964). By using synthetic copper sulfide almost double the rate of bacterial oxidation was observed when particle size reduced from 104 to 64 μm (Ehrlich and Fox 1967). For large scale in-situ operations, the exposure of the sulfide minerals can be accomplished by blasting of the ore body. The studies of nuclear underground explosion indicated that fragmentation of large volumes of massive ore bodies could be realized cheaply and safely by this mean (Hardwick 1979; Hardwick 1967).
Toxicity of Heavy Metals

Many heavy metals in low concentrations are necessary constituents of the nutrient medium, but in higher concentrations they have a toxic effect. *T. ferrooxidans* requires Cu for rusticyanin; Fe for cytochromes; Mg for energy-transducing enzyme activities (Tuovinen 1990). The metal toxicity for acidophilic thiobacilli has been evaluated in numerous laboratory studies using synthetic mineral-salt solutions. Silver and molybdenum are particularly toxic to *T. ferrooxidans* (Hofmann and Hendrix 1976). High tolerance limits of *T. ferrooxidans* by soluble copper [55.0 g/L], nickel [50.0 g/L] and zinc [120 g/L] (Sakaguchi et al. 1976; Torma et al. 1972) have been reported in literatures. Different strains of the same species may show completely different sensitivities to heavy metals. It is often possible to adapt individual strains to higher concentrations of metals or to specific substrates by gradually increasing the concentration of metals or substrates (Martin et al. 1983). The toxicity of uranium for *T. ferrooxidans* as a limitation of the process was investigated by several authors. Duncan and Bruynesteyn (1971) observed strains of *T. ferrooxidans* active in uranium mines even at concentrations as high as 10.0 g/L uranium. Tuovinen and Kelly (1974c) reported that *T. ferrooxidans* can be resistant to at least 10 mM UO$_2^{2+}$.
MATERIALS AND METHODS

Materials

Sandstone Uranium Ore

A representative [25 tons] sample of low-grade sandstone uranium ore was collected from Baghalchur mine site, Dera Ghazi Khan. The ore was well mixed by repeated coning and quartering and finally, a homogenized sample [1.0 kg] was prepared by using laboratory sample divider [Retsch 520-355, Germany] for chemical and mineralogical analyses.

Mill Tailings Residue

A representative mill tailings residue sample [500 kgs] was collected from Tailings Pond, Ore-Processing Plant, D.G. Khan. It was well mixed and a homogenized sample [1.0 kg] was prepared for uranium analysis.

Elemental Sulfur and Sulfur Slag

Elemental sulfur and sulfur slag (a waste of sulfuric acid plant) samples were obtained from BC-1 Project, D.G. Khan and were used as external energy substrates for growth of acidophilic iron- and sulfur-oxidizing Thiobacillus bacteria in bioleaching studies of sandstone uranium ore and tailings residue. Elemental sulfur and sulfur slag were crushed to a particle size of 0.2-3.0 mm by using laboratory disc mill [Fritsch Pulverisett 13-103, Germany] and ground to pass through -100 mesh sieve opening [<149 \(\mu\)m] by using laboratory vibrating cup mill [Fritsch Pulverisett 09-003, Germany] for oxidative and bioleaching studies of uranium ore and tailings residue.

Pyrite Mineral and Sufidic Ore

Research grade pyrite sample was obtained from Ward’s Natural Science Establishment, Inc., Rochester, NY and was ground to a -200 mesh size [<74 \(\mu\)m]
particle size] fraction using laboratory vibrating cup mill for carrying out microbial leaching experiments. A sulfidic ore containing 8.86% pyrite (FeS₂) was also obtained from the Geological Survey of Pakistan and was processed as above. The sulfidic ore was also used as external energy substrate for T. ferrooxidans during leaching studies of sandstone ore.

Microorganisms

Strains of Thiobacillus ferrooxidans and T. thiooxidans were isolated from uranium tailings liquid samples by using standard enrichment techniques. Both these strains were employed for bacterial oxidation of elemental sulfur, sulfur slag, pyrite and uranium bioleaching experiments. Standard strains of T. ferrooxidans [ATCC 13661] and T. thiooxidans [ATCC 8085] obtained from the American Type Culture Collection, USA, and were also used for some comparative studies with indigenous strains of T. ferrooxidans and T. thiooxidans.

All the chemicals used for these studies were of analytical grade or otherwise stated.

Methods

Sieve Analysis

Sieve analysis of homogenized ore was carried out by using laboratory sieving machine [Retsch 365-035, Germany] into different ASTM mesh size fractions. Each ore fraction was weighed to determine the particle size and uranium content distribution at different mesh size fractions.

Bromoform Separation

Heavy and light minerals were separated from sandstone ore sample by using bromoform (tribromomethane) heavy liquid separation technique (Muller 1977). The separation of minerals was carried out by taking known weight [100 g] of ore in a
beaker of suitable size containing 600 mL of bromoform [density 2.89 g/mL at 20°C]. The mineral grains were stirred into the liquid to ensure complete wetting and those with densities greater than that of liquid settled down to form a "sink" product [heavy minerals]. After separation, the "float" product [light minerals] remaining on the liquid surface were skimmed off with a fine wire mesh scoop. Each product was separately filtered and washed with acetone to remove the final traces of bromoform.

Isodynamic Magnetic Separation

Heavy mineral fraction of ore sample obtained by bromoform separation technique was separated into mono-mineral fractions by using Frantz Isodynamic Magnetic Separator [L-1, S.G. Frantz, NY] at different electromagnetic field and slope.

Stereomicroscopic Analysis

Loose grains of various mineral fractions obtained from bromoform and isodynamic separation techniques were examined under a stereomicroscope [Leitz-Wild M32, Heebrugg, Switzerland] to identify the minerals and their percentage distribution in the ore sample. The ore grains were mounted on the Ilford Nuclear Emulsion Plate of size 3 X 4 inches and thickness of 50 μm. The identification of both opaque and transparent minerals depending upon the physical properties of minerals: crystal structure, shape, color, luster, streak, cleavage, fracture, Moho’s hardness, fluorescence and microchemical tests was made. The identification of transparent minerals was confirmed by refractive index.

The black coated grains of radioactive mineral present in sandstone uranium ore was carefully separated and then hand picked under a binocular microscope for x-ray diffraction (XRD) work.
X-Ray Diffraction Analysis

The radioactive mineral grains as obtained above were ground with an agate mortar. X-ray diffraction (XRD) analysis of topfill powder mount was conducted by using CuKα radiation and a vertical, wide-range goniometer [Philips PW 131690] equipped with a diffracted-beam monochromator and a Θ compensating slit. The specimen was scanned from 10-70°2Θ in increments of 0.05° with 4-second step time.

Chemical Analysis of Uranium Ore
Sample Preparation

The homogenized uranium ore sample obtained by coning and quartering technique was ground to fine powder of particle size [≤200 mesh] by using laboratory vibrating cup mill. The method used for sample preparation of ore for chemical analysis was as follows:

Triplicate samples [0.50 g] of ore were taken in a platinum dish, moistened with 2-3 drop of deionized water and added 5 mL of 15 M HNO₃ and 5 mL of 40% HF. The contents of the dish were heated on hot plate [Sybron Type 220 Hot Plate, Thermolyne, USA] and were evaporated to dryness and the same procedure was repeated with same volumes of 15 M HNO₃ and 40% HF to complete dryness. The residue of the dish was attacked with 2 mL of 72% HClO₄ and heated on a hot plate until the volume of HClO₄ was less than 1 mL. The solution was allowed to cool and added in 20 mL of 5 M HNO₃ to dissolve the residue, after that it was filtered through Whatmann filter paper into a 100 mL volumetric flask. The filter paper was burnt to ash in a platinum dish and sodium carbonate [0.30 g] was added to fuse for 5 minutes. The fused contents of the dish were melted and dissolved in 20 mL of 5 M HNO₃. The solution was added to the same 100 mL volumetric flask and diluted to mark with addition of deionized water. Similarly, the solution of tailings residue sample was prepared for uranium content.
Uranium Determination

Uranium contents of sandstone ore and leached residues samples were determined by a modified spectrophotometric method (Johnson and Florence 1971). In this method, an aliquot of ore solution [containing 1-150 µg U] as prepared above was taken in 50 mL screw-cap test tube and added aluminum nitrate solution [15 mL, 1g/mL] as masking agent. In this test tube, 10 mL of MIBK [Methylisobutylketone] was added and the contents of test tube were stirred vigorously for 5 minutes. Organic phase [2 mL] was taken into a dry 25 mL volumetric flask and was added 1.0 mL of complexing solution [25g of 1,2-diaminocyclohexane tetraacetic acid (CyDTA), 5g of NaF, and 65g of sulphosalicylic acid in 800 mL of water and neutralized to pH 7.85 with 40% NaOH, and diluted to 1 litre], 4 mL of 0.05% Bromo-PADAP solution [2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol] (0.35g of reagent in 100 mL of reagent-grade ethanol), and 1 mL of triethanolamine buffer pH 8.35 [1.49g of triethanolamine in 800 mL of water, neutralized to pH 8.35 with perchloric acid, allowed to stand overnight] in that order, mixing thoroughly after each addition. The flask was stoppered and allowed to stand for 40 minutes and then added 16 mL of ethanol and diluted to volume with addition of deionized water. The intensity of UO$_2$$^{2+}$-Bromo-PADAP red color complex was measured by a spectrophotometer [Shimadzu UV-120-02, Japan] at 578 nm against a reagent blank carried through extraction procedure.

Uranium content of bacterial leach liquors was determined by a spectrophotometric method (Bhatti et al. 1991b) using Arsenazo-III as chromogenic reagent.

Ferrous and Total Iron Determination

Ferrous iron contents of sandstone ore and sulfur slag samples were determined by a titrimetric method (Jeffery 1975), while ferrous iron contents of bacterial leach liquors and tailings liquid samples were determined by a method as described by Norris and Kelly (1978). In this method, an aliquot [2 mL] was taken
described by Norris and Kelly (1978). In this method, an aliquot [2 mL] was taken in 50 mL titration flask and followed by the addition of 2-3 drops of 10M H₂SO₄; 0.1 mL of 1,10-phenanthroline-ferrous sulfate as indicator. Added 10 mL deionized water and titrated with 1 mM ceric sulfate solution to the red-pale blue transition end point. One other modified spectrophotometric method (Christian 1986) was also used to estimate ferrous iron content in process solutions. In this method, an appropriate amount of aliquot was taken in 50 mL volumetric flask and followed by the addition of 20-25 mL deionized water. An aqueous solution of 1,10-phenanthroline monohydrate [1 mL, 1% w/v] was added into the flask. The contents of flask were buffered by the addition of sodium acetate solution [8 mL, 10% w/v] and diluted to the mark with deionized water. The absorbance of red complex of Fe(C₆H₈N₂)₃³⁺ was measured against a reagent blank at 510 nm.

Total iron contents of sandstone ore, bacterial leach liquors, tailings liquid and other process solution samples was analyzed by atomic absorption spectrophotometry [Varian SpectrAA-20ABQ, Australia].

Elemental Analysis of Uranium Ore

Chemical analysis of elements, such as Al, Ba, Co, Cu, Mn, Mo, Ni, P, Pb, S, Th, Ti, V, Y, Yb, Zn, and Zr present in sandstone ore solution or suitable dilutions thereof, were determined by atomic absorption spectrophotometric method (Agemian and Chau 1975).

Induced Coupled Plasma Spectroscopic Analysis

Induced Coupled Plasma (ICP) emission spectroscopic technique was employed for complete chemical analysis of uranium concentrate (yellow cake) to check its purity. The impurities were determined by emission spectrograph (JY 24 ICP Spectroanalyzer, France) by carrier distillation methodology after conversion to U₃O₈.
Acid Determination

Sulfuric acid produced from oxidation of elemental sulfur and sulfur slag by \( T. \text{ferrooxidans} \) and \( T. \text{thiooxidans} \) was determined by standard acid-base titration using phenolphthalein as an indicator.

Free Acid Determination

Free acid in bacterial leach liquors was detected by precipitating hydrolyzable cations with \( \text{KNO}_3 \)-\( K_2 \text{Fe(CN)}_6 \) solution and titrating against standard \( \text{NaOH} \) solution in methanol till pH reached to 5.0 (Rodden 1950).

Sulfur Determination

Elemental sulfur content of sulfur and sulfur slag was determined by using a method as described in the Manual of Pakistan Standard Specification for Sulfur (Anonymous 1966). In this method, pulverized sample of sulfur and/or slag was taken in a thimble and the elemental sulfur content was extracted in carbon disulfide \([\text{CS}_2]\) by using soxhlet apparatus.

Chemical Analysis of Uranium Mine Water

Uranium mine water samples were collected from various mine sites at Baghalchur, in sterilized containers for microbial and chemical analysis. Chemical analysis for \( K^+ \), \( Na^+ \), \( SO_4^{2-} \), \( Mg^{2+} \), \( PO_4^{3-} \) etc., which are usual ingredients of mineral salts media of ore leaching bacteria was performed of these samples. Flame photometric technique [Jenway PFP7, UK] was applied to determine \( K^+ \), \( Na^+ \) and \( Mg^{2+} \) ions present in mine water, while spectrophotometric methods were applied to detect \( SO_4^{2-} \) (Mateen et al. 1985) and \( PO_4^{3-} \) (Hanif et al. 1984).

Uranium contents present in these water samples were determined by a spectrophotometric method (Bhatti et al. 1991b).
Microbiological Growth Media

Liquid Media

Iron medium (9KFe\(^{2+}\))

The iron liquid medium (9KFe\(^{2+}\)) used for isolation and growth of *T. ferrooxidans* was a 9K mineral salts medium described by Silverman and Lundgren (1959) and contained [g/L]: (NH\(_4\))\(_2\)SO\(_4\), 3.00; MgSO\(_4\).7H\(_2\)O, 0.50; K\(_2\)HPO\(_4\), 0.50; KCl, 0.10; Ca(NO\(_3\))\(_2\), 0.01. These salts were dissolved in 800 mL of distilled water and the pH was adjusted to 2.0 by adding 1M H\(_2\)SO\(_4\). Aliquots of basal salts solution were sterilized by autoclaving at 121°C and 4 kPa for 20 minutes.

Ferrous sulfate [FeSO\(_4\).7H\(_2\)O] solution was prepared by dissolving 44.42g in 200 mL distilled water whose pH was adjusted to 2.0 with 1M H\(_2\)SO\(_4\). It was sterilized separately by passing through membrane filters [0.22 μm Millipore GVWP filters]. The sterilize ferrous sulfate solution was added aseptically to the cooled basal salts medium to give final ferrous iron concentration of 160 mM.

Sulfur medium (9KS\(^0\))

Sulfur medium (9KS\(^0\)) used for isolation and growth of *T. thiooxidans* was 9K mineral salts medium in which ferrous sulfate was replaced by elemental sulfur [10g/L]. The medium was prepared as described earlier (Silverman and Lundgren 1959). The pH of medium was adjusted to 2.5 with 1M H\(_2\)SO\(_4\) and 100 mL of aliquots were taken into 250 mL Erlenmeyer flasks. Finely ground (≤149 μm particle size) elemental sulfur was added to each flask (1% w/v pulp density), which were then plugged with cotton wool. Medium sterilization was done by tyr rallization by heating the flasks at 121°C for 30 minutes for three successive days (Alder 1976). This medium was used for performing growth studies of *T. ferrooxidans* and bioleaching of uranium ore and tailings residue.
Sulfur slag medium

Sulfur slag medium was the same as described above, except sulfur was replaced with finely ground (≤149 μm) sulfur slag [10g/L]. This media was also employed for performing growth studies of *T. thiooxidans* and *T. ferrooxidans* and bioleaching experiments with uranium ore and tailings residue.

Pyrite medium

Pyrite was sterilized by sodium hypochlorite solution and then air-dried overnight in a vacuum desiccator. The mineral salts solution used in pyrite mineral oxidation by *T. ferrooxidans* was that described by Tuovinen and Kelly (1973) and contained [g/L]: (NH_4)_2SO_4, 0.40; MgSO_4·7H_2O, 0.40 and K_2HPO_4, 0.40. Medium pH was adjusted to 2.0 with 5M H_2SO_4 and autoclaved at 121°C for 20 minutes. When the medium was cooled to ambient temperature, then pyrite mineral [5% w/v] was aseptically added into it.

Tetrathionate medium

Tetrathionate medium was prepared with the basal salts solution as previously described by Silverman and Lundgren (1959) in iron liquid medium. The medium pH was adjusted to 4.0 with dilute sulfuric acid and sterilized by autoclaving. Potassium tetrathionate (3.06 g K_2S_2O_6 dissolved in 200 mL distilled water) was sterilized separately by filtration and added into 9K basal salts solution to get final concentration of 10 mM K_2S_2O_6. The growth of acidophilic thiobacilli was observed by drop in pH of the medium due to acid production from oxidation of tetrathionate and the appearance of turbidity and microscopic observation.

Glucose medium

Glucose medium was prepared as described by Guay and Silver (1975), and containing [g/L]: (NH_4)_2SO_4, 3.00; K_2HPO_4, 0.50; MgSO_4·7H_2O, 0.50; KCl, 0.10; Ca(NO_3)_2, 0.012 and FeSO_4·7H_2O, 0.01mg. These salts were dissolved in 900 mL of distilled water and pH of the medium was adjusted to 3.5 with dilute H_2SO_4.
Aliquots of basal salts solution in Erlemeyer flasks were sterilized by autoclaving at 121°C for 20 minutes.

Glucose solution [10% wt/v] was sterilized separately by filtration [0.22 µm Millipore GVWP filters], and aseptically into basal salts solution as prepared above.

**Solid Media**

**Gelrite-FeSO₄ medium**

A new efficient "Gelrite-FeSO₄" solid medium was developed and successfully employed for isolation and enumeration of *T. ferrooxidans* in mine water, tailings liquid, microbially leached solutions and solid samples (Khalid et al. 1993). Gelrite-FeSO₄ solid medium was routinely used in the present studies and it was prepared as follows: [three separate solutions were prepared initially, and mixed after separate sterilization].

1. **Solution A** (Ferrous sulfate solution), was prepared by dissolving 50.0g FeSO₄·7H₂O in 200 mL distilled water and the pH was adjusted to 2.2 with 5M H₂SO₄. It was sterilized by filtration [0.22 µm Millipore GVWP filters] and collected in a pre-sterilized bottle.

2. **Solution B** (Mineral salts medium), which was a modified form of 9K medium (Silverman and Lundgren 1959), and containing (g/L): (NH₄)₂SO₄, 3.00; MgSO₄·7H₂O, 0.50; K₂HPO₄, 0.10; KCl, 0.10; Ca(NO₃)₂, 0.02. All ingredients were dissolved in 500 mL distilled water and the pH was adjusted to 2.3 with 5M H₂SO₄. This solution was sterilized by autoclaving for 20 minutes at 4 kPa and 121°C.

3. **Solution C** (Gelling solution), 4.0g of Gelrite [Kelco Division of Merck, USA] or Gelan-Gum (Gel-Gro) [ICN Biochemicals, USA] was soaked in 300 mL distilled water for 20 minutes and then autoclaved for 20 minutes at 4 kPa and 121°C to sterilize it.
When all these solutions had cooled down to 70-75°C, solution B was mixed with solution C aseptically, and then solution A was added to get final concentration of gelrite of 0.4% w/v and FeSO₄·7H₂O of 5% w/v. These solutions were stirred constantly during mixing and the temperature near 70°C. The final pH of this medium should be 2.3-2.5. Approximately 25-30 mL of this mixture was poured into pre-sterilized petri-dishes, which were used for microbiological investigations.

**Thiosulfate Medium (Agarose-S₂O₃²⁻ medium)**

Thiosulfate solid medium was routinely used for isolation and enumeration of *T. thiooxidans* during present study and was prepared as follows: [three separate solutions were prepared separately, and mixed after separate sterilization].

1. **Solution A** [Sodium thiosulfate solution], was prepared by dissolving 10.0 g Na₂S₂O₃·5H₂O in 100 mL distilled water and it was separately sterilized by filtration [0.22 μm Millipore GVWP filters] and collected in a pre-sterilized bottle.

2. **Solution B** [Mineral salts solution], which was prepared in a modified form according to 9K medium (Silverman and Lundgren 1959). It contained (g/L): (NH₄)₂SO₄, 3.00; MgSO₄·7H₂O, 0.50; K₂HPO₄, 0.50; KCl, 0.10; Ca(NO₃)₂, 0.02. All ingredients were dissolved in 600 mL distilled water and the final pH was adjusted to 4.0-4.5 with dilute H₂SO₄. This solution was sterilized by autoclaving for 20 minutes at 4 kPa and 121°C.

3. **Solution C** (Gelling solution), 4.0 g of purified agarose [Sigma Chemical Company, USA] was soaked in 300 mL distilled water for 20 minutes and then autoclaved for 20 minutes at 4 kPa and 121°C to sterilize it.

When all these solutions had cooled down to 45-50°C, solution B was mixed to solution C aseptically, and then solution A was added to a final concentration of agarose of 0.4% w/v and Na₂S₂O₃·5H₂O of 1% w/v. These solutions were stirred.
constantly during mixing and the final pH of the medium should be 4.2-4.5 and temperature near 50°C. Approximately 25-30 mL of this mixture was poured into pre-sterilized petri-dishes, which were used for further investigations.

**Glucose Medium (Agarose-Glucose medium)**

Glucose solid medium was prepared as described earlier for the preparation of thiosulfate solid medium, except that thiosulfate solution was replaced by glucose solution (10% wt/vol).

**Isolation and Characterization of Acidophilic Iron- and Sulfur-Oxidizing *Thiobacillus* Bacteria**

**Sampling Procedures**

The tailings liquid samples were collected in pre-sterilized [autoclaving at 121°C, for 15 minutes] polypropylene screw cap bottles from Tailings Pond, Uranium Ore-Processing Plant, Dera Ghazi Khan. The bottles were thoroughly rinsed with tailings liquid samples and filled to half of the capacity. While the tailings residue samples were collected in polyethylene bags. All these samples were stored at 4°C and preliminary isolation experiments were carried out immediately on return to the laboratory.

**Isolation and Enumeration of *Thiobacillus ferroxidans***

After sampling, 1.0 mL aliquot of each tailings liquid sample was inoculated separately into liquid iron (9KFe^{2+}) medium as prepared earlier (Silverman and Lundgren 1959). These flasks were incubated at 30°C and 100 rpm. The presence of iron-oxidizing bacteria in liquid iron medium was indicated by the formation of ferric iron [Fe^{3+}] and the medium becoming brick red in color. A serial dilutions of each culture was prepared using sterile acidified (pH 2.5) 9K mineral salts solution as a diluent; 0.1 mL amounts of each dilution was spread on solid Ge rite-FeSO_{4} plates. The inoculated plates were incubated at 30°C in a sealed polyvinyl-bag to
keep the medium from drying. Plates were examined with the naked eye and then under a stereomicroscope [Leitz-Wild M32, Heerbrugg, Switzerland] to observe the colony size, shape, colour, and other morphological features. Single colonies of iron-oxidizing bacteria (*T. ferrooxidans*) were picked from the plates by using a sterile inoculation loop and each one inoculated into 25 mL capacity culture vials containing 10 mL liquid iron medium of pH 2.0 and was crushed to release the bacteria. All the cultures were incubated at 30°C until the medium colour changed to brick red indicating ferrous iron [Fe²⁺] oxidation by iron-oxidizing bacteria. To check the purity of isolated cultures, cells growing in liquid iron medium were spread on solid Gelrite-FeSO₄ plates. After 5-7 days, the plates were examined for uniformity of colony morphology and the presence of any contaminants. Single colonies were also subcultured into separate liquid iron and sulfur media as described earlier [Silverman and Lundgren 1959]. The cells of iron-oxidizing bacterium (*T. ferrooxidans*) was viewed under a phase-contrast microscope [Nikon YB-Ph-11, Tokyo, Japan] to examine cell size, shape, motility and the tendency to form filaments. Other tailings liquid samples were employed directly on Gelrite-FeSO₄ plates, which were incubated at 30°C.

For tailings residue sample, 1.0g of each sample was added into a 250 mL Erlenmeyer flask containing 100 mL sterile 9K mineral salts medium of pH 4.0 and mixed thoroughly using vortex mixer. A volume of 0.1 mL of this solution was spread on solid Gelrite-FeSO₄ plates and incubated at 30°C.

**Isolation and Enumeration of *Thiobacillus thiooxidans***

One mL aliquot of each tailings liquid sample was inoculated into liquid sulfur medium (9KS⁹) of pH 2.5 and incubated at 30°C and 100 rpm. The presence of sulfur-oxidizing bacteria (*T. thiooxidans* and *T. ferrooxidans*) in liquid sulfur media was indicated by a drop in pH of the medium due to the production of sulfuric acid. When the pH of medium dropped to less than 1.0, an aliquot of 1.0 mL was taken and subcultured into fresh sulfur liquid medium. Finally, after 5-6 subculturing of this strain, it was centrifuged at 10,000 rpm in a refrigerated centrifuge for 10
minutes. The cells pellet was re-suspended in sterile distilled water (20 mL) of pH 4 adjusted with 1M H₂SO₄. A dilution series of isolated culture was prepared using sterile acidified (pH 4) mineral salts solution as a diluent: 0.1 mL amounts of each dilution were spread on solid tetrathionate plates and incubated at 30°C. To avoid drying-up, the plates were kept in a sealed polyvinyl bag. Plates were examined with naked eye and under a stereomicroscope to record morphological features of colonies, such as size, shape, and colour. Single colonies were picked from the plates by using a sterile loop and each one inoculated separately into 25 mL capacity culture vials containing 10 mL tetrathionate liquid media of pH 4.0 and cultured to release the bacteria. All cultures were incubated at 30°C until the medium became milky and pH dropped to less than 1.0 due to oxidation of tetrathionate by sulfur-oxidizing bacteria. To check the purity of isolated strains, cells growing in liquid tetrathionate medium were spread on solid Gelrite-FeSO₄ and glucose plates and observed after 5-7 days of incubation, for the presence of any contaminants of iron-oxidizer (T. ferrooxidans) or glucose-oxidizer (T. acidophilus) bacteria respectively, were picked and streaked onto solid tetrathionate, glucose and Gelrite-FeSO₄ media to check its growth on glucose and ferrous iron media. Single colonies were also subcultured into ferrous iron, tetrathionate, and glucose liquid media.

Taxonomical investigations (Gram staining and microscopy) and physiological tests viz., the ability to oxidize elemental sulfur [S⁰], reduced sulfur compounds like thiosulfate [(S₂O₃)²⁻] and tetrathionate [(S₄O₆)²⁻], and glucose in liquid media were performed for isolated strains of T. ferrooxidans and T. thiooxidans.

Direct Bacterial Counts

Total bacterial numbers were counted with a bacterial counting chamber [Pettroff-Hauser Bacterial Counter, Arther and H. Thomas Company, Philadelphia, depth 0.02 mm, area 0.04 mm²]. Total microbial populations were counted by placing chamber under a phase-contrast microscope. Viable numbers were estimated by spreading appropriate dilutions on solid media. This method utilizes a serial dilutions and statistically determines the number of bacteria present in a culture and
generally used to provide a comparison to counts made using Geirite-FeSO₄ plates.

Maintenance of Cultures

*Thiobacillus ferroxidans* was routinely maintained in fresh liquid iron medium (9KFe²⁺) pH 2.0 at 30°C by following oxidation of Fe²⁺ after every 5-7 days of incubation. While *T. thiooxidans* was maintained by subculturing into fresh liquid sulfur medium (9KS⁰) after every two weeks of incubation.

Preparation of Inocula

*Thiobacillus ferroxidans* was grown in liquid iron medium (9KFe⁺) as prepared earlier (Silverman and Lundgren 1959) in a 10 litre working volume of top drive fermentor [B Braun Biostat E, Germany]. The fermentor was aerated with air [1.3 L/min] and CO₂ [0.1 L/min] and was agitated at 200 rpm at 30°C. Aactively growing culture of *T. ferroxidans* was used to inoculate and when ferrous sulfate was completely exhausted, the culture broth was centrifuged at 10,000 rpm in a refrigerated centrifuge [Beckman J2-21, USA] for 15 minutes. The pellet, containing cells and ferric salts, was resuspended in sterile water of pH 2.0 adjusted with 5.0 M H₂SO₄, transferred into a separating funnel and allowed to stand overnight at 4°C. The upper milky layer, which contained cells, was removed aseptically and centrifuged at 10,000 rpm for 10 minutes. The pellet containing cell’s was resuspended in sterile distilled water (100 mL) of pH 2.5 adjusted with 5.0 H₂SO₄ and was used for as inoculum of *T. ferroxidans* for oxidation studies of ferrous iron, pyrite and leaching studies with sandstone ore amended with sulfidic ore as external energy substrate. This inoculum was found to contain 4.5 X 10⁶ cells/mL of *T. ferroxidans*.

For bioleaching studies of uranium ore, both strains of *T. ferroxidans* and *T. thiooxidans* were grown separately in liquid sulfur medium (9KS⁰) in a 10 litre working volume of top drive fermentor. The fermentor was operated as above. These actively growing strains of *T. ferroxidans* and *T. thiooxidans* were harvested
after 7-days of incubation, the unused sulfur was removed by filtration through Whatman No. 1 filter paper and the filtrate was centrifuged at 10,000 rpm in a refrigerated centrifuge for 10 minutes. The cells pellet was resuspended in sterile distilled water (100 mL) of pH 2.50 adjusted with 5M H₂SO₄ and was used as inoculum for oxidation studies of elemental sulfur, sulfur slag and bioreaching of sandstone uranium ore.

**Oxidation of Ferrous Iron by *T. ferrooxidans***

The oxidation of ferrous sulfate (Fe²⁺ iron) by both isolated and standrad strains *T. ferrooxidans* was carried out in 250 mL Erlenmeyer flasks containing 90 mL liquid iron medium (9KFe²⁺) of pH 2.0 as described earlier (Silverman and Lundgren 1959). These flasks were inoculated with 10 mL suspension containing 4.5 X 10⁶ cells/mL of *T. ferrooxidans* and incubated at 30°C on an orbital shaking incubator [OSI-503L, Ogawa Seiki, Japan]. Periodically, samples were aseptically drawn to monitor pH and oxidation of Fe²⁺ to Fe³⁺ by these strains.

**Oxidation of Pyrite by *T. ferrooxidans***

The oxidation of pyrite by *T. ferrooxidans* was carried out in 250 mL Erlenmeyer flasks containing 90 mL liquid pyrite medium as described earlier (Tuovinen and Kelly 1973). These flasks were inoculated with 10 mL suspension containing 5 X 10⁸ cells/mL of pre-grown pyrite culture of *T. ferrooxidans*, and the flasks were incubated at 30°C on an orbital shaking incubator. Periodically, samples were removed for monitoring the pH and for the analysis of total iron release from pyrite oxidation. An aliquot of 10 mL was drawn from each flask and filtered through 0.22 μm Millipore membrane filter to remove the mineral particles. The filtrate was mixed with 6N HCl [1:1 ratio] to prevent the precipitation of ferric compounds and stored at room temperature. The samples, or suitable dilutions thereof, were analyzed for total iron using atomic absorption spectrophotometry.
Oxidation of Elemental Sulfur and Sulfur Slag by *T. ferrooxidans* and *T. thiooxidans*

Oxidation of elemental sulfur and sulfur slag was carried out in 250 mL Erlenmeyer flasks containing separately 90 mL liquid sulfur (9KS⁶) and sulfur slag media as described earlier (Silverman and Lundgren 1959). These flasks were inoculated separately with 10 mL suspension containing 10⁸-10⁹ cells/mL of *T. ferrooxidans* and *T. thiooxidans* and flasks were incubated at 30°C and 100 rpm in a incubating shaker. Periodically, samples were aseptically drawn to monitor pH and sulfuric acid production from bacterial oxidation of elemental sulfur and sulfur slag. Bacterial oxidation studies of sulfur and slag were also performed by standard strains of *T. ferrooxidans* (ATCC 13361) and *T. thiooxidans* (ATCC 8085).

Similarly, growth studies were performed on liquid sulfur [9KS⁶] and sulfur slag media as described earlier using mixed cultures of *T. ferrooxidans* and *T. thiooxidans* under the same experimental conditions as described for above experiments.

Bioleaching Studies of Sandstone Uranium Ores

Shake Flask Leaching Studies

Shake flask leaching experiments were carried out in 250 mL Erlenmeyer flasks containing uranium ore [10.0g] and 90 mL liquid sulfur (9KS⁶) medium as described earlier (Silverman and Lundgren 1959) by using indigenous microflora of *T. ferrooxidans* and *T. thiooxidans*. These flasks were inoculated with 10 mL suspension containing 10⁸-10⁹ cells/mL of locally isolated strains of *T. ferrooxidans* and *T. thiooxidans*. The flasks were incubated at 30°C and 100 rpm on orbital shaking incubator. Periodically, leach liquor samples were aseptically taken for monitoring pH and the analysis for free acid and uranium solubilization from sandstone ore. In these studies, bioleaching studies of uranium ore was also conducted with sulfur slag and pyrite media as external energy source for these *Thiobacillus* bacteria.
In shake flask studies, various leaching parameters like pulp density, particle size, initial pH adjustment of medium, inoculum size, nature and proportion of external energy substrates, and leaching time, on bioleaching of sandstone uranium ores were investigated.

**Tank Leaching Studies**

Tank leaching experiments of low-grade sandstone uranium ore were performed in a 10-litres working volume of top-drive fermentor (Model B Braun Biostat E). Sulfur was used as external energy substrate for *T. ferrooxidans* and *T. thiooxidans* bacteria in 9K mineral salts medium of initial pH 2.5. A mixed culture of indigenous strains of *T. ferrooxidans* and *T. thiooxidans* were grown in liquid 9K50 mineral salts medium (as described earlier) for 5-7 days to produce sufficient amount of sulfuric acid to neutralize major acid consuming gangue minerals like calcite and/or dolomite present in sandstone ore. Initially, a 10% [wt/vol] pulp density was used and increased stepwise 15%, 20%, 30%, 40%, and 50% [wt/vol]. The bioreactor was aerated with air [1.3 L/min] and CO₂ [0.1 L/min] and was agitated at 150 rpm at 30.0±2.0°C. Periodically, leach liquor samples were taken for monitoring pH and uranium solubilization from ore. The leach liquor samples were also examined microscopically for growth of *T. ferrooxidans* and *T. thiooxidans*.

**Air-Lift Percolator Column Studies**

Four air-lift percolation columns made of glass [40 cm height and 6 cm dia] were used in these studies and were placed in a cabinet thermostatically maintained at 30°C. Uranium ore [1kg] amended with sulfur slag [5% w/w] as external energy source for *Thiobacillus* bacteria, was placed on the fitted glass bottom. The 9K mineral salts medium [1 L] without Fe²⁺ iron was pumped air flow in order to maintain a good circulation of the solution. The pH-value of solution was adjusted and kept at 2.0-2.5 by addition of dilute sulfuric acid and make-up water was added when necessary to compensate for evaporation. After reaching pH values of 2.0-2.5 in the effluent solution, the columns were inoculated with pure and mixed cultures.
of *T. ferrooxidans* and *T. thiooxidans*. Periodically, solution samples were pipetted and were assayed for pH and solubilization of uranium from ore. The leachate was examined microscopically in wet mounts for the presence of bacteria.

**PVC Column Leaching Studies**

Four polyvinyl chloride (PVC) columns 25.0 cm in diameter and 2.0 meter height, were used during these studies. The columns had flat acrylic bottoms which were fitted with 10 mm dia outlets in the center and were labelled as A, B, C and D. The ore was manually charged in the columns. Each column was loaded with 100 kg of low-grade sandstone uranium ore containing 0.023% U₃O₈ amender, with 5% (w/w) elemental sulfur except column D in which sulfur slag 5% (w/w) was used as external energy source for acidophilic *Thiobacillus* bacteria. An initial inoculum (10% v/v) containing 10⁸ cells per mL of mixed culture of *T. ferrooxidans* and *T. thiooxidans* was used for each column. Each column was fed from separate reservoir which contained 200-Litres of mine water with and without certain treatments (initial pH adjustment, nutrient supply) as lixiviant. The column A was only irrigated from mine water as collected from mine site without any treatment, while column B was pumped with mine water whose pH was adjusted with H₂SO₄ to a value of 3.5. Other two columns C and D were irrigated with mine water of adjusted pH 3.50 to which 3.0 g/L (NH₄)₂SO₄ as nitrogen source for bacteria was added. The nine water was pumped at a rate of 1.5 L/hr in each column. Periodically, the column effluent samples were taken from each column for pH monitoring and the analysis for uranium solubilization from ore. Autotrophic sulfur- and iron-oxidizing bacteria in these column effluents were also enumerated by employing solid Gelrite-FeSO₄, tetrathionate and thiosulfate media as delineated earlier. The presence of some mixotrophs like *T. acidophilus* were also monitored by using liquid and solid media described by Guay and Silver (1975).
Microbial Heap Leaching Studies

Heap Construction

A heap of 20.0 tons ore capacity (5 X 3 X 2 meter) was established at N BGE Campus according to the general procedure as described follow: The site was prepared by cleaning and levelling to a gentle slope of 2.0% towards the solution collection end. A layer of sand was spread on the prepared surface to have a smooth base free of projections or rough stones or pebbles. A PVC sheet (0.15 mm thickness) was used as acid proof lining material to prepare an impermeable surface. The edges of the pad were raised about 30 cm on three sides and the sheet was embedded underneath. A layer of ore mixed with sulfur slag was placed over the PVC sheet to protect it from any damage or puncture. The perforated PVC pipes (5 cm dia) were placed along the slope of the pad at a spacing of one meter to act as solution collectors and these pipes were discharged in PVC lined common collecting launder prepared at the lower end of the heap base. The pebbles or stones (5-10 mm) were placed manually along the pipes to prevent the perforation from blocking. The diagrammetrical representation of microbial heap leaching process for sandstone uranium ore was illustrated in Figure 4.

Initially, the ore was mixed with crushed sulfur slag (10% wt/wt; containing 54.0% sulfur) and was dumped on the heap by hand-moving trolley. The few trolleys of ore amended with sulfur slag were dumped carefully to protect the PVC pipes and lining. The maximum height of the heap was kept at two meters. When the desired height (2.0 meter) was achieved, the top of the heap was levelled manually. The top was divided into two segments having one meter length and 15.0 cm depth to act as ponds for uniform distribution of solution over the heap.
Figure 4: A diagrammetrical representation of heap leaching recovery of uranium from sandstone ore. (AC = Air compressor, B = Inoculum preparation, BL = Bacterial leach liquor, CV = Control valve, RC = Resin column, P = Water pump).
Heap Operation

An inoculum (600-Liters) of mixed culture of *T. ferrooxidans* and *T. thiooxidans* was prepared in a fibre-glass tank (BL) using elemental sulfur (1.0 wt vol) as energy substrate. Ammonium sulfate (3.0 g L) as nitrogen nutrient was dissolved in subsoil water, and the pH was adjusted to 6.5±0.1 with 10.0 M H₂SO₄. The medium was air-bubbled with compressed air (AC) to supply oxygen, CO₂ and to agitate it. The inoculum (6-7 days old) so prepared was sprinkled on top of the heap intermittently once a week, which passed through the ore pile and collected in a tank (BL). The microbial leach liquor thus obtained was passed through an anion-exchange resin column (RC) containing Amberlite IRA-400 resin to recover solubilized uranium content and the barren solution (liquid tailings) was again used for inoculum preparation with certain nutrients supply, if required.

Periodically, samples were taken to analyze soluble uranium content and the isolation and enumeration of autotrophic and heterotrophic *Thiobacillus* bacteria in microbial heap leach liquors.

Adsorption of Uranium by Anion-Exchange Resins

Packing of Resin Column

A glass column [100 cm in height and 5 cm in dia(e)] was packed with Amberlite IRA-400 [Rohm and Hass Product, France] anion-exchange resin [styrene-DVB: Cl form: strongly basic; 20-50 mesh] to recover soluble uranium from bacterial leach liquors. Amberlite IRA-400 resin [1.0 L(t)] was soaked in 2-Litre of deionized water for one-hour and then it was packed in a glass column having an outlet (1 cm e) at the bottom. Precautions were taken that there should be no air-bubbles in the column. Similarly, two other strongly basic anion-exchange resins like Duolite® A-147 (Duolite International, France) and Dowex 1 X 8 (Dow Europe, Switzerland) were also tested for uranium adsorption accordingly.
Loading of Uranium

A reservoir [500 litre capacity] containing bacterial leach liquor was connected to column packed with Amberlite IRA-400 resin by a rubber or flexible tube. The leach liquor containing soluble uranium content was passed through the resin column at a flow rate of 5 mL/min using a peristaltic pump "Masterflex" (Cole-Parmer Instruments, USA). These resin columns were operated until "saturation point (S)" for uranium recovery was achieved from the feeding solution. Samples were taken after adequate volume of microbial leach solution has passed and were analyzed for uranium content in resin columns effluents to check the uranium adsorption or loading capacity (E), breakthrough bed volume (B) and saturation point (S) for these resins.

Elution of Uranium From Loaded Adsorbed Resins

A reservoir [10-litre capacity] containing 2 M NH₄HCO₃ solution as an eluent was connected to resin column (Amberlite IRA-400) loaded with uranium by a rubber or flexible tube. This eluent was passed through the resin column at a flow rate of 5 mL/min using a peristaltic pump "Masterflex" (Cole-Parmer Instruments, USA). Samples of strip solutions were taken after adequate volume of eluent has passed and were analyzed for uranium. Similarly, the optimal stripping efficiency for uranium of ammonium bicarbonate [1.0-2.0 M NH₄HCO₃ solutions] and ammonium sulfate [1.0-2.0 M (NH₄)₂SO₄ solutions] with and without 3.95 g/L NH₄HCO₃ were tested as eluents accordingly.

Production of Uranium Concentrate (Yellow Cake)

Uranium concentrate [yellow cake] was produced by precipitating the strip solution with ammonia [NH₃] gas. The yellow precipitate of uranium as ammonium diuranate [(NH₄)₂U₂O₇] was filtered, washed and subsequently dried at 110°C in an electric oven [Binder, Germany]. Uranium content of yellow cake samples were determined by spectrophotometric method (Johnson and Florence 1971). The Induced-Coupled Plasma (ICP) Emission Spectrometry (JY24 ICP Spectroanalyzer,
Jobin Yvon, France, was employed for detailed chemical analyses of a typical yellow cake sample to check the purity.
RESULTS

Mineralogy of Low-Grade Sandstone Uranium Ore

Sandstone Uranium Ore

Baghalchur sandstone uranium was light grey, mostly medium to fine grained, soft, friable, poorly sorted and in general, resembled to river sand.

Sieve Analysis

Sieve analysis of Baghalchur sandstone uranium ore was grouped into eight sieve fractions depending upon weights of ore fractions collected and results are reported in Table 5. It was found that the ore contained 46.90% (wt/wt) of ore fraction with a particle size of $\geq 595$ $\mu$m ($\geq 30$ ASTM mesh size) which was a major fraction and almost the weight of ore fraction was found to decrease concomittantly with particle size.

Table 5
Particle Size Distribution at Different Mesh Size Fractions of Baghalchur Sandstone Uranium Ore

<table>
<thead>
<tr>
<th>ASTM Mesh Size Number</th>
<th>ASTM Particle Size ((\mu)m)</th>
<th>Weight of Fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 30$</td>
<td>$\geq 595$</td>
<td>46.90</td>
</tr>
<tr>
<td>- 30 to + 40</td>
<td>- 595 to + 420</td>
<td>19.14</td>
</tr>
<tr>
<td>- 40 to + 60</td>
<td>- 420 to + 250</td>
<td>8.68</td>
</tr>
<tr>
<td>- 60 to + 100</td>
<td>- 250 to + 149</td>
<td>5.04</td>
</tr>
<tr>
<td>- 100 to + 150</td>
<td>- 149 to + 105</td>
<td>3.00</td>
</tr>
<tr>
<td>- 150 to + 200</td>
<td>- 105 to + 74</td>
<td>2.30</td>
</tr>
<tr>
<td>- 200 to + 230</td>
<td>- 74 to + 63</td>
<td>1.92</td>
</tr>
<tr>
<td>$\leq 300$</td>
<td>$\leq 53$</td>
<td>4.20</td>
</tr>
</tbody>
</table>
When the uranium content of these ore fractions were determined by a spectrophotometric method (Jhonson and Florence 1971), it was observed that the distribution of uranium content was much higher in the fine size ore fraction as compared to coarse one Figure 5. Therefore, the uranium content was higher (0.046% $U_3O_8$) in the particle size of $\leq$53 $\mu$m ($\leq$300 ASTM mesh size) and was lower (0.01% $U_3O_8$) in the particle size of $\geq$595 $\mu$m ($\geq$30 ASTM mesh size). However, the average uranium content of the ore sample was 0.023% $U_3O_8$ on dry matter basis Figure 5.

Figure 5: Distribution of uranium content at different particle size fraction of Baghalchur sandstone uranium ore.
**Light and Heavy Minerals Separation**

Light and heavy minerals analysis of representative sandstone uranium ore sample was conducted by bromoform separation technique (Muller 1977) and the results obtained are reported in Figure 6. These results indicated that the sandstone ore sample contained 86.75% light minerals, 8.14% heavy minerals, and 5.52% clay minerals fractions. The chemical analysis results of clay minerals fraction of ore revealed that it contained major portion of uranium content (0.021% U₃O₈) which was 91.30% of the total uranium content present in the ore sample (0.023% U₃O₈), whereas the light and heavy minerals fractions together contained only 8.70% of the total uranium content of the ore [Figure 7].

![Graph showing mineral fractions of sandstone ore](image)

**Figure 6**: Bromoform separation of light, heavy, and clay minerals of sandstone uranium ore.
Magnetite Separation

The ore sample was air-dried and subjected to magnetite separation using hand magnet. Highly magnetic minerals can be separated by using any suitable permanent bar or horse-shoe type magnet, of which the poles are being covered by thin paper to facilitate subsequent release of mineral grains. The magnetite (Fe₃O₄) mineral grains was weighed and calculated in bulk of the ore sample, which gave the value of 0.21% [Figure 6]. Magnetite and pyrrhotite (Fe₃S) minerals are considered highly magnetic minerals (Muller 1977).

![Pie chart showing Clay Fraction and Sand Fraction](chart.png)

**Figure 7:** Uranium distribution in clay and others minerals fractions of sandstone ore.
Isodynamic Magnetic Separation

Heavy mineral fraction of the ore sample was separated into mono-mineral fraction using isodynamic magnetic separator (Frantz magnetic separator) by applying different electromagnetic field current and side slope [Incline feed]. The magnetic susceptibility of individual mineral species will vary, however, due to compositional variation or to the presence of minute inclusions of differing susceptibility. The minerals are paramagnetic [attracted towards electromagnetic field], diamagnetic [repelled reverse to electromagnetic field] and ferromagnetic which carrying residual magnesium after removal from the magnetic field.

The results obtained from Isodynamic Magnetic Separation technique are shown in Table 6. It was observed that magnetic minerals at 0.40 Ampere (A) electromagnetic field current and 20° slide slope of the Isodynamic Magnetic Separator; ilmenite and garnet minerals were separated. At electromagnetic field current of 0.40-0.80 A and 20° slide slope of the separator; minerals like biotite, actinolite, epidote, hornblende, tourmaline, chlorite, and hematite were separated. Whereas at field current of 1.0 A and 20° side slope, muscovite (a dioctahedral mica mineral) was separated. Non-magnetic minerals like rutile, calcite, zircon, quartz, feldspar, and radioactive mineral grains were separated at 1.5 A field current and 5° side slope of the Isodynamic Magnetic Separator and grains of pyrite (FeS₂) mineral were separated by applying 1.5 A electromagnetic field current at reverse side slope; that was due to diemagnetic property of this mineral (pyrite) which was repelled by electromagnetic field current.
Table 6
Mineral Analyses of Sandstone Ore by Frantz Isodynamic Magnetic Separation Technique

<table>
<thead>
<tr>
<th>Type of Minerals</th>
<th>Electromagnetic Field Current [Ampere (A)]</th>
<th>Side Slope</th>
<th>Minerals identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic Minerals:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.40</td>
<td>20°</td>
<td></td>
<td>Ilmenite, Garnet</td>
</tr>
<tr>
<td>0.40-0.80</td>
<td>20°</td>
<td></td>
<td>Actinolite, Biotite, Epidote, Hornblende, Tourmaline, Chlorite, Hematite</td>
</tr>
<tr>
<td>0.80-1.00</td>
<td>20°</td>
<td></td>
<td>Muscovite</td>
</tr>
<tr>
<td>Non-Magnetic Minerals:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.50</td>
<td>5°</td>
<td></td>
<td>Calcite, Feldspar, Rutile, Zircon, Quartz, Tyuyamunite</td>
</tr>
<tr>
<td>Diamagnetic Minerals:</td>
<td></td>
<td>Reverse side</td>
<td>Pyrite</td>
</tr>
</tbody>
</table>

Stereomicroscopic Analysis

The light and heavy mineral fractions of sandstone ore obtained by bromoform separation were examined under a stereomicroscope [Leitz-Wild M32, Switzerland]. Various mineral constituents of the uranium ore sample were identified and their respective visual percentage were obtained by grain counting under the stereomicroscope. The computation of these results have been reported in Table 7. It was observed that the major constituents of the Baghalchur sandstone ore were quartz (SiO$_2$), calcite (CaCO$_3$), feldspar (KAlSi$_3$O$_8$),
hornblende [(Ca, Mg, Fe, Na, Al)\textsubscript{7.8}(Al, Si\textsubscript{3}O\textsubscript{22})(OH)\textsubscript{2}], chlorite [(Mg, Fe\textsuperscript{2+}, Fe\textsuperscript{3+})\textsubscript{6}AlSi\textsubscript{3}O\textsubscript{10}(OH)\textsubscript{8}], epidote [Ca\textsubscript{2}Fe\textsuperscript{3+}Al\textsubscript{2}Si\textsubscript{3}O\textsubscript{12}(OH)], muscovite [KAl\textsubscript{3}Si\textsubscript{3}O\textsubscript{10}(OH)\textsubscript{2}], biotite [K(Mg, Fe)\textsubscript{3}AlSi\textsubscript{3}O\textsubscript{10}(OH)\textsubscript{2}], and clay minerals and refractory fragments. However, the pyrite (FeS\textsubscript{2}), actinolite [Ca\textsubscript{2}(Mg, Fe)Si\textsubscript{8}O\textsubscript{22}(OH)\textsubscript{2}], pyroxene [ABS\textsubscript{2}O\textsubscript{6}, where A = Ca, Na, Mg, Fe\textsuperscript{2+} and B = Mg, Al, Mn, Fe\textsuperscript{2+}, Fe\textsuperscript{3+}], ilmenite [FeTiO\textsubscript{3}], limonite [Fe\textsubscript{2}O\textsubscript{6}6H\textsubscript{2}O], rutile [TiO\textsubscript{2}] minerals were among minor constituents. Muscovite, biotite, chloride, actinolite, hornblende, epidote, pyroxene, and tourmaline are the mica minerals present in sandstone uranium ore.

Quartz (SiO\textsubscript{2}) was the most abundant constituent of the sandstone uranium ore and represented 59.0% of the frame grains. Zircon (ZrSiO\textsubscript{4}), garnet (Fe\textsubscript{3}Al\textsubscript{2}Si\textsubscript{3}O\textsubscript{12}) and tourmaline [(XY\textsubscript{3}Al\textsubscript{6}(OH))\textsubscript{4}(BO\textsubscript{3})\textsubscript{3}(Si\textsubscript{6}O\textsubscript{18}): where X = Na, Ca and Y = Al, Fe\textsuperscript{3+}, Fe\textsuperscript{2+}, Li, Mg] minerals were present in trace amounts. Hematite (Fe\textsubscript{2}O\textsubscript{3}) and magnetite (Fe\textsubscript{3}O\textsubscript{4}) minerals were found as iron oxides which constituted the major portion of the heavy minerals present in the ore. Uranium mineral was present as black-coated grains which represented the essential radioactivity of the sandstone ore.

X-Ray Diffraction Analysis

Uranium mineral was present as black-coated grains in an appreciable amount (1.32%) and the mineral identified by X-ray diffraction (XRD) was tyuyamanite [Ca(VO\textsubscript{4})\textsubscript{2}(UO\textsubscript{2})\textsubscript{2}5-8 H\textsubscript{2}O]. Tyuyamanite is a uranium-vanadium complex in which uranium is present in hexavalent state [U\textsuperscript{6+}], which is readily soluble in dilute acid or carbonate-bicarbonate solution.
Table 7
Stereomicroscopic Mineral Analysis of Baghalchur Low-Grade Sandstone Uranium Ore

| Mineral         | Mineral Formula                                                                 | Mineral Fraction (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>SiO₂</td>
<td>59.00</td>
</tr>
<tr>
<td>Feldspar</td>
<td>MAI₅Si₃O₈</td>
<td>5.80</td>
</tr>
<tr>
<td>Calcite</td>
<td>CaCO₃</td>
<td>3.50</td>
</tr>
<tr>
<td>Pyrite</td>
<td>FeS₂</td>
<td>0.10</td>
</tr>
<tr>
<td>Chlorite</td>
<td>(Mg,Fe²⁺,Fe³⁺)₆AlSi₃O₁₀(OH)₈</td>
<td>2.90</td>
</tr>
<tr>
<td>Muscovite</td>
<td>KAl₅Si₉O₁₀(OH)₂</td>
<td>2.00</td>
</tr>
<tr>
<td>Biotite</td>
<td>K(Mg,Fe)₃AlSi₃O₁₀(OH)₂</td>
<td>1.82</td>
</tr>
<tr>
<td>Actinolite</td>
<td>Ca₂(Mg,Fe)₂Si₈O₂₂(OH)₂</td>
<td>0.16</td>
</tr>
<tr>
<td>Hornblende</td>
<td>(Ca,Mg,Fe,Na,Al)₁₋₈(Al,Si)₈O₂₂(OH)₂</td>
<td>3.17</td>
</tr>
<tr>
<td>Pyroxene</td>
<td>ABSi₂O₆</td>
<td>0.50</td>
</tr>
<tr>
<td>Epidote</td>
<td>Ca₂Fe³⁺Al₂Si₃O₁₂(OH)</td>
<td>3.00</td>
</tr>
<tr>
<td>Tourmaline</td>
<td>XY₃Al₆(OH)₄(BO₃)₃(Si₆O₁₈)</td>
<td>Traces</td>
</tr>
<tr>
<td>Garnet</td>
<td>Fe₃Al₂Si₃O₁₂</td>
<td>Traces</td>
</tr>
<tr>
<td>Ilmenite</td>
<td>FeTiO₃</td>
<td>0.62</td>
</tr>
<tr>
<td>Limonite</td>
<td>Fe₂O₆.6H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>Rutile</td>
<td>TiO₂</td>
<td>0.02</td>
</tr>
<tr>
<td>Hematite</td>
<td>Fe₂O₃</td>
<td>0.50</td>
</tr>
<tr>
<td>Magnetite</td>
<td>Fe₃O₄</td>
<td>0.21</td>
</tr>
<tr>
<td>Zircon</td>
<td>ZrSiO₄</td>
<td>Traces</td>
</tr>
<tr>
<td>Tuyuyamunite</td>
<td>Ca(VO₄)₂(UO₂)₂·5-8 H₂O</td>
<td>1.32</td>
</tr>
<tr>
<td>Clay minerals</td>
<td></td>
<td>4.83</td>
</tr>
<tr>
<td>Refractory fragments</td>
<td></td>
<td>9.14</td>
</tr>
</tbody>
</table>

A = Ca,Na,Mg,Fe²⁺
B = Mg,Al,Mn,Fe²⁺,Fe³⁺
M = K⁺, Na⁺, Ca²⁺
X = Na, Ca
Y = Al,Fe³⁺,Fe²⁺,Li,Mg.
Chemical Analyses of Sandstone Uranium Ore

Sandstone uranium ore used in present bioleaching studies conducted in shake flask, tank, bioreactor, air-lift percolators, continuous leaching, column and heap leaching studies had an average uranium content of 0.023\% $\text{U}_3\text{O}_8$ (230 ppm $\text{U}_3\text{O}_8$) on dry matter basis. The uranium content of the ore was analyzed by a spectrophotometric method (Johnson and Florence 1971). The chemical analysis of this ore was accomplished and reported in Table 8. The vanadium (V) and phosphorus (P) contents were found to be 0.027\% (as $\text{V}_2\text{O}_5$) and 0.11\% (as $\text{P}_2\text{O}_5$), respectively. Total iron content ($\text{Fe}_i$) of the ore was 2.60\%; of which 1.65\% and 0.95\% was present as ferric ($\text{Fe}^{3+}$) and ferrous iron ($\text{Fe}^{2+}$), respectively. The vanadium, phosphorus and total iron contents of the ore sample was analyzed by atomic absorption spectrophotometry, while the ferrous iron ($\text{Fe}^{2+}$) content was analyzed by a titrimetric method (Jeffery 1975) using potassium dichromate solution (0.05 N $\text{K}_2\text{Cr}_2\text{O}_7$) in the presence of barium diphenylamine sulfonate as indicator.

Table 8

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{U}_3\text{O}_8$</td>
<td>0.023</td>
</tr>
<tr>
<td>$\text{V}_2\text{O}_5$</td>
<td>0.027</td>
</tr>
<tr>
<td>$\text{P}_2\text{O}_5$</td>
<td>0.110</td>
</tr>
<tr>
<td>Iron (Total)</td>
<td>2.60</td>
</tr>
<tr>
<td>Iron (Ferrous)</td>
<td>0.95</td>
</tr>
<tr>
<td>Iron (Ferric)</td>
<td>1.65</td>
</tr>
<tr>
<td>Moisture</td>
<td>3.60</td>
</tr>
</tbody>
</table>
Chemical results of some other metals ions present in the ore sample obtained by atomic absorption spectrophotometry have been reported in Table 9. These results revealed that barium (Ba), stronsium (Sr) and manganese (Mn) were in appreciable amounts in the sandstone uranium ore. Thorium (Th), Yiterbium (Yb) and zirconium (Zr) were present in trace amounts (less than 10 ppm). However, the metals like B, Co, Cr, Cu, Mo, Ni, Pb, Ti, Y, and Zn were present in the concentration range of 10-53 ppm. The amount of sulfur (510 ppm) detected in the ore sample was the main constituent of pyrite (FeS₂) mineral present in it.

<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>Concentration [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barium</td>
<td>Ba</td>
<td>230</td>
</tr>
<tr>
<td>Bismuth</td>
<td>Bi</td>
<td>N.D.</td>
</tr>
<tr>
<td>Boron</td>
<td>B</td>
<td>20</td>
</tr>
<tr>
<td>Chromium</td>
<td>Cr</td>
<td>10</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Co</td>
<td>30</td>
</tr>
<tr>
<td>Copper</td>
<td>Cu</td>
<td>12</td>
</tr>
<tr>
<td>Lead</td>
<td>Pb</td>
<td>18</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mn</td>
<td>320</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Mo</td>
<td>N.D.</td>
</tr>
<tr>
<td>Nickel</td>
<td>Ni</td>
<td>7</td>
</tr>
<tr>
<td>Stronsium</td>
<td>Sr</td>
<td>370</td>
</tr>
<tr>
<td>Sulfur</td>
<td>S</td>
<td>510</td>
</tr>
<tr>
<td>Thorium</td>
<td>Th</td>
<td>4</td>
</tr>
<tr>
<td>Titanium</td>
<td>Ti</td>
<td>10</td>
</tr>
<tr>
<td>Yiterrium</td>
<td>Y</td>
<td>12</td>
</tr>
<tr>
<td>Yiterbium</td>
<td>Yb</td>
<td>2</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zn</td>
<td>53</td>
</tr>
<tr>
<td>Zirconium</td>
<td>Zr</td>
<td>6</td>
</tr>
</tbody>
</table>

N.D. = Not detectable.
Chemical Analysis of Sulfur and Sulfur Slag

Chemical analyses of sulfur and sulfur slag have been accomplished and reported in Table 10. Sulfur and sulfur slag were found to contain 99.6% and 70.4% elemental sulfur ($S^0$) respectively. Total iron was found to be 12.5%; of which 5.3% was present as ferrous iron in sulfur slag (sulfur mud), while no iron content was detected in sulfur. The ash contents of sulfur slag and sulfur were found to be 30.0% and 0.1%, respectively, which indicated the presence of inorganic material.

Table 10

Chemical Analysis of Sulfur and Sulfur Slag on Dry Basis

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfur</td>
<td>$Sulfur$ Slag</td>
</tr>
<tr>
<td>Elemental Sulfur [$S^0$]</td>
<td>99.6</td>
</tr>
<tr>
<td>Total Iron</td>
<td>N.D.</td>
</tr>
<tr>
<td>Ferrous Iron</td>
<td>N.D.</td>
</tr>
<tr>
<td>Ferric Iron</td>
<td>N.D.</td>
</tr>
<tr>
<td>Ash</td>
<td>0.1</td>
</tr>
<tr>
<td>Carbon</td>
<td>0.1</td>
</tr>
</tbody>
</table>

N.D. = Not Detected.
n.d. = not determined.
Chemical and Microbiological Analyses of Tailings Liquid Samples

Chemical analyses of three tailings liquid samples were carried out for uranium content by using a spectrophotometric method (Johnson and Florence 1971). Results obtained are reported in Table 11. The pH-values of these samples were within the range of 2.0-2.9, which contained a small amount of dissolved uranium content varying from 3.1-4.5 mg/L $U_3O_8$.

Table 11
Chemical Analyses of Uranium Tailings Liquid Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
<th>pH</th>
<th>$U_3O_8$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLP-1</td>
<td>In-Process (Fresh)</td>
<td>2.0</td>
<td>4.50</td>
</tr>
<tr>
<td>TLP-2</td>
<td>Out Flow</td>
<td>2.9</td>
<td>3.10</td>
</tr>
<tr>
<td>TLP-3</td>
<td>One month old</td>
<td>2.5</td>
<td>3.61</td>
</tr>
</tbody>
</table>

The presence of Fe$^{2+}$ and Fe$^{3+}$ iron contents in these samples differed from sample to sample as shown in Figure 8. The ferrous iron concentration was much higher (1.485 g/L) in fresh tailings liquid [TLP-1] sample as compared to samples collected from outflow [TLP-2] and one month old [TLP-3] sites of the tailings pond. It was obvious from these results that the ferric iron concentration was increased in tailings samples, TLP-2 (0.75 g/L) and TLP-3 (0.65 g/L) and simultaneously, ferrous iron content was decreased in both of these samples with the passage of time.
Figure 8: Chemical analyses of mill tailings liquid samples for iron contents.
Yellowish-dark brown precipitation of Fe\textsuperscript{3+} was observed at the sampling sites of the tailings pond for samples TLP-2 and TLP-3, which indicated the formation of basic ferric sulphate (jarosite\textsuperscript{3+}) at pH-value of \( \approx 2 \). Therefore, the environmental conditions (pH, and the availability of Fe\textsuperscript{3+} as energy substrate) of the tailings-dam favoured the growth and occurrence of acidophilic *Thiobacillus* iron-oxidizing (*T. ferrooxidans*) bacteria which could be isolated from different sites of tailings pond.

### Table 12

**Microbiological Analysis of Uranium Tailings Liquid Samples**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Microbial Population (cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>T. ferrooxidans</em> (I)</td>
</tr>
<tr>
<td>TLP-1</td>
<td>(3.1 \times 10^3)</td>
</tr>
<tr>
<td>TLP-2</td>
<td>(9.2 \times 10^1)</td>
</tr>
<tr>
<td>TLP-3</td>
<td>(8.0 \times 10^2)</td>
</tr>
</tbody>
</table>

*cfu* stands for colony forming unit.

(I) = Gelrite-FeSO\textsubscript{4} medium (Khalid et al. 1993).

(II) = Agarose-S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} medium.

(III) = Agarose-Glucose medium.

N.D. = Not detectable.
The indigenous microflora of iron-oxidizing bacteria (*T. ferrooxidans*) present in tailings liquid showed a slight variation in their microbial population on solid Geırite-FeSO₄ medium (Khalid et al. 1993). In tailings liquid samples, the microbial population (cfu/mL) of *T. ferrooxidans* was 3.1 X 10⁶, 9.2 X 10⁶, and 8.0 X 10⁵ in TLP-1, TLP-2 and TLP-3 samples, respectively. The number of iron-oxidizing bacteria were relatively greater in tailings liquid samples TLP-1 (3.1 X 10⁶ cfu/mL), and TLP-2 (9.2 X 10⁶ cfu/mL); which were collected from the in-process (fresh) and outlet-flow sites of the ore-processing unit, respectively. The presence of Fe²⁺ iron as energy source for iron-oxidizing bacteria was available in tailings liquid samples of TLP-1 and TLP-2 as compared to TLP-3 (Figure 8). Hence, the microbial population of *T. ferrooxidans* was less in tailings liquid sample (TLP-3) collected from one-month old tailings site of Tailings Dam as compared to TLP-1 and TLP-2 samples (Table 12).

The isolation and enumeration of sulfur-oxidizers (*T. thiooxidans*) and glucose-oxidizing heterotrophs were carried out growing onto solid Agarose-S₂O₃²⁻ and Agarose-Glucose media, respectively, in these samples. The growth of sulfur-oxidizers and heterotrophs were observed in TLP-2 and TLP-3 samples (Table 12). The microbial population of sulfur-oxidizing bacteria was slightly greater in number in TLP-2 (2.5 X 10⁵ cfu/mL) as compared to TLP-3 (1.2 X 10⁵ cfu/mL). But the microbial population of glucose-oxidizers was much greater in TLP-3 (4.0 X 10⁵ cfu/mL) as compared to TLP-2 (3.5 X 10⁴ cfu/mL), which might be due to the availability of carbon source present in the form of dead biomass.

The growth of some filamentous fungi was also detected in tailings liquid samples (TLP-2 and TLP-3), when grown on agarose-glucose medium but their identification was not undertaken.
Physico-Chemical and Microbiological Analyses of Mill Tailings Residues

Physical, chemical and microbiological analyses of uranium mill tailings residue samples collected from various sites of the tailings pond have been reported in Table 13. Chemical analyses data revealed that uranium concentration was higher (0.0049% \( \text{U}_3\text{O}_8 \)) in fresh dried tailings residue sample designated as TS-1, as compared with other residue samples (Table 13). The tailings residue sample used in shake flask and column bioleaching experiments had an average uranium content of 0.0025% \( \text{U}_3\text{O}_8 \) (25.0 ppm \( \text{U}_3\text{O}_8 \)) on dry matter basis. The physical appearance of this residue was light grey similar to sandstone uranium ore. The pH-values of TS-2, TS-3 and TS-4 tailings residue samples were relatively acidic as compared to TS-1 (fresh dried of pH 6.1), which favoured the growth of acidophilic Thiobacillus bacteria. The drop in pH-values of the solid samples was might be due to acid production (\( \text{H}_2\text{SO}_4 \)) by the metabolic activity of these bacteria.

The microbial population of iron- and sulfur-oxidizers (\( T. \text{ferrooxidans} \)) were relatively much higher in the tailings solid samples TS-2 and TS-3, which were collected from one-month and two-months old dried tailings residue pile as compared to those of TS-1 and TS-4 residue samples, which were collected from fresh and four-months old dried tailings piles, respectively.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Description of Sample</th>
<th>Physical Appearance</th>
<th>pH of Solid Slurry</th>
<th>$U_3O_8$ (%)</th>
<th>Iron-Oxidizing Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS-1</td>
<td>Fresh dried</td>
<td>Grey</td>
<td>6.1</td>
<td>0.0049</td>
<td>+</td>
</tr>
<tr>
<td>TS-2</td>
<td>One month old dried</td>
<td>Grey</td>
<td>4.8</td>
<td>0.0034</td>
<td>+++</td>
</tr>
<tr>
<td>TS-3</td>
<td>Two months old dried</td>
<td>Grey</td>
<td>3.2</td>
<td>0.0017</td>
<td>+++</td>
</tr>
<tr>
<td>TS-4</td>
<td>Four months old dried</td>
<td>Yellowish brown</td>
<td>2.9</td>
<td>0.0019</td>
<td>++</td>
</tr>
</tbody>
</table>

+ = Sparse growth; ++ = Intermediate growth; +++ = Heavy growth
Chemical and Microbiological Analyses of Uranium Mine Water Samples

Chemical analyses of uranium mine water samples collected from various mine sites at Baghalchur were conducted for K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), SO\(_4^{2-}\), PO\(_4^{3-}\) etc., which are usual ingredients of 9K mineral salts medium (Lundgren and Silverman 1959), described for the growth and isolation of \textit{T. ferrooxidans} and the results thus obtained have been reported in Table 14. The pH-values of all mine water samples were neutral to slightly alkaline in nature and found in the pH range of 6.9-8.0. The cations, such as Na\(^+\) and Ca\(^{2+}\) were present in appreciable amounts; whereas K\(^+\) and Mg\(^{2+}\) cations were present relatively less amounts as compared to the ingredients of 9K mineral salts medium in all mine water samples. The anions, such as SO\(_4^{2-}\) and PO\(_4^{3-}\) were also determined in these samples. Phosphate ion was present in a very minute quantity in all samples. The dissolved uranium contents were detected in very small amounts (0.71 to 2.24 mg/L \text{U}_3\text{O}_8) in all these mine water samples.

Table 14

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>pH</th>
<th>\text{U}_3\text{O}_8 (mg/ L)</th>
<th>Ca(^{2+})</th>
<th>SO(_4^{2-})</th>
<th>K(^+)</th>
<th>Na(^+)</th>
<th>PO(_4^{3-})</th>
<th>Mg(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW-1</td>
<td>B/Chur South, Incline 3</td>
<td>7.4</td>
<td>1.70</td>
<td>27.8</td>
<td>438</td>
<td>7.7</td>
<td>195</td>
<td>N.D.</td>
<td>15.5</td>
</tr>
<tr>
<td>MW-2</td>
<td>B/Chur South, D4A3</td>
<td>7.8</td>
<td>0.71</td>
<td>16.0</td>
<td>340</td>
<td>6.8</td>
<td>204</td>
<td>N.D.</td>
<td>8.2</td>
</tr>
<tr>
<td>MW-3</td>
<td>B/Chur South, Incline 4</td>
<td>7.8</td>
<td>0.91</td>
<td>15.3</td>
<td>268</td>
<td>5.9</td>
<td>198</td>
<td>0.1</td>
<td>7.3</td>
</tr>
<tr>
<td>MW-4</td>
<td>B/Chur South, Incline 5</td>
<td>8.0</td>
<td>1.60</td>
<td>12.6</td>
<td>168</td>
<td>6.8</td>
<td>158</td>
<td>0.3</td>
<td>9.4</td>
</tr>
<tr>
<td>MW-5</td>
<td>B/Chur North, GD3</td>
<td>6.9</td>
<td>1.50</td>
<td>22.7</td>
<td>620</td>
<td>7.2</td>
<td>236</td>
<td>N.D.</td>
<td>16.8</td>
</tr>
<tr>
<td>MW-6</td>
<td>B/Chur North, Incline 2</td>
<td>7.7</td>
<td>2.24</td>
<td>17.5</td>
<td>320</td>
<td>7.6</td>
<td>177</td>
<td>0.5</td>
<td>11.7</td>
</tr>
</tbody>
</table>

9K Standard Medium

<table>
<thead>
<tr>
<th>pH</th>
<th>\text{U}_3\text{O}_8 (mg/ L)</th>
<th>Ca(^{2+})</th>
<th>SO(_4^{2-})</th>
<th>K(^+)</th>
<th>Na(^+)</th>
<th>PO(_4^{3-})</th>
<th>Mg(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>-</td>
<td>2.0</td>
<td>17,714</td>
<td>270</td>
<td>-</td>
<td>273</td>
<td>49.0</td>
</tr>
</tbody>
</table>

N.D. = Not detected.
The presence of indigenous microflora of acidophilic iron-oxidizing bacteria (*T. ferrooxidans*) in mine water samples was in the range of 2.0-6.0 cfu/mL (Table 15). While growing on Gelrite-FeSO₄ medium (Khalid et al. 1993). But no sulfur-oxidizing (*T. thiooxidans*) and glucose-oxidizing heterotrophs were observed in these mine water samples, when solid Agarose-S₂O₇²⁻ and Agarose-Glucose media were employed for their growth and isolation, respectively. However, the presence of sulfur-grown bacteria were observed on solid Agar-S₂O₇²⁻ medium of pH 6.60, which was described for the isolation and growth of *T. thiooxidans*.

**Table 15**

Microbiological Analyses of Uranium Mine Water Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Growth on solid media (cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gelrite-FeSO₄</td>
</tr>
<tr>
<td></td>
<td>No. Colony Morphology</td>
</tr>
<tr>
<td>MW-1</td>
<td>4 Yellowish brown, circular</td>
</tr>
<tr>
<td></td>
<td>shape and small size</td>
</tr>
<tr>
<td>MW-2</td>
<td>N.D. -</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>MW-3</td>
<td>6 Yellowish brown, circular</td>
</tr>
<tr>
<td></td>
<td>shape and medium size</td>
</tr>
<tr>
<td>MW-4</td>
<td>N.D. -</td>
</tr>
<tr>
<td>MW-5</td>
<td>2 Dark brown in centre with</td>
</tr>
<tr>
<td></td>
<td>outer yellowish ring</td>
</tr>
<tr>
<td>MW-6</td>
<td>5 Dark brown, circular shape,</td>
</tr>
<tr>
<td></td>
<td>and small size</td>
</tr>
</tbody>
</table>

N.D. = Not detectable
Isolation and Characterization of Acidophilic Iron- and Sulfur-Oxidizing *Thiobacillus* Bacteria

**Isolation of Iron- and Sulfur-Oxidizer (*Thiobacillus ferrooxidans*)**

For isolation of acidophilic iron- and sulfur-oxidizing (*T. ferrooxidans*) bacteria from tailings liquid sample (TLP-2), an appropriate amount (100 μL) of liquid sample was inoculated onto solid Gelrite-FeSO₄ medium (Khalid et al. 1993). After 5-7 days of incubation at 30°C, reddish-brown colonies of iron-oxidizing bacteria were developed on the plates. These reddish-brown colonies growing on Gelrite-FeSO₄ plates were divided into two types depending upon their morphological characteristics (Figure 9). One type strain of *T. ferrooxidans* (TFe-1) showed relatively large and irregular reddish-brown colonies, and the other type strain of *T. ferrooxidans* (TFe-2) exhibited minute and circular reddish-brown colonies. Each type of single colony was picked and cultivated separately into liquid iron medium (9KFe²⁺) as described earlier. After 3-5 days of incubation at 30°C under shaking condition, the medium became reddish-brown due to bacterial oxidation of Fe²⁺ to Fe³⁺. The harvested cells of iron-oxidizing bacteria were inoculated onto solid Gelrite-FeSO₄ plates. Such ordinary purification procedures were repeated several times, finally pure cultures of the two-types were obtained. Colonies of isolated strain of *T. ferrooxidans* ([TFe-1] on Gelrite-FeSO₄ plate were large in size (1.0-4.0 mm) and irregular (auriculate with unequal projections), while the colonies of *T. ferrooxidans* (TFe-2) were very minute (0.2-0.7 mm), circular, and entire (Figure 10).

The reddish-brown colonies of isolated strain of *T. ferrooxidans* (TFe-2) were morphologically similar to those of axenic strain of *T. ferrooxidans* (ATCC 13661), when growing on solid Gelrite-FeSO₄ medium (Figure 11).
Figure 9: Reddish-brown colonies of indigenous strains of iron-oxidizing bacteria growing on Gelrite-FeSO$_4$ medium isolated from tailings liquid samples.
Figure 10: Colonies of iron-grown (9KFe$^{2+}$) indigenous strain of iron-oxidizing bacteria onto Gelrite-FeSO$_4$ medium. (A) *T. ferrooxidans* (TFe-1) and (B) *T. ferrooxidans* (TFe-2).
Figure 11: Colonies of isolated and standard strains of *T. ferrooxidans* on FeSO₄-Gelrite medium. (A) *T. ferrooxidans* (TFe-2) and (B) *T. ferrooxidans* (ATCC 13661).
Isolation of Sulfur-Oxidizer (*Thiobacillus thiooxidans*)

For the isolation of acidophilic sulfur-oxidizing bacteria (*T. thiooxidans*) from tailings liquid sample (TLP-2), an appropriate amount (100 μL) of liquid sample was streaked onto solid Agarose-S$_2$O$_7^{2-}$ medium. Two types of colonies were observed after 12-15 days of incubation at 30°C (Figure 12). Of which, one-type (TTh-1) of colonies were off-white, circular in shape and relatively medium in size, which became pale-yellow in color after 3-4 weeks of incubation. A characteristics smell of elemental sulfur (S⁰) was observed from these colonies. The other type (TTh-2) of colonies were milky white, circular shape and relatively small in size. Each type of single colony was picked and inoculated separately into liquid sulfur (9KSm⁰) and glucose media for further screening. The flasks were incubated on a shaking incubator at 30°C. After 5-7 days of incubation, the sulfur medium became turbid and milky white exhibiting the growth of microbial populations by both strains designated as TTh-1 and TTh-2. No heterotrophic growth of sulfur-oxidizing strain (TTh-1) was observed in liquid glucose medium. The growth of TTh-2 strain was positive in liquid glucose medium. Further purification and characterization of strain TTh-2 was not undertaken.

The harvested cells of sulfur-oxidizing strain (TTh-1) were inoculated onto solid Agarose-S$_2$O$_7^{2-}$ medium to isolate pure strain of sulfur-oxidizing bacteria (*T. thiooxidans*). Pale-yellow colonies of sulfur-oxidizing bacteria (*T. thiooxidans*) were observed after two-weeks of incubation time at 30°C (Figure 13). Finally, pure colonies of *T. thiooxidans* strain was obtained. These colonies were minute in size, circular in shape, entire, convex or pulvinate, smooth and glistening. The pale yellow colonies were easily dissolved in CS$_2$, indicating the formation of elemental sulfur (S⁰) crystals as intermediate product resulting from the microbial oxidation of thiosulfate. Single colonies were picked and inoculated into liquid iron (9KFe$^{2+}$), tetrathionate, and sulfur. No growth was occured in iron liquid and Gelrite-FeSO$_4$ solid media.
Figure 12: Colonies of indigenous strain of *T. thiooxidans* on Agarose-\(S_2O_3^{2-}\) medium. (A) Colonies after 2-weeks of incubation and (B) Colonies after 4-weeks of incubation showing the formation of elemental sulfur as intermediate product of thiosulfate oxidation.
Figure 13: Colonies of indigenous and axenic strains of *T. thiooxidans* on Agarose-\(S_2O_3^{2-}\) medium after 2-weeks of incubation. (A) *T. thiooxidans* (TTh-1) and (B) *T. thiooxidans* (ATCC 8085)
Colonies of TTh-1 were morphologically similar to those of the axenic strain of *T. thiooxidans* (ATCC 8085) [Figure 12]. *T. thiooxidans* (TTh-1) was morphologically and physiologically very similar to *T. ferrooxidans* (TFe-2) in all respects except its lack of ability to oxidize Fe²⁺ and pyrite.

**Cell Morphology and Characterization of Isolated Strains**

The phase-contrast microscopic observations of isolated strains of *T. ferrooxidans* (TFe-1 and TFe-2) and *T. thiooxidans* (TTh-1) revealed that these strains were Gram-negative, motile, and single rod-shaped bacteria. *T. ferrooxidans* (TFe-2) oxidized Fe²⁺ to Fe³⁺, pyrite, sulfur and reduced sulfur compounds like thiosulfate and tetrathionate. The bacterial oxidation of pyrite, sulfur and reduced sulfur compounds produced sulfuric acid which followed a drop in initial pH-value of the medium. *T. thiooxidans* (TTh-1) oxidized only sulfur and reduced sulfur compounds to sulfuric acid through its metabolic activity. The oxidation of various energy substrates by indigenous and standard strains of *T. ferrooxidans* and *T. thiooxidans* have been cited in Table 16.

<table>
<thead>
<tr>
<th>Table 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth of Acidophilic <em>Thiobacillus</em> Bacteria on Different Energy Substrates</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Growth on substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S⁰</td>
</tr>
<tr>
<td><em>T. ferrooxidans</em> (TFe-1)</td>
<td>+</td>
</tr>
<tr>
<td><em>T. ferrooxidans</em> (TFe-2)</td>
<td>+</td>
</tr>
<tr>
<td><em>T. ferrooxidans</em> (ATCC 13661)</td>
<td>+</td>
</tr>
<tr>
<td><em>T. thiooxidans</em> (TTh-1)</td>
<td>+</td>
</tr>
<tr>
<td><em>T. thiooxidans</em> (ATCC 8085)</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Growth observed
- = No growth observed.
Growth Studies of *Thiobacillus* Bacteria

Oxidation of Ferrous Iron (Fe$^{2+}$) by *T. ferrooxidans*

The ferrous iron oxidation by isolated strain of *T. ferrooxidans* (TFe-2) and standard strain of *T. ferrooxidans* (ATCC 13361) was conducted in shake flasks containing iron liquid medium (9KFe$^{2+}$) containing 160 mM FeSO$_4$ of pH-value of 2.0 (Silverman and Lundgren 1959). It was observed that ferrous iron (Fe$^{2+}$) was completely oxidized to ferric iron (Fe$^{3+}$) by both strains of *T. ferrooxidans* during 60-hrs of incubation time at 30°C and 100 rpm (Figure 14). In chemical control flasks, only a negligible amount of ferrous iron was oxidized due to air-oxidation under same experimental conditions.

Figure 14: Oxidation of ferrous iron (Fe$^{2+}$) by *T. ferrooxidans* (TFe-2) and *T. ferrooxidans* (ATCC 13661).
Oxidation of Pyrite (FeS₂) by *T. ferrooxidans*

The oxidation of pyrite by isolated strain of *T. ferrooxidans* (TFe-2) and axenic strain of *T. ferrooxidans* (ATCC 13361) was conducted in shake flasks containing pyrite medium as described earlier. Results revealed that iron was released from pyrite both by chemical and bacterial oxidation process (Figure 15a). The release of iron (Feᵢ) from pyrite was much more pronounced (about 3-folds) by inoculated system as compared to chemical control. It was observed that 14.66 g/L Feᵢ (total iron) was released from the bacterial oxidation of pyrite by indigenous strain of *T. ferrooxidans* (TFe-2) in 20-days of incubation at 30°C and 100 rpm. The release of total iron from pyrite was relatively higher (16.0 g/L Feᵢ) in standard strain of *T. ferrooxidans* (ATCC 13361) as compared to the locally isolated strain of *T. ferrooxidans* (TFe-2). In chemical sterile control sample, only 5.15 g/L Feᵢ was dissolved in the leach suspension.

A significant change in the initial pH-value of the inoculated medium was indicative during bacterial oxidation of pyrite. The pH was dropped from 2.0 to 1.0 due to sulfuric acid production from pyrite oxidation by *T. ferrooxidans* strains (Figure 15b).
Figure 15: Oxidation of pyrite by isolated strain of *T. ferrooxidans* (TFe-2) and axenic strain of *T. ferrooxidans* (ATCC 13661). (A) Release of iron (Fe$_{\text{r}}$) during pyrite oxidation (B) Change in initial pH-value of the medium.
Oxidation of Elemental Sulfur By *T. ferrooxidans* and *T. thiooxidans*

Both isolated strains of *T. ferrooxidans* (TFe-7) and *T. thiooxidans* (TTh-1) produced 10.80 and 13.52 g/L H$_2$SO$_4$, respectively, from bacterial oxidation of elemental sulfur in 20-days of incubation at 30°C and 100 rpm (Figure 16a). A mixed culture of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) produced 18.60 g/L H$_2$SO$_4$ from sulfur oxidation under the same experimental conditions. In sterile control samples, the concentration of sulfuric acid was determined as 0.74 g/L H$_2$SO$_4$, which was in fact, the amount of sulfuric acid used to adjust initial pH-value 2.50 of the liquid sulfur medium [9KS$^0$] (Figure 16b).

In inoculated systems, there was a drastic change in initial pH-value of the liquid sulfur media as a result of sulfuric acid production from bacterial oxidation of sulfur. The initial pH-value of medium inoculated with *T. ferrooxidans* (TFe-2) was changed from 2.50 to 1.0 in 20-days of incubation (Figure 16b). A mixed culture of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) dropped the pH of the medium from 2.5 to 0.68.
Figure 16: Oxidation of elemental sulfur by isolated strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1). (A) Production of sulfuric acid (B) Change in pH-value.
Oxidation of Sulfur Slag By *T. ferrooxidans* and *T. thiooxidans*

The bacterial oxidation of sulfur slag by isolated strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) produced 20.8 and 22.0 g/L H₂SO₄ respectively, in 20-days of incubation at 30±2.0°C and 100 rpm (Figure 17a). Similarly, a mixed culture of these acidophilic bacteria produced 27.0 g/L H₂SO₄ from bacterial oxidation of sulfur slag under same experimental conditions. Similar trend in the change of initial pH-values of the inoculated media were observed as resulted in bacterial sulfur oxidation experiments. The initial pH-value of the medium inoculated with *T. ferrooxidans* (TFe-2) was dropped from 2.50 to 1.0; whereas the pH-value of medium inoculated with *T. thiooxidans* (TTh-1) showed 0.70 as a consequence of acid production (Figure 17b). Similarly, the initial pH-value of the medium inoculated with a mixed culture of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) was dropped from 2.5 to 0.62. However, in case of sterile control samples, the pH-value of the medium remained almost constant throughout these experimental studies, because no acid was produced by this system.

Growth studies of isolated and axenic strains of *T. thiooxidans* was also conducted in shake flasks on liquid sulfur and slag media at 30°C and 100 rpm under shaking conditions. The indigenous strain of *T. thiooxidans* showed a comparable sulfuric acid production to the axenic strain of *T. thiooxidans* (ATCC 8085) (Figure 18).

A greater amount of sulfuric acid was produced from the oxidation of sulfur slag as compared to media supplemented with elemental sulfur by strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) (Figure 19).
Figure 17: Oxidation of sulfur slag by isolated strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1). (A) Sulfuric acid production (B) Change in pH-value of the medium.
Figure 18: A comparison of sulfuric acid produced from bacterial oxidation of sulfur and sulfur salg by isolated strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1).
When the growth studies of actively S'-grown strains of *T. thiooxidans* (TTh-1) and *T. thiooxidans* (ATCC 8085) were conducted in shake flasks containing liquid sulfur medium (9KS")}, it was observed that the indigenous strain of *T. thiooxidans* (TTh-1) showed a comparable results of sulfuric acid production (Figure 19) from the bacterial oxidation of elemental sulfur with the axenic strain of *T. thiooxidans* (ATCC 8085). *T. thiooxidans* (TTh-1) produced 39.80 g/L H₂SO₄ with a drop in initial pH-value of 2.50 to 0.68 in 30-days incubation; whereas 42.60 g/L H₂SO₄ was produced from sulfur oxidation by *T. thiooxidans* (ATCC 8085).

**Figure 19:** A comparison of sulfuric acid production from the oxidation of sulfur by indigenous and axenic strains of *T. thiooxidans*. 
The optical density curves of S²-free cell suspensions of *T. thiooxidans* (TTh-1) and *T. thiooxidans* (ATCC 8085) measured at 410 nm using Shimadzu UV/VIS 120-02 spectrophotometer have been presented in Figure 20. *T. thiooxidans* (TTh-1) showed a comparable growth pattern with the axenic strain of *T. thiooxidans* (ATCC 8085).

**Figure 20:** Optical density showing the growth of *T. thiooxidans* (TTh-1) and *T. thiooxidans* (ATCC 8085) on sulfur liquid medium.
Optimization of Leaching Parameters for Bioleaching of Sandstone Uranium Ore

Shake Flask Studies

In leaching experiments performed in shake flasks, the isolated strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) solubilized 91.0% and 88.0% U$_3$O$_8$, respectively, from low-grade sandstone uranium ore (0.023% U$_3$O$_8$) in 20-days of incubation at 30°C and 100 rpm (Figure 21a). Sandstone ore (10g) was added into 250-ml Erlenmeyer flasks containing 7-days sulfur grown culture of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) in order to neutralize the calcite (CaCO$_3$) content of ore matrix by sulfuric acid produced from bacterial oxidation of sulfur. The pH-values of leach suspensions increased due to the presence of calcite content of the sandstone uranium ore. The change in pH-values for different leaching experiments after inoculation with *T. ferrooxidans* and *T. thiooxidans* (Figure 21b).

In both inoculated systems, the initial pH-value of the medium that was inoculated with *T. ferrooxidans* (TFe-2) was dropped from 2.5 to 1.5 due to sulfuric acid produced from sulfur oxidation after 7-days of incubation. As the ore was added into these flasks, the calcite content of the ore matrix was neutralized by the sulfuric acid to some extent. Therefore, the pH-value of the leach suspension was increased from 1.5 to 4.30 in inoculated system of *T. ferrooxidans*. However, the pH-value again dropped from 4.3 to 1.3 during 30-days of incubation, as bacteria produced more acid from sulfur oxidation (Figure 21b). This drop in the pH-value of the inoculated system resulted a uranium recovery of 91.0% U$_3$O$_8$ from sandstone ore (Figure 21a).

Similarly, the isolated strain of *T. thiooxidans* (TTh-1) solubilized 88.0% U$_3$O$_8$ from sandstone uranium ore under identical experimental conditions. In this case, when sandstone ore was added into flasks containing 7-days pre-grown culture of *T. thiooxidans* (TTh-1), the pH-values was raised from 1.5 to 3.7 due to chemical reaction between calcite content of the ore matrix and sulfuric acid produced from
Figure 21: Solubilization of $U_3O_8$ from Baghalchur sandstone uranium ore by indigenous microflora of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTTh-1). (A) Uranium recovery ($\%U_3O_8$) and (B) pH-profile.
bacterial oxidation of sulfur (Figure 21b). As the bacteria started to produce sulfuric acid from oxidation of sulfur through their metabolic activity, the pH of the bacterial leach liquor was again dropped from 3.7 to 1.10 in 30-days of incubation. This drop in pH of the medium resulted in uranium solubilization from the ore. In both inoculated systems, uranium recovery of more than 80.0% U₃O₈ was obtained from ore by *T. ferrooxidans* and *T. thiooxidans* during 20-days of incubation. Uranium concentration in the bacterial leach liquor samples was 140-210 mg/L U₃O₈ after 20-days of sampling. In sterile control sample, when the ore was added into flasks, the initial pH-value 2.5 was increased to 6.8 due to calcite content of the ore and remained as such throughout the experiment. Uranium recovery of 2.0% U₃O₈ was obtained in this case under the same experimental conditions of temperature and shaking.

In these shake flask studies, a sufficient amount of un-consumed sulfuric acid was also detected as free acid in the presence of hydrolyzable cations in bacterial leach liquor samples [Figure 22a]. Therefore, the leach suspensions of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) indicated the presence of 10.4 and 13.0 g/L H₂SO₄, respectively, after 30-days of incubation. No free acid was detected in sterile control system.

During bioleaching of sandstone uranium ore, a significant amount of iron (Fe₃) was also solubilized by the chemical reaction between H₂SO₄ and iron minerals like hematite (Fe₂O₃), magnetite (Fe₃O₄) and pyrite (FeS₂) etc., present in the ore matrix (Figure 22b).

The presence of bacteria in the inoculated flasks and their absence in the uninoculated ones was confirmed by microscopic examination of ore slurries and leach liquors daily and by culturing 1.0-mL sample of last ore-suspensions into separate flasks containing each 10-mL of liquid sulfur [9KS⁰] and iron [9KFe²⁺] media.
Figure: 22: Free acid production and iron solubilization during bioreaching of uranium ore by *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1). (A) free acid and (B) iron [Fe₆] solubilization.
The indigenous strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) exhibited leaching data of uranium recovery that was comparable to those of axenic strains of *T. ferrooxidans* (ATCC 13661) and *T. thiooxidans* (ATCC 8085) (Table 17). The axenic strains of *T. ferrooxidans* (ATCC 13661) and *T. thiooxidans* (ATCC 8085) solubilized 89.48% and 91.17% U₃O₈, respectively, from sandstone uranium ore (10% ore pulp density) during 30-days of incubation at 30±2.0°C and 100 rpm. Similarly, a uranium recovery of 86.00% and 86.52% U₃O₈ was obtained by *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) from sandstone ore, respectively, under the same experimental conditions. Uranium concentration in the bacterial leach liquor samples was in the range of 105.80-117.70 mg/L U₃O₈. In chemical control samples, only 1.67% U₃O₈ solubilized from sandstone uranium ore.

Table 17

<table>
<thead>
<tr>
<th>Strains</th>
<th>Physical Appearance of Leach Liquor</th>
<th>pH</th>
<th>U₃O₈ (mg/L)</th>
<th>U₃O₈ Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Colorless</td>
<td>7.40</td>
<td>0.77</td>
<td>1.67</td>
</tr>
<tr>
<td><em>T. thiooxidans</em> (TTh-1)</td>
<td>Light green</td>
<td>1.60</td>
<td>107.70</td>
<td>86.52</td>
</tr>
<tr>
<td><em>T. thiooxidans</em> (ATCC 8085)</td>
<td>Light green</td>
<td>1.30</td>
<td>117.70</td>
<td>91.17</td>
</tr>
<tr>
<td><em>T. ferrooxidans</em> (TFe-2)</td>
<td>Light green</td>
<td>1.70</td>
<td>105.80</td>
<td>86.00</td>
</tr>
<tr>
<td><em>T. ferrooxidans</em> (ATCC 13661)</td>
<td>Light green</td>
<td>1.40</td>
<td>113.30</td>
<td>89.48</td>
</tr>
</tbody>
</table>

N.D. = Not detected.

In sterile control samples, not more than 2.0% U₃O₈ was solubilized under the same leaching conditions. In chemical control samples, the pH-values of the media were found to raise from an initial pH-values of 2.5 to 7.60; as the sandstone uranium ore was added into the medium (Figure 23a,b).
Figure 23: pH-profiles of bioleaching of sandstone uranium ore by indigenous and standard strains of *T. ferrooxidans* and *T. thiooxidans*. (A) pH-profiles of *T. ferrooxidans* strains (B) pH-profiles of *T. thiooxidans* strains.
Effect of External Energy Substrates

In batch tests, the effect of different substrates like elemental sulfur, sulfur slag and pyrite (a low-grade sulfidic ore containing pyrite) on uranium recovery from sandstone ore was carried out by indigenous strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1). An ore pulp density of 10.0% (wt/vol) was added into these flasks and incubated at 30±2.0°C and 100 rpm. Results revealed that 86.7%, 92.0%, and 84.3% U₃O₈ was solubilized from sandstone ore amended with elemental sulfur, sulfur slag and pyrite, respectively, during 30-days of incubation by mixed culture of *T. thiooxidans* and *T. ferrooxidans* (Figure 24). A higher uranium recovery (92% U₃O₈) was obtained from sandstone ore amended with sulfur slag as compared with elemental sulfur and pyrite. It was due to the presence of iron (Fe²⁺ and Fe³⁺) in sulfur slag, which catalyzed the chemical reaction for uranium solubilization from ore.

![Graph showing uranium recovery (%) for different strains](image)

**Figure 24:** Effect of different external energy substrates (sulfur, slag and pyrite) on uranium solubilization from sandstone uranium ore.
Effect of Inoculum Size

Studies on the effect of different inoculum size (1.0-10.0% vol/vol: containing 8.0±0.5 X 10^6 cells/mL) of a mixed culture of T. ferrooxidans (TFe-2) and T. thiooxidans (TTh-1) on uranium solubilization from sandstone ore were carried out in shake flasks. An ore pulp density of 10.0% (wt/vol) was used for these bioremediation studies. All flasks were prepared in triplicate, including abiotic controls, and were incubated on an orbital shaking incubator at 30.0±2.0°C and 100 rpm for 20-days of incubation. Results revealed that the inoculum size influenced the rate of uranium recovery from ore. Therefore, a uranium recovery of 35.2% U_3O_8 was obtained from the ore inoculated with 1.0% (vol/vol) inoculum size of (Figure 25). However, the uranium recovery of 82.7-88.9% U_3O_8 was solubilized from ore suspensions inoculated with 2.0-10.0% (vol/vol) inoculum size of these strains.

![Figure 25: Uranium solubilization at different inoculum sizes of a mixed culture of indigenous strains of T. ferrooxidans (TFe-2) and T. thiooxidans (TTh-1).](image-url)
Uranium Leaching Tests with Sulfuric Acid

In the microbial leaching tests, the leaching of uranium was apparently associated with the production of sulfuric acid from the oxidation of sulfur and/or sulfur slag by *T. ferrooxidans* and *T. thiooxidans*. Therefore, batch tests were carried out to determine the amount of acid that could solubilized maximum uranium from the ore. Maximum uranium solubilization (80.0% $\text{U}_3\text{O}_8$) was obtained from the acid leaching test containing 130.0 kg $\text{H}_2\text{SO}_4$/ton of sandstone ore during 4-hrs of leaching time (Figure 26). The concentration of uranium in the acid leach liquor was 434.62 mg/L $\text{U}_3\text{O}_8$ at pH-value of 1.52.

![Graph showing uranium solubilization vs. sulfuric acid consumption](image)

**Figure 26**: Chemical leaching of sandstone uranium ore at different concentrations of sulfuric acid.
The physical appearance of acid leach liquors was colorless for acid concentrations of 0-60 kg H₂SO₄ ton of ore and light green in the acid concentrations more than 60 kg/ton of ore (Table 18). The pH-values of the acid leach liquors were found more acidic with increase of sulfuric acid concentration. The optimal pH-value for maximum uranium recovery from sandstone ore was 1.70 to 1.90.

Table 18

Sulfuric Acid Leaching of Sandstone Uranium ore

Wt of sandstone uranium ore = 100 gm
Volume of water taken = 100 mL
Leaching time = 4.0 hr at 70 rpm
Room temperature = 32.5°C

<table>
<thead>
<tr>
<th>Sulfuric Acid Consumption (Kg/ton of ore)</th>
<th>Physical appearance of Leach Liquor</th>
<th>pH</th>
<th>U₃O₈ (mg/L)</th>
<th>U₃O₈ (% Recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Colorless</td>
<td>7.60</td>
<td>1.50</td>
<td>0.27</td>
</tr>
<tr>
<td>10</td>
<td>Colorless</td>
<td>6.90</td>
<td>4.90</td>
<td>0.90</td>
</tr>
<tr>
<td>20</td>
<td>Colorless</td>
<td>4.50</td>
<td>35.13</td>
<td>6.40</td>
</tr>
<tr>
<td>40</td>
<td>Colorless</td>
<td>2.38</td>
<td>101.80</td>
<td>18.50</td>
</tr>
<tr>
<td>60</td>
<td>Colorless</td>
<td>2.21</td>
<td>112.10</td>
<td>20.40</td>
</tr>
<tr>
<td>80</td>
<td>Light green</td>
<td>2.10</td>
<td>152.60</td>
<td>27.75</td>
</tr>
<tr>
<td>100</td>
<td>Light green</td>
<td>1.80</td>
<td>324.40</td>
<td>59.00</td>
</tr>
<tr>
<td>120</td>
<td>Light green</td>
<td>1.70</td>
<td>364.10</td>
<td>66.20</td>
</tr>
<tr>
<td>130</td>
<td>Light green</td>
<td>1.52</td>
<td>434.62</td>
<td>80.00</td>
</tr>
<tr>
<td>140</td>
<td>Light green</td>
<td>1.41</td>
<td>387.20</td>
<td>70.40</td>
</tr>
<tr>
<td>160</td>
<td>Light green</td>
<td>1.35</td>
<td>387.00</td>
<td>70.00</td>
</tr>
<tr>
<td>180</td>
<td>Light green</td>
<td>1.32</td>
<td>423.10</td>
<td>76.93</td>
</tr>
<tr>
<td>200</td>
<td>Light green</td>
<td>1.28</td>
<td>415.40</td>
<td>75.53</td>
</tr>
</tbody>
</table>
Sulfuric acid leaching of sandstone uranium ore was an exothermic reaction in which the temperature of leaching system was raised from 32.5°C to 48.0°C with the addition of different concentrations of sulfuric acid (Figure 27). The rise in temperature of the leaching system was directly proportional to the acid concentration.

Figure 27: Temperature profile of sulfuric acid leaching of sandstone uranium ore.
An experiment was carried out to determine the amount of sulfuric acid required to adjust the pH-value 2.50±0.10 of the ore suspension (10.0% wt/vol ore pulp density) being used for microbiological leaching of sandstone uranium ore. Results of the batch tests revealed that an acid volume of 13.0±0.10 mL of 1.0 N H$_2$SO$_4$ was required to adjust the pH-value of 2.50±0.10 of the leaching system containing 10.0g of sandstone uranium ore containing 3.50% calcite. This acid volume corresponds to 3.61±0.1 mL 36 N H$_2$SO$_4/ 100g$ of the ore.

Effect of Different Proportions of Sulfur and Sulfur Slag

The effect of different proportions of elemental sulfur to low-grade sandstone ore was carried out in shake flasks to determine the optimum [S$^{6-}$/ore] ratio for uranium recovery by indigenous strains of T. ferrooxidans (TFe-2) and T. thiooxidans (TTh-1). Uranium ore [100g] was added into 500-ml Erlenmyer flasks (containing 1-10g of elemental sulfur in 9K mineral salts medium without ferrous iron) of 7-days grown mixed culture of these strains. A significant amount of sulfuric acid (8.0-10.0g/L H$_2$SO$_4$) was produced from bacterial oxidation of sulfur. Uranium recovery of 0.29-65.2% U$_3$O$_8$ was obtained for different proportions of sulfur to uranium ore in 15-days of incubation (Figure 28a). Therefore, the optimal recovery was observed from these shake flask leaching experiments that the elemental to uranium ore ratios [S$^{6-}$/ore] in the range of 4:100 to 6:100 (wt/wt) was found to solubilize maximum uranium recovery (63.0%-64.0% U$_3$O$_8$) from ore in 15-days of incubation at 30°C and 100 rpm. The pH-values of the bacterial leach liquor samples were in the pH range of 1.8-6.90 (Figure 28b). The optimal pH-value of 1.8-2.0 was observed for maximum uranium recovery from sandstone ore.

Similarly, the effect of different proportions of sulfur slag to low-grade uranium ore was conducted in shake flasks. These results revealed that uranium solubilization of 0.52% to 68.0% U$_3$O$_8$ was achieved from sandstone ore amended with various proportions of sulfur slag during 15-days of incubation by mixed culture of T. ferrooxidans (TFe-2) and T. thiooxidans (TTh-1) (Figure 28a). A maximum
uranium recovery of 68.0% \( \text{U}_3\text{O}_8 \) was obtained from slag to ore ratio of 5:100 to 6:100 (wt/ wt).

Physical appearance of bacterial leach liquor samples were found to light-green due to the presence of soluble salts of iron like \( \text{FeSO}_4 \) and were very similar to acid leach liquor of uranium ore-processing plant (Table 19). The pH-values of bacterial leach liquors were observed in the pH range of 1.5-1.9 as optimal values. A significant amount of free acid (\( \text{H}_2\text{SO}_4 \)) remained un-reacted in the leaching systems.

<table>
<thead>
<tr>
<th>Sulfur to Ore Ratio [( \text{SO}_3: \text{Ore} )]</th>
<th>Physical Appearance of Leach Liquor</th>
<th>pH of Leach Liquor</th>
<th>( \text{U}_3\text{O}_8 ) Leached (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1:100]</td>
<td>Colorless</td>
<td>6.90</td>
<td>0.0080</td>
</tr>
<tr>
<td>[2:100]</td>
<td>Very light green</td>
<td>2.80</td>
<td>56.22</td>
</tr>
<tr>
<td>[3:100]</td>
<td>Very light green</td>
<td>2.30</td>
<td>120.00</td>
</tr>
<tr>
<td>[4:100]</td>
<td>Very light green</td>
<td>2.00</td>
<td>176.70</td>
</tr>
<tr>
<td>[5:100]</td>
<td>Light green</td>
<td>1.92</td>
<td>175.40</td>
</tr>
<tr>
<td>[6:100]</td>
<td>Light green</td>
<td>1.90</td>
<td>175.00</td>
</tr>
<tr>
<td>[7:100]</td>
<td>Light green</td>
<td>1.90</td>
<td>172.00</td>
</tr>
<tr>
<td>[8:100]</td>
<td>Light green</td>
<td>1.90</td>
<td>165.00</td>
</tr>
<tr>
<td>[9:100]</td>
<td>Light green</td>
<td>1.93</td>
<td>172.30</td>
</tr>
<tr>
<td>[10:100]</td>
<td>Light green</td>
<td>1.80</td>
<td>180.00</td>
</tr>
</tbody>
</table>
Figure 28: Uranium solubilization at different proportions of sulfur and sulfur slag by *Thiobcillus* bacteria. (A) $\text{U}_3\text{O}_8$ Recovery [%] and (B) pH-profile.
Effect of Pulp Density

Effect of different ore pulp densities (wt/vol) on uranium solubilization from Baghalchur sandstone was carried out in shake flasks. A mixed culture of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) was used for these studies. Initially, the bacterial cells were grown in 9K mineral salts medium containing different amounts of sulfur according to weight of uranium ore to be added. A sufficient amount of sulfuric acid was produced from bacterial oxidation of sulfur which dropped the initial pH-value of 2.5 to 1.5. Sansstone ore (1.0-100% wt/vol) was added into respective flasks containing 5-days grown culture of the acidophilic thiobacilli. These flasks were again incubated at 30±2.0°C and 100 rpm on an orbital shaking incubator for further 10-days.

Maximum uranium recovery of 83.50% $\text{U}_3\text{O}_8$ was obtained from ore-pulp density of 1.0% (wt/vol) during 15-days of incubation with a pH-value of the bacterial leach liquor was 1.30. It was observed that uranium extraction rate was inversely proportional to the ore pulp density (Figure 29). Subsequent continuous laboratory testing indicated that it might be due to nutrient limitation or insufficient biological adaptation (toxic effect on the growth of the acidophilic *Thiobacillus* bacteria). But at low ore pulp density, an economical recovery of uranium from low-grade uranium ores by microbial biotechnology would not be feasible at industrial scale. Therefore, the influence of ore concentration on bacterial growth and uranium recovery had to be investigated.
Figure 29: Effect of ore pulp density on bioleaching of sandstone uranium ore.
Effect of Particle Size

Batch test experiments were conducted to study the effect of particle size on uranium recovery from sandstone ore by indigenous strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1). Uranium ore [10% wt/vol pulp density] was added into flasks containing 7-days grown mixed culture of these acidophilic thiobacilli. Sulfur was used as energy substrate for the bacteria. Results revealed that a maximum uranium recovery of 89.12% $\text{U}_3\text{O}_8$ was obtained from ore fraction of particle size of $\leq 53$ $\mu$m during 20-days of incubation at 30.0 $\pm$ 2.0°C and 100 rpm (Table 20). In sterile control sample, uranium recovery of 2.60% $\text{U}_3\text{O}_8$ was resulted from ore fraction of particle size of $\leq 53$ $\mu$m.

<table>
<thead>
<tr>
<th>ASTM Particle Size (µm)</th>
<th>Control (pH)</th>
<th>$\text{U}_3\text{O}_8$ Recovery (%)</th>
<th>Inoculated (pH)</th>
<th>$\text{U}_3\text{O}_8$ Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ore sample</strong></td>
<td>6.70</td>
<td>1.80</td>
<td>1.70</td>
<td>75.63</td>
</tr>
<tr>
<td>$\geq 595$</td>
<td>6.40</td>
<td>0.70</td>
<td>1.70</td>
<td>65.30</td>
</tr>
<tr>
<td>+297</td>
<td>6.42</td>
<td>0.94</td>
<td>1.60</td>
<td>68.80</td>
</tr>
<tr>
<td>+177</td>
<td>6.52</td>
<td>0.94</td>
<td>1.70</td>
<td>72.70</td>
</tr>
<tr>
<td>+149</td>
<td>6.70</td>
<td>1.76</td>
<td>1.90</td>
<td>78.23</td>
</tr>
<tr>
<td>$\leq 53$</td>
<td>6.43</td>
<td>2.60</td>
<td>1.80</td>
<td>89.12</td>
</tr>
</tbody>
</table>

Pulp density (10% wt/vol).
Shake Flask Leaching Studies of Talings Residue

Bioleaching recovery of uranium from tailings residue (containing 0.0025% U₃O₈) was carried out in shake flasks. A mixed culture of indigenous strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) was used. Results revealed that maximum uranium recovery of 75.0% and 89.5% U₃O₈ was obtained from tailings residue amended with 5.0% and 10% (wt/wt) sulfur slag respectively, during 30-days of incubation at 30±2.0°C and 100 rpm (Figure 30a). In sterile control samples, only 1.80% U₃O₈ was recovered from residue under the same leaching experimental conditions.

The pH-values of the bacterial leach liquors was ≤2.0 after 10-days of incubation (Figure 30b). As a consequence of this drop in pH of the leaching medium, a significant amount of uranium was solubilized from the residue.
Figure 30: Bioreaching of tailings residue by indigenous strains of *Thiobacillus* bacteria. (A) Uranium recovery (%) (B) pH-profile.
Tank Leaching Studies

In tank bioleaching studies, a uranium recovery of 87.30% $U_3O_8$ was obtained from low-grade sandstone uranium ore by a mixed culture of *T. ferrooxidans* (TFe-2) and *T. thiiooxidans* (TTh-1) during 20-days at 30.0±2.0°C and 200 rpm. Initially, an ore pulp density of 10.0% (wt/vol) was used and then stepwise increased to 50.0% (wt/vol) in liquid sulfur medium (9K$S^-$). Uranium concentration of the bacterial leach liquor was 780.0 m g/L $U_3O_8$ (Table 21). Physical appearance of bacterial leach liquor was light green due to the presence iron salts like FeSO$_4$ and resembled to acid leach liquor. In sterile sample, only 2.43% $U_3O_8$ was solubilized under the same experimental conditions of ore pulp density, shaking and temperature.

### Table 21
**Tank Leaching Studies of Baghalchur Sandstone Uranium Ore**

<table>
<thead>
<tr>
<th>Description</th>
<th>Physical appearance of Leach Liquor</th>
<th>pH</th>
<th>$U_3O_8$ (mg/L)</th>
<th>$U_3O_8$ (%)</th>
<th>Iron Analyses (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$Fe_{(aq)}$</td>
</tr>
<tr>
<td>Control</td>
<td>Colorless</td>
<td>7.84</td>
<td>16.3</td>
<td>2.43</td>
<td>N.D.</td>
</tr>
<tr>
<td>Inoculated</td>
<td>Light green</td>
<td>1.70</td>
<td>780.0</td>
<td>87.30</td>
<td>1163</td>
</tr>
</tbody>
</table>

N.D. = Not detectable.

The pH-value of the bacterial leach liquor collected on 20th-day of experiment was 1.70; which resulted the uranium solubilization from sandstone ore. This change in pH-value of the medium was due to sulfuric acid production from bacterial oxidation of sulfur. The presence of bacteria was confirmed microscopically by examination of the daily ore slurry suspension and by culturing 1.0-mL sample of the last day into 10.0-mL of liquid sulfur medium.
Air-Lift Percolator Column Leaching Studies

These leaching experiments were performed as a model for microbial heap leaching studies of sandstone uranium ore. A set of four air-lift columns designated as PC-1, PC-2, PC-3 and PC-4 was used for these studies. A maximum uranium recovery of 65.38% U₃O₈ was obtained from column PC-4 inoculated with a mixed culture of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) in 60-days, as compared with columns PC-2 and PC-3 inoculated with pure strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) (Table 22). In sterile control column PC-1, only 6.32% U₃O₈ was solubilized at pH-value of 8.10. A diagrammatic presentation of the air-lift percolator column leaching experiment is shown in Figure 31.

Table 22
Air-Lift Percolators Studies of Baghalchur Sandstone Uranium Ore

<table>
<thead>
<tr>
<th>Column</th>
<th>Description</th>
<th>Physical appearance of Leach Liquor</th>
<th>pH</th>
<th>U₃O₈ Recovery (％)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-1</td>
<td>Control</td>
<td>Colorless</td>
<td>8.10</td>
<td>6.32</td>
</tr>
<tr>
<td>PC-2</td>
<td><em>T. ferrooxidans</em></td>
<td>Light green</td>
<td>2.24</td>
<td>59.60</td>
</tr>
<tr>
<td>PC-3</td>
<td><em>T. thiooxidans</em></td>
<td>Light green</td>
<td>2.16</td>
<td>54.77</td>
</tr>
<tr>
<td>PC-4</td>
<td><em>T. ferrooxidans</em> +</td>
<td>Light green</td>
<td>1.93</td>
<td>65.38</td>
</tr>
</tbody>
</table>


Figure 30: A diagrammatic presentation of air-lift percolator column leaching experiments of sandstone uranium ore.
PVC Column Leaching Studies

A detailed description of the microbial column leaching studies of sanstone uranium ore have been summarized in Table 23. Uranium recovery of 78.0% and 90.0% U₃O₈ was obtained from PVC columns labelled as C and D, respectively, in 150-days of leaching time (Figure 32). In both these columns, the initial pH-value of 3.50 of the feeding mine water was adjusted with dilute sulfuric acid and 3.0 g/L (NH₄)₂SO₄ was added as nitrogen source for growth of *T. ferrooxidans* and *T. thiooxidans*. It was not possible to keep the columns at constant temperature during experimental studies. Initially, the daily average temperature was about 23°C (September to November) during day-time; rising to a maximum of 25-48°C (April to June) and falling to 10°C during the months of December and January.

<table>
<thead>
<tr>
<th>Description</th>
<th>PVC Columns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content</td>
<td>A</td>
</tr>
<tr>
<td>Energy Source</td>
<td>S⁰</td>
</tr>
<tr>
<td>Nutrient Supply</td>
<td>(NH₄)₂SO₄, 3.0 g/L</td>
</tr>
<tr>
<td>Adjustment of pH 3.5 of mine water as inoculum</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 32: $U_3O_8$ recovery from Baghalchur low-grade sandstone uranium ore in PVC column leaching studies by indigenous microflora of *T. frrooxidans* and *T. thiooxidans*. 
During column leaching studies of sandstone ore, it was observed that when uranium bearing solution was recycled through the columns, in which calcite was not completely neutralized, it resulted co-precipitation of uranium the column ore bed. When sulfuric acid was added into mine water to adjust pH-value of 3.5, it provided better conditions to keep dissolve uranium in recycle solution and favoured the bacterial growth of acidophilic thiobacilli. This could be the probable reason, why more uranium was leached out from columns B, C and D as compared to column A (Figure 32). It was found that about 48.0% $\text{U}_3\text{O}_8$ was leached out from column A which was loaded with ores amended with elemental sulfur and was irrigated with mine water of initial pH-value of 7.40; which was used as inoculum of indigenous microflora of *Thiobacillus* bacteria. However, when the initial pH of this mine water was adjusted to 3.50, the uranium leaching after 150 days was found to have increased to 66.0% $\text{U}_3\text{O}_8$ in column B. Since a further fortification of irrigation medium by ammonium sulfate (3.0 g/L) could also augment the uranium leachability from 66.0% to 78.0% $\text{U}_3\text{O}_8$, it indicated that with mine water the leaching process was operated under nitrogen limiting conditions. These results demonstrated that by using acidified mine water (pH-value of 3.50); the uranium leachability was found much higher. Since a further fortification of irrigation medium by ammonium sulfate could also augment the uranium leachability from 78.0% to 90.0% $\text{U}_3\text{O}_8$, it indicated that with mine-water, the process was operating under nitrogen limiting conditions. The column containing ore amended with 10-12% sulfur slag and irrigated with mine water of initial pH-value of 3.50, exhibited 90.0% leachability of uranium which was the highest so far obtained during these investigations.

The experimental set-up of microbial column leaching experiments of sandstone uranium ore is shown in Figure 33.
Figure 33: Photograph showing the experimental set-up of PVC columns leaching experiments of sandstone uranium ore.
Column Effluents Analyses

Chemical and microbiological analyses of PVC column effluents taken after 100-days of leaching time have been reported in Table 24. Analytical data of column effluents revealed that higher concentration of uranium 156.4 mg/L $U_3O_8$ was detected in column effluent collected from column labelled as D with a pH-value of 2.40. Similarly, the uranium concentration in the column effluents of A, B, and C were 66.0, 79.0, and 142.8 mg/L $U_3O_8$, respectively. The presence of soluble ferric iron as well as Fe(111)-precipitate (Jarosite?) was observed in columns effluents of C and D. Physical appearance of a microbial leach liquor of column D is shown in Figure 34. The yellow-brown color of the leach liquor indicates the presence of soluble iron content (mainly $Fe^{3+}$-iron).

<table>
<thead>
<tr>
<th>PVC Column</th>
<th>Physical Appearance of Column Effluent</th>
<th>pH</th>
<th>$U_3O_8$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Colorless, turbid</td>
<td>4.40</td>
<td>66.0</td>
</tr>
<tr>
<td>B</td>
<td>Pale yellow, turbid</td>
<td>3.10</td>
<td>79.0</td>
</tr>
<tr>
<td>C</td>
<td>Dark yellow, turbid</td>
<td>2.70</td>
<td>142.8</td>
</tr>
<tr>
<td>D</td>
<td>Yellowish-brown, turbid</td>
<td>2.40</td>
<td>156.4</td>
</tr>
</tbody>
</table>

The microbial population of iron-oxidizing bacteria (T. ferrooxidans) in columns effluents showed a slight variation in number of cells when growing on solid Gelrite-FeSO$_4$ medium (Khalid et al. 1993). The microbial population of T. ferrooxidans was $30 \times 10^4$, $20 \times 10^5$, $24 \times 10^5$, and $28 \times 10^5$ (cfu/ mL) in columns
effluents of A, B, C, and D, respectively (Table 25). However, the number of iron-oxidizing bacteria were relatively greater in effluent obtained from column D (28 X 10^4 cfu/mL) as compared to effluents of columns A, B, and C. The higher number of cells of *T. ferrooxidans* in column D might be due to the presence of Fe^{2+} iron present in the sulfur slag used as energy substrate for iron-oxidizing bacteria which was additionally available in column D.

<table>
<thead>
<tr>
<th>PVC Column</th>
<th>Microbial Population (cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron-Oxidizers</td>
</tr>
<tr>
<td>A</td>
<td>30 X 10^4</td>
</tr>
<tr>
<td>B</td>
<td>20 X 10^5</td>
</tr>
<tr>
<td>C</td>
<td>24 X 10^5</td>
</tr>
<tr>
<td>D</td>
<td>28 X 10^5</td>
</tr>
</tbody>
</table>

The presence of sulfur-oxidizers (*T. thiooxidans*) in these column effluents were observed by growing on solid Agarose-S_{2}O_{3}^{2-} medium of pH 4.0-4.5 as described earlier. The microbial population of sulfur-oxidizing bacteria was slightly greater in number in column effluent of D (40 X 10^7 cfu/mL) as compared to columns effluents of A, B, and C (Table 25). Glucose-oxidizing heterotrophs were observed in columns effluents by growing on solid Agarose-Glucose medium of pH 4.0-4.50. The microbial population of glucose-oxidizers was much greater in number in column effluent of A (12 X 10^4 cfu/mL) as compared to those of columns B, C, and D. It was observed that the growth of heterotrophs was pH-depandant, that at lower pH-value (towards acidic) of the effluent, the number of cells were found less as was indicated in column D. Heterotrophic growth of fungus with black-spores was also observed in all column effluents.
Figure 34: Photograph showing the physical appearance of a microbial leach liquor obtained from PVC column D during bioleaching studies of sandstone uranium ore.
Core Samples Analyses

The core samples of 2.50 cm diameter (\(\phi\)) were taken from PVC columns with the help of a soil plunger and samples corresponding to depth of 00-30; 30-60; and 60-90 cms (from top to bottom of the columns) after 150-days of leaching experiments. pH, moisture content, solubilization of uranium and iron were monitored and variations in these physical parameters have been tabulated (Table 26). The pH-values of core-slurries (10.0g of core in 10.0 mL of distilled water) obtained from these columns at 00-30 cm depth (at the top of column) were changed significantly from an initial pH-values of 7.80 to 2.0-3.0. Similarly, the pH-values of the core-slurries taken at depth of 30-60 cm and 60-90 cm were also changed in the pH range of 2.3-4.7. A significant change in pH-value of the core samples taken at depth of 00-30 cm (from top of column) in columns B, C, and D was observed due to the irrigation of acidified mine water (pH 3.5-0) and also probably the availability of maximum oxygen at the top surface of column which enhanced the microbial population of acidophilic Thiobacillus bacteria. Therefore, bacterial activity enhanced the rate of oxidation of sulfur and sulfur slag to produce \(\text{H}_2\text{SO}_4\). As the depth of the ore-bed in columns increased, the availability of air/oxygen was limited.

Physical appearance of core samples obtained from columns A and B was similar to the ore feed (light grey), whereas core samples of columns C and D was rusty-brown in color indicating the formation of a secondary iron mineral called as jarosite with a general chemical formula of \([\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6]\).
### Table 26
Physico-Chemical Analyses of Core Samples obtained from PVC Column
Leaching Studies of Sandstone Uranium Ore after 150-days

<table>
<thead>
<tr>
<th>PVC Column</th>
<th>Depth (cm)</th>
<th>Moisture (%)</th>
<th>pH of Core Slurry</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>00-30</td>
<td>15.4</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>16.4</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>17.4</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*Physical appearance of core sample was gray.*

| B           | 00-30      | 16.0         | 2.2               |
|            | 30-60      | 17.6         | 3.4               |
|            | 60-90      | 19.8         | 4.0               |

*Physical appearance of core sample was gray.*

| C           | 00-30      | 15.7         | 2.1               |
|            | 30-60      | 17.6         | 2.9               |
|            | 60-90      | 18.5         | 3.2               |

*Physical appearance of core sample was rusty brown (jarosite?)*

| D           | 00-30      | 19.4         | 2.0               |
|            | 30-60      | 20.3         | 2.3               |
|            | 60-90      | 22.0         | 2.7               |

*Physical appearance of core sample was rusty brown (jarosite?)*

N.D = Not determined.

Microbial populations of iron- and sulfur-oxidizers and heterotrophs present in these core samples were observed by growing on various solid media and the results thus obtained have been summarized in Table 26. It was found that in PVC columns C and D, which were loaded with ore amended with sulfur and sulfur slag, respectively, the microbial populations of iron-oxidizing bacteria (*T. ferrooxidans*) were significantly higher in number of cells as compared with columns A and B. The distribution of iron-oxidizing bacteria (*T. ferrooxidans*) at various depths of 00-30,
30-60, and 60-90 cm in PVC column D were present 20 \times 10^4, 33 \times 10^5, and 28 \times 10^4 cfu/g of core sample, respectively. The solid Gelrite-FeSO_4 medium (Khalid et al. 1993) was used for the isolation and enumeration of iron-oxidizing bacteria.

### Table 27

<table>
<thead>
<tr>
<th>Depth (Cm)</th>
<th>Number of Microbial Populations (cfu / g of core sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column A</td>
</tr>
<tr>
<td>00-30</td>
<td>24 \times 10^4</td>
</tr>
<tr>
<td>30-60</td>
<td>20 \times 10^4</td>
</tr>
<tr>
<td>60-90</td>
<td>10 \times 10^4</td>
</tr>
</tbody>
</table>

Note = Colonies are taken as average of triplicate. Number of colonies calculated on dry basis.

The isolation and enumeration of S-oxidizing bacteria (T. thiooxidans) in core samples were carried out by using Agarose-S_2O_3^2- medium of pH 4.0-4.5. The microbial population found in core samples have been presented in Table 28. The microbial populations of these bacteria were predominantly in PVC columns B, C, and D as compared to column A, which was filled with sandstone ore amended with elemental sulfur. The higher number of microbial cells in columns B, C, and D might be due to adjustment of initial pH-value of 3.50 of the feeding mine water. Similarly, the addition of ammonium sulfate (3.0 g/L) in the feeding mine water of PVC columns C and D also increased the microbial population of iron- and sulfur-oxidizing bacteria (Tables 27 and 28).
Table 28
Isolation and Enumeration of Sulfur-Oxidizers in Core Samples

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Number of Microbial Populations (cfu/g of core sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column A</td>
</tr>
<tr>
<td>0-30</td>
<td>30 X 10⁷</td>
</tr>
<tr>
<td>30-60</td>
<td>20 X 10⁷</td>
</tr>
<tr>
<td>60-90</td>
<td>16 X 10⁷</td>
</tr>
</tbody>
</table>

Note: Colonies are taken as average of triplicate. Number of colonies calculated on dry basis.

In addition to iron- and sulfur-oxidizing bacteria present in core samples, the isolation and enumeration of glucose-oxidizing bacteria (heterotrophs) were also observed by growing on solid agarose-glucose medium of pH 4.0-4.5. Microbial population of glucose-oxidizing heterotrophs were higher in PVC column A (18 X 10⁴ cfu/g of core sample) for core sample taken at the depth of 00-30 cm as compared to column D (9 X 10⁴ cfu/g of core sample). The number of cells were increased at columns depths of 30-60, and 60-90 cm in column A (Table 29). In columns B and C, the microbial population of these bacteria were not observed at depths of 00-30 cm; whereas the columns B contained microbial cells at depth of 30-60 cm (9 X 10³ cells/g of core sample) and C at depth of 60-90 cm (26 X 10⁴ cells/g of core) were isolated. The growth of these heterotrophs was observed pH-dependent and the availability of carbon source in the form of dead cell biomass.

Microbial populations of sulfur-oxidizing bacteria growing at pH 6.60 were isolated and enumerated in these core samples growing on solid Agar-S₂O₃⁻² medium (Vishniac and Santer 1957) of pH 6.60 as described for the isolation and growth of T. thioparus. The microbial population of these bacteria were found higher in all PVC columns A, B, C, and D (Table 30).
### Table 29
Isolation and Enumeration of Glucose-Oxidizing Heterotrophs in Core Samples

<table>
<thead>
<tr>
<th>Depth (Cm)</th>
<th>Column A</th>
<th>Column B</th>
<th>Column C</th>
<th>Column D</th>
</tr>
</thead>
<tbody>
<tr>
<td>00-30</td>
<td>$18 \times 10^4$</td>
<td>-</td>
<td>-</td>
<td>$9 \times 10^4$</td>
</tr>
<tr>
<td>30-60</td>
<td>$28 \times 10^4$</td>
<td>$9 \times 10^3$</td>
<td>-</td>
<td>$4 \times 10^4$</td>
</tr>
<tr>
<td>60-90</td>
<td>$46 \times 10^4$</td>
<td>-</td>
<td>$26 \times 10^5$</td>
<td>$15 \times 10^4$</td>
</tr>
</tbody>
</table>

Note = Colonies are taken as average of triplicate. Number of colonies calculated on dry basis.

### Table 30
Isolation and Enumeration of Sulfur-Oxidizers (*T. thioparus*) in Core Samples

<table>
<thead>
<tr>
<th>Depth (Cm)</th>
<th>Column A</th>
<th>Column B</th>
<th>Column C</th>
<th>Column D</th>
</tr>
</thead>
<tbody>
<tr>
<td>00-30</td>
<td>$24 \times 10^9$</td>
<td>$13 \times 10^9$</td>
<td>$19 \times 10^7$</td>
<td>$43 \times 10^6$</td>
</tr>
<tr>
<td>30-60</td>
<td>$22 \times 10^9$</td>
<td>$18 \times 10^9$</td>
<td>$31 \times 10^9$</td>
<td>$19 \times 10^4$</td>
</tr>
<tr>
<td>60-90</td>
<td>$10 \times 10^9$</td>
<td>$50 \times 10^9$</td>
<td>$41 \times 10^6$</td>
<td>$25 \times 10^6$</td>
</tr>
</tbody>
</table>

Note = Colonies are taken as average of triplicate. Number of colonies calculated on dry basis.
PVC Column Leaching Studies of Tailings Residue

Descriptions of the microbial column leaching experiments on tailings residue (containing 0.0025% U₃O₈) are summarized in Table 31. The results of these studies have been reported in Table 32. Uranium recovery of 67.47% and 84.08% U₃O₈ was obtained from PVC columns I and J respectively, in 100-days of leaching time. In both these columns, the tailings residue was amended with sulfur slag an external energy substrate for T. ferrooxidans and T. thiooxidans. In addition, 3.0 g/L (NH₄)₂SO₄ was dissolved in feeding mine water as nitrogen source for these bacteria. The lowest uranium recovery of 19.96% U₃O₈ was observed from column G which was loaded with mill tailings residue and irrigated with mine water of pH-value 7.40 containing tailings liquid (10% vol/ vol) as inoculum of indigenous microflora of thiobacilli.

Table 31

<table>
<thead>
<tr>
<th>Description</th>
<th>PVC Column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
</tr>
<tr>
<td>Content</td>
<td>Residue</td>
</tr>
<tr>
<td>Energy Source</td>
<td>-</td>
</tr>
<tr>
<td>Nutrient Supply (NH₄)₂SO₄, 3g/L</td>
<td>-</td>
</tr>
<tr>
<td>Adjustment of pH 3.5</td>
<td>-</td>
</tr>
<tr>
<td>of mine water as inoculum</td>
<td></td>
</tr>
</tbody>
</table>
When 3.0 g/L $\text{(NH}_4\text{)}_2\text{SO}_4$ was dissolved in mine water as nitrogen nutrient and used as inoculum for column H, the uranium leaching was increased to 37.01% $\text{U}_3\text{O}_8$, after 100-days of leaching time. These results demonstrated that by adding ($\text{NH}_4\text{)}_2\text{SO}_4$ as nutrient supply for leaching bacteria, the uranium leachability was almost doubled (Table 32). It further indicated that with mine-water, the process was operated under nitrogen limiting conditions.

### Table 32

**Physico-Chemical Analyses of Columns Samples of Tailings Residues after 100-Days**

<table>
<thead>
<tr>
<th>Column</th>
<th>Physical Appearance of Leach Liquor</th>
<th>$\text{pH}$</th>
<th>Total $\text{U}_3\text{O}_8$ Leached (gm)</th>
<th>$\text{U}_3\text{O}_8$ Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>Colorless, clear</td>
<td>4.12</td>
<td>0.4492</td>
<td>19.96</td>
</tr>
<tr>
<td>H</td>
<td>Colorless, turbid, white particles suspension</td>
<td>3.68</td>
<td>0.8327</td>
<td>37.01</td>
</tr>
<tr>
<td>I</td>
<td>Bright yellow, turbid</td>
<td>2.87</td>
<td>1.5180</td>
<td>67.47</td>
</tr>
<tr>
<td>J</td>
<td>Yellowish, turbid, brick red suspension</td>
<td>2.30</td>
<td>1.8918</td>
<td>84.08</td>
</tr>
</tbody>
</table>

The $\text{pH}$-values of the effluents obtained from column leaching studies was found to be within a range of 2.3-4.12 in all these columns. The $\text{pH}$-values of effluents obtained from columns I and J were found to be 2.87 and 2.30, respectively; and thus indicated the maximum uranium solubilization from tailings residue.
Physical appearance of microbial leach liquors collected from columns I and J was turbid and bright-yellow indicating the presence of Fe\textsuperscript{3+} released from residue and sulfur slag by the bacterial action. In addition, Fe(111)-precipitates were also observed in these column effluents. The effluent of column H was observed colorless, turbid, and contained white solid particles suspension; whereas the column effluent of G was colourless.

**Microbial Heap Leaching Studies**

The experimental set-up and a view of 20-tons microbial heap leaching process of low-grade sandstone uranium ore established at NIBGE campus are shown in Figures 4 and 35. The results of progressive microbial heap leaching recovery of uranium from sandstone ore have been presented in Figure 36a (where the soluble uranium content present in the microbial leach liquor was plotted against time course). The uranium concentration of microbial leach liquor increased from 10.02 mg/L to 33.60 mg/L U\textsubscript{3}O\textsubscript{8} within 15-days of heap operation and then started decreasing gradually (Figure 36a). After 25-days of operation, U\textsubscript{3}O\textsubscript{8} concentration fell below to 6.62 mg/L U\textsubscript{3}O\textsubscript{8}. At that time, fresh inoculation was applied to enhance the uranium recovery. The concentration of uranium in microbial leach liquor again increased to 23.80 mg/L U\textsubscript{3}O\textsubscript{8} and then started decreasing gradually to less than 10.0 mg/L U\textsubscript{3}O\textsubscript{8}. It was also observed that uranium recovery was increased within 24-hrs after the sprinkling of inoculum at the top of the heap. It might be due to low pH-value of the inoculated solution and the presence of higher number of microbial populations of *T. ferrooxidans* and *T. thiooxidans*.

The pH-values of microbial leach liquors were presented in Figure 36b. The pH-value of inoculum was in the range of 2.40-2.70 due to acid production of acidophilic *Thiobacillus* bacteria. When the inoculum was sprinkled on the top of the heap, it sickered through the ore body and was collected at the bottom of the heap. The pH-value of the solution increased (i.e pH more than 6.0) due to the presence of calcite in the ore matrix.
Figure 35: A view of 20-tons microbial heap leaching experiment of low-grade sandstone uranium ore at NIBGE campus.
Figure 36: Microbial heap leaching of low-grade sandstone uranium ore. (A) Uranium solubilization ($U_3O_8$ mg/L) and (B) pH-profile of microbial heap leach liquor.
Microbial populations of iron- and sulfur-oxidizers and heterotrophs present in some heap inocula were determined by growing on various solid media and the results thus obtained have been summarized in Table 33. It was observed that in inocula samples designated as INOC-1, INOC-2, and INOC-3, the number of iron-oxidizing bacteria present were $18 \times 10^4$, $35 \times 10^4$, and $22 \times 10^2$ (cfu/ mL), respectively. The microbial population of sulfur-oxidizing bacteria (T. thiooxidans) was slightly higher ($50 \times 10^5$ cfu/ mL) in inoculum sample of INOC-1 as compared to iron-oxidizing bacteria ($18 \times 10^4$ cfu/ mL) present in this sample.

Table 33

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>pH</th>
<th>Temp. (°C)</th>
<th>Microbial Populations (cfu/ mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T. ferrooxidans (I)</td>
</tr>
<tr>
<td>INOC-1</td>
<td>2.70</td>
<td>18-30°C</td>
<td>$18 \times 10^4$</td>
</tr>
<tr>
<td>INOC-2</td>
<td>2.40</td>
<td>23-35°C</td>
<td>$35 \times 10^4$</td>
</tr>
<tr>
<td>INOC-3</td>
<td>2.70</td>
<td>28-45°C</td>
<td>$22 \times 10^2$</td>
</tr>
</tbody>
</table>

(I) = Gelrite-FeSO$_4$ medium (Khalid et al. 1993)
(II) = Agarose-S$_2$O$_3$ medium of pH-value of 4.0-4.5.
(III) = Agarose-Glucose medium of pH-value of 4.0-4.5.
N.D. = Growth not observed.

The growth of glucose-oxidizing heterotrophs were observed $60 \times 10^2$ and $47 \times 10^3$ cfu/ mL, in inocula samples of INOC-1 and INOC-3, respectively. No heterotroph was observed in inoculum sample of INOC-2 (Table 33).
Heap Effluents Analyses

Chemical and microbiological analyses of some heap effluents (leach liquors) have been presented in Table 34. Uranium concentrations of 10.02, 24.12, 18.30, and 12.08 mg/L $\text{U}_3\text{O}_8$ was present in heap effluent samples designated as HPL-1, HPL-2, HPL-3, and HPL-4, respectively. The pH-values of these effluents were in the pH range of 2.70 to 6.70.

Table 34
Physico-Chemical and Microbiological Analyses of Microbial Heap Leach Liquors

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Temp.</th>
<th>$\text{U}_3\text{O}_8$</th>
<th>Microbial Populations (cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(°C)</td>
<td>(mg/L)</td>
<td>$T. \text{ferrooxidans}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(I)</td>
</tr>
<tr>
<td>HPL-1</td>
<td>5.80</td>
<td>10-25</td>
<td>10.02</td>
<td>$45 \times 10^2$</td>
</tr>
<tr>
<td>HPL-2</td>
<td>6.70</td>
<td>18-30</td>
<td>24.12</td>
<td>$24 \times 10^4$</td>
</tr>
<tr>
<td>HPL-3</td>
<td>2.70</td>
<td>22-35</td>
<td>18.30</td>
<td>$28 \times 10^4$</td>
</tr>
<tr>
<td>HPL-4</td>
<td>4.10</td>
<td>28-45</td>
<td>12.08</td>
<td>$10 \times 10^3$</td>
</tr>
</tbody>
</table>

(1) = Gelrite-FeSO$_4$ medium (Khalid et al. 1993)
(II) = Agarose-$\text{S}_2\text{O}_3^-$ medium of pH-value of 4.0-4.5.
(III) = Agarose-Glucose medium of pH-value of 4.0-4.5.
N.D. = Not detectable.

The isolation and enumeration of microbial population of iron- and sulfur-oxidizers and heterotrophs present in microbial heap effluents were carried out by growing on various solid media (Table 34). The microbial population of iron-oxidizing bacteria ($T. \text{ferrooxidans}$) was $45 \times 10^2$, $24 \times 10^4$, $28 \times 10^4$, and $10 \times 10^3$ (cfu/mL) in heap effluents of HPL-1, HPL-2, HPL-3 and HPL-4 respectively, when growing on solid Gelrite-FeSO$_4$ medium (Khalid et al. 1993). The growth of iron-
oxidizing bacteria was found to temperature dependent. These bacteria were found in higher numbers (24 \times 10^4 to 28 \times 10^4 cfu/mL) at temperature between 18-35°C during the months of March and April, as observed in the heap effluents of HPL-2 and HPL-3. But less number of cells were observed in heap effluent samples of HPL-1 and HPL-4 taken during the months of January (temperature between 10-25°C) and July (temperature between 28-45°C), respectively (Table 34).

The presence of sulfur-oxidizers (T. thiooxidans) in these heap effluents (HPL-1 to HPL-4) were observed by growing on solid Agarose-S₂O₅²⁻ medium of pH 4.0-4.5. The microbial population of T. thiooxidans was 26 \times 10^3, 55 \times 10^3, 12 \times 10^3, and 14 \times 10^2 (cfu/mL) in heap effluents of HPL-1, HPL-2, HPL-3 and HPL-4, respectively. The temperature also influenced the load of microbial population of sulfur-oxidizing bacteria in these samples. The number of cells were higher in samples of HPL-1, HPL-2, and HPL-3, but was found less in number in sample HPL-4 (14 \times 10^2 cfu/mL).

The growth of heterotrophs were observed in the columns effluents by growing on solid Agarose-Glucose medium of pH 4.0-4.50. The microbial population of glucose-oxidizers was observed much greater in heap effluents of HPL-2 and HPL-4, which were found 23 \times 10^4 and 11 \times 10^4 cfu/mL, respectively as compared to HPL-1 (38 \times 10^2 cfu/mL). The growth of heterotrophs might be dependent on the availability of carbon source in the form of dead cells biomass. (Table 34). The growth of fungus with black-spores was also observed in all heap effluents growing on solid glucose-agarose medium.

The physical appearance of heap effluents was observed colorless to pale-yellow and contained solid particles suspension.
Heap Core Samples Analyses

Uranium recovery of 4.90% $U_3O_8$ was obtained (calculated on the basis of heap effluents) from the microbial heap process during 150-days. But on the basis of chemical data of leached residues (core samples taken at depths of 00-100 cm), an average uranium recovery of 31.64% $U_3O_8$ was leached out during 150-days of heap operation (Table 35). Heap core samples was taken at various depths of 00-20, 20-40, 40-60, 60-80, and 80-100 cm (from top to bottom of the heap) with the help of a soil plunger. These results have been calculated on dry matter basis and reported in Table 35.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Depth</th>
<th>pH of Core Slurry</th>
<th>$U_3O_8$ in Core (%)</th>
<th>$U_3O_8$ Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core-1</td>
<td>00-20</td>
<td>2.49</td>
<td>0.0065</td>
<td>59.38</td>
</tr>
<tr>
<td>Core-2</td>
<td>20-40</td>
<td>4.06</td>
<td>0.0129</td>
<td>19.06</td>
</tr>
<tr>
<td>Core-3</td>
<td>40-60</td>
<td>5.74</td>
<td>0.0122</td>
<td>23.75</td>
</tr>
<tr>
<td>Core-4</td>
<td>60-80</td>
<td>6.40</td>
<td>0.0121</td>
<td>24.38</td>
</tr>
<tr>
<td>Core-5</td>
<td>80-100</td>
<td>6.65</td>
<td>0.0121</td>
<td>24.38</td>
</tr>
</tbody>
</table>

Average $U_3O_8$ recovery (%) = 31.64

The average pH-values of triplicate core slurry samples were reported.

It was observed from the chemical results of core samples that uranium solubilization was higher at the top (59.38% $U_3O_8$) of the heap core sample designated as Core-1; which was taken from top to bottom at the depth of 00-20 cm.
(Table 35). But the uranium recovery was found to be 19.06% to 24.38% U₃O₈ in the core samples taken at the depths of 20-100 cm. For pH-values of core samples, a water slurry [10g core sample in 10 mL distilled water] was used. It was observed from these findings that the pH-values of the core slurry samples were acidic (pH 2.49) at the top (00-30 cm) of the heap and the values increased to 6.65 towards the depth (80-100 cm) of the heap (Table 35). This variation in the pH-values might be due to acidic pH-values of inocula neutralized the calcite content of the ore at the top of the heap. Another possible reason might be higher metabolic activity of acidophilic thiobacilli at the top of heap due to sufficient supply of atmospheric oxygen.

The isolation and enumeration of microbial population of iron- and sulfur-oxidizers and heterotrophs present in core samples taken at various depths of heap were observed by growing on various solid media as described earlier and the results thus obtained have been summarized in Table 36. The microbial population of *T. ferrooxidans*, *T. thiooxidans* and heterotrophs present in the core samples was found higher in number of cells at the top of the heap and less at the bottom (60-80 cm)

| Sample  | Depth (cm) | Number of Microbial Populations/ gm of ore slurry |  
|---------|------------|-------------------------------------------------|---|
|         |            | *T. ferrooxidans* | *T. thiooxidans* | Heterotrophs |
| Core-1  | 00-20      | 95 X 10⁴         | 24 X 10³          | 38 X 10⁴     |
| Core-2  | 20-40      | 71 X 10³         | 29 X 10³          | 51 X 10³     |
| Core-3  | 40-60      | 15 X 10³         | 11 X 10⁴          | 45 X 10³     |
| Core-4  | 60-80      | 11 X 10³         | 10 X 10⁵          | 46 X 10³     |

The average number of cells of triplicate samples were reported.
Heap Sludge Samples Analyses

During microbial heap leaching process of sandstone uranium ore, an off-white fluffy solid material (sludge) was observed emerging along with heap effluents. Five numbers heap sludge samples were collected, filtered, and dried in an oven at 110°C. These sludge samples had a texture like talc powder and labelled as HSG-1 to HSG-5. A significant amount of uranium (0.0517% to 0.6283% $U_3O_8$) was present in these samples (Table 37). It seemed that a major portion of the uranium leached so far during heap leaching process had precipitated/entrapped into these sludge samples.

Chemical analyses data of these sludge samples revealed that uranium beneficiation was 2.25 to 27.32 folds with respect to original uranium concentration present in the ore sample. Further mineralogical studies of these sludge samples are required to understand the uranium precipitation phenomena.

### Table 37

<table>
<thead>
<tr>
<th>Sample</th>
<th>$U_3O_8$ on dry basis (%)</th>
<th>$U_3O_8$ Beneficiation w.r.t original ore (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSG-1</td>
<td>0.1880</td>
<td>8.18</td>
</tr>
<tr>
<td>HSG-2</td>
<td>0.6283</td>
<td>27.32</td>
</tr>
<tr>
<td>HSG-3</td>
<td>0.0517</td>
<td>2.25</td>
</tr>
<tr>
<td>HSG-4</td>
<td>0.0980</td>
<td>4.26</td>
</tr>
<tr>
<td>HSG-5</td>
<td>0.1545</td>
<td>6.72</td>
</tr>
</tbody>
</table>

w.r.t stands for with respect to
The formation of off-white sludge and precipitation of uranium in these sludge samples might be due to the presence of high calcium content (150.0 mg/L) in subsoil water being used for inocula preparation for microbial heap leaching process. However, no such problem was encountered during microbial column leaching studies of sandstone uranium ore earlier since mine water was employed as inoculum. The mine water sample contained less calcium content (27.6 mg/L Ca\(^{2+}\)) as compared with NIBGE water (Table 38).

### Table 38

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Water Sample Analyses (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIBGE Water</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>150.0</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>-</td>
</tr>
<tr>
<td>K(^+)</td>
<td>14.4</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>225.0</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>182.0</td>
</tr>
<tr>
<td>CO(_3)^{2-}</td>
<td>36.0</td>
</tr>
<tr>
<td>HCO(_3)^{-}</td>
<td>524.0</td>
</tr>
<tr>
<td>Total dissolved solid</td>
<td>16400.0</td>
</tr>
<tr>
<td>EC (µS/cm)</td>
<td>2750.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.8</td>
</tr>
</tbody>
</table>

EC stands for electrical conductivity.
N.D. = Not detectable.
Down-Stream Processing of Uranium

Adsorption of Uranium by Strongly Basic Anion-Exchange Resins

Studies were conducted to separate uranium by different anion-exchange resins from microbi ally leached solutions. These solutions were produced from Baghalchur low-grade sandstone uranium ores by the action of *Thiobacillus* bacteria. Adsorption of uranium by different anion-exchange resins such as Amberlite IRA-400 (Rohm and Haas, France), Duolite® A-147 (Duolite International, France), and Dowex 1 X 8 (Dow Europe, Switzerland) packed in glass columns were investigated in details. It was found that Amberlite IRA-400 was capable of adsorbing 97.60% \( U_2O_8 \) present in the microbial leach solution as compared to Dowex-1 and Duolite® A-147 anion-exchange resins, which absorbed 82.0% and 87.0% \( U_2O_8 \), respectively (Table 39). The uranium concentration present in the feeding solution was 0.12 g/L \( U_2O_8 \) having pH-value of 2.0±0.1.

Table 39

**Uranium Loading Capacities of Different Strongly Basic Anion-Exchangers (Cl⁻ form)**

<table>
<thead>
<tr>
<th>Resin</th>
<th>Breakthrough Bed-Volume (B)</th>
<th>Saturation Point of resin (S)</th>
<th>Loading Capacity of Uranium (E) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Litre)</td>
<td>(Litre)</td>
<td>( U_2O_8 )</td>
</tr>
<tr>
<td>Amberlite IRA-400</td>
<td>300</td>
<td>440</td>
<td>97.60</td>
</tr>
<tr>
<td>Dowex 1 X 8</td>
<td>200</td>
<td>530</td>
<td>82.0</td>
</tr>
<tr>
<td>Duolite® A-147</td>
<td>140</td>
<td>230</td>
<td>87.0</td>
</tr>
</tbody>
</table>
Uranium adsorption or loading capacity (E), breakthrough bed volume (B) and saturation point (S) for Amberlite IRA-400, Dowex-1 and Duolite® A-147 anion-exchange resins anion-exchange resin was investigated in details and summarized in Table 39. A typical curve showing uranium adsorption by Amberlite IRA-400 resin have shown in Figure 37.

![Diagram](image)

**Figure 37:** Typical uranium adsorption curve of Amberlite IRA-400 anion-exchange resin. [S = Saturation point; B = Breakthrough volume].
Elution Studies of Uranium

The optimal recovery of uranium adsorbed by Amberlite IRA-400 anion-exchange resin was investigated in detail by employing various solutions of ammonium sulfate and ammonium bicarbonate as eluents. These results revealed that 2.0 M NH₄HCO₃ solution of pH-value of 7.94 showed the maximum uranium elution efficiency (E) and stripped out 99.80% U₃O₈ from the uranium loaded resin column (Table 40). The uranium elution efficiency of 97.3% and 98.5% U₃O₈ was stripped out by 1.0 M and 1.5 M NH₄HCO₃ solutions, respectively.

Table 40

Elution Studies of Uranium with Ammonium Sulfate and Ammonium Bicarbonate Solutions

<table>
<thead>
<tr>
<th>Uranium Eluting Solution</th>
<th>pH of Eluting Solution</th>
<th>U₃O₈ Eluting Efficiency [E] (％)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 M (NH₄)₂SO₄ Solution</td>
<td>5.34</td>
<td>81.2</td>
</tr>
<tr>
<td>1.5 M (NH₄)₂SO₄ Solution</td>
<td>5.40</td>
<td>90.0</td>
</tr>
<tr>
<td>2.0 M (NH₄)₂SO₄ Solution</td>
<td>5.56</td>
<td>95.0</td>
</tr>
<tr>
<td>1.0 M (NH₄)₂SO₄ containing 3.95 g/L NH₄HCO₃</td>
<td>7.41</td>
<td>95.7</td>
</tr>
<tr>
<td>1.5 M (NH₄)₂SO₄ containing 3.95 g/L NH₄HCO₃</td>
<td>7.46</td>
<td>99.8</td>
</tr>
<tr>
<td>2.0 M (NH₄)₂SO₄ containing 3.95 g/L NH₄HCO₃</td>
<td>7.60</td>
<td>99.8</td>
</tr>
<tr>
<td>1.0 M NH₄HCO₃ Solution</td>
<td>8.05</td>
<td>97.3</td>
</tr>
<tr>
<td>1.5 M NH₄HCO₃ Solution</td>
<td>8.03</td>
<td>98.5</td>
</tr>
<tr>
<td>2.0 M NH₄HCO₃ Solution</td>
<td>7.94</td>
<td>99.8</td>
</tr>
</tbody>
</table>
However, a 2.0 M \((\text{NH}_4)_2\text{SO}_4\) solution showed 96.0% \(\text{U}_3\text{O}_8\) elution efficiency as compared to 1.0 M and 1.5 M \((\text{NH}_4)_2\text{SO}_4\) solutions which stripped out 81.20% and 90.0% \(\text{U}_3\text{O}_8\), respectively. The pH-values of \((\text{NH}_4)_2\text{SO}_4\) solutions were ranged between 5.34-5.56, but the pH-values were increased to 7.41-7.60 with the addition of 3.95 g/L \(\text{NH}_4\text{HCO}_3\). Therefore, the uranium elution efficiency of 1.0 M, 1.5 M, and 2.0 M \((\text{NH}_4)_2\text{SO}_4\) solutions increased with the addition of 3.95 g/L \(\text{NH}_4\text{HCO}_3\) (Table 40).

Strip solutions obtained by 2.0 M \(\text{NH}_4\text{HCO}_3\) solution was found to contain 15.50-25.60 g/L \(\text{U}_3\text{O}_8\) (Table 41). The pH-values of these strip solutions were found in the pH range of 8.95-9.30. The physical appearance of the strip solutions was pale yellow to bright yellow depending on the concentration of uranium present in the solution. The self-crystallization of uranium was also observed in some strip solution samples of 2.0 M \(\text{NH}_4\text{HCO}_3\) solution. Physical appearance of a typical strip solution has been shown in Figure 38.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Physical appearance of Strip Solution</th>
<th>pH of Strip Solution</th>
<th>(\text{U}_3\text{O}_8) (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strip Solution-1</td>
<td>Pale Yellow</td>
<td>9.30</td>
<td>15.50</td>
</tr>
<tr>
<td>Strip Solution-2</td>
<td>Pale Yellow</td>
<td>8.95</td>
<td>16.50</td>
</tr>
<tr>
<td>Strip solution -3</td>
<td>Bright Yellow</td>
<td>9.10</td>
<td>25.60</td>
</tr>
</tbody>
</table>

Table 40

Chemical Analyses of Uranium Strip Solutions using 2.0 M \(\text{NH}_4\text{HCO}_3\) as Eluent
Figure 38: Photograph showing the physical appearance of a typical strip solution
Production of Uranium Concentrate (Yellow Cake)

Yellow cake was produced by precipitating the strip solution obtained from the uranium elution studies. Ammonia (NH₃) gas was injected to strip solution to precipitate uranium as ammonium diuranate [(NH₄)₂U₂O₇], commonly known as yellow cake. The precipitate was filtered, washed with water and subsequently dried at 110°C in an electric oven. The end-product (uranium concentrate) was found to had a texture like talc-powder and was readily soluble in acidified water of pH 2.0 heating at 70°C on a hot-plate. No acid insoluble matter was detected. Uranium analyses of the yellow cake samples have been reported in Table 42.

\[
2 \text{UO}_2\text{SO}_4 + 6 \text{NH}_4\text{OH} \rightarrow (\text{NH}_4)_2\text{U}_2\text{O}_7↓ + 2 (\text{NH}_4)_2\text{SO}_4 + 3 \text{H}_2\text{O}
\]

Ammonium diuranate
(yellow cake)

<table>
<thead>
<tr>
<th>Sample</th>
<th>U₃O₈ on dry matter basis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BYC-1</td>
<td>74.80</td>
</tr>
<tr>
<td>BYC-2</td>
<td>82.53</td>
</tr>
<tr>
<td>BYC-3</td>
<td>89.60</td>
</tr>
<tr>
<td>BYC-4</td>
<td>77.30</td>
</tr>
</tbody>
</table>

Table 42

Uranium Analyses of Yellow Cake Samples

Physical appearance of a typical yellow cake sample (BYC-3) containing 89.60% U₃O₈ on dry matter basis was shown in Figure 39.
Figure 39: Physical appearance of a typical yellow cake sample.
The Induced-Coupled Plasma Emission Spectrographic analyses (ICPES) of the yellow cake sample BYC-3 was carried out to check its purity. Results of the ICP analyses have been reported in Table 43. The yellow cake sample contained 75.01% U (89.60% U3O8) on dry matter basis with minor impurities of other metal ions (Table 43). Boron (B) was present in a concentration of 4.0 μg/g of the sample.

When the ICP data of yellow cake sample BYC-3 was compared with the analytical data of an Australian yellow cake sample produced by acid leaching process (Allman et al. 1968), it was found that the bacterially produced yellow cake was much pure and contained less amounts of metal ions impurities as compared with the Australian yellow cake (Table 44). The purity of the yellow cake sample BYC-3 to some extent meet the specifications of the Canadian UO2 powder (Chalder 1961) (Table 44).
<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>Concentration (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>Al</td>
<td>45</td>
</tr>
<tr>
<td>Arsenic</td>
<td>As</td>
<td>ND</td>
</tr>
<tr>
<td>Antimony</td>
<td>Sb</td>
<td>ND</td>
</tr>
<tr>
<td>Boron</td>
<td>B</td>
<td>4</td>
</tr>
<tr>
<td>Barium</td>
<td>Ba</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Beryllium</td>
<td>Be</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Bismuth</td>
<td>Bi</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Calcium</td>
<td>Ca</td>
<td>ND</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Cd</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Co</td>
<td>ND</td>
</tr>
<tr>
<td>Chromium</td>
<td>Cr</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Copper</td>
<td>Cu</td>
<td>2</td>
</tr>
<tr>
<td>Iron</td>
<td>Fe</td>
<td>25</td>
</tr>
<tr>
<td>Indium</td>
<td>In</td>
<td>ND</td>
</tr>
<tr>
<td>Lead</td>
<td>Pb</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Mg</td>
<td>85</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mn</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Mo</td>
<td>ND</td>
</tr>
<tr>
<td>Nickel</td>
<td>Ni</td>
<td>ND</td>
</tr>
<tr>
<td>Sodium</td>
<td>Na</td>
<td>95</td>
</tr>
<tr>
<td>Silver</td>
<td>Ag</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Silicon</td>
<td>Si</td>
<td>320</td>
</tr>
<tr>
<td>Strontium</td>
<td>Sr</td>
<td>ND</td>
</tr>
<tr>
<td>Tin</td>
<td>Sn</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Titanium</td>
<td>Ti</td>
<td>ND</td>
</tr>
<tr>
<td>Uranium</td>
<td>U</td>
<td>75.01%</td>
</tr>
<tr>
<td>Vanadium</td>
<td>V</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zn</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

ND = Not detectable.
<table>
<thead>
<tr>
<th>Constituents</th>
<th>Bacterially Produced Yellow Cake</th>
<th>Australian Rum Jungle Yellow Cake*</th>
<th>Canadian UO₂ Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>45</td>
<td>270</td>
<td>25</td>
</tr>
<tr>
<td>Boron</td>
<td>4</td>
<td>&lt;5</td>
<td>0.3</td>
</tr>
<tr>
<td>Calcium</td>
<td>N.D</td>
<td>550</td>
<td>50</td>
</tr>
<tr>
<td>Cadmium</td>
<td>&lt;0.4</td>
<td>&lt;5</td>
<td>0.2</td>
</tr>
<tr>
<td>Copper</td>
<td>2</td>
<td>150</td>
<td>10</td>
</tr>
<tr>
<td>Iron</td>
<td>25</td>
<td>2200</td>
<td>50</td>
</tr>
<tr>
<td>Silica</td>
<td>325</td>
<td>1000</td>
<td>50</td>
</tr>
<tr>
<td>Sodium</td>
<td>95</td>
<td>28000</td>
<td>50</td>
</tr>
<tr>
<td>Vanadium</td>
<td>&lt;5</td>
<td>&lt;13</td>
<td>100</td>
</tr>
<tr>
<td>Zinc</td>
<td>&lt;50</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Uranium (% U₃O₈)</td>
<td>89.6</td>
<td>78.6</td>
<td>-</td>
</tr>
</tbody>
</table>

* = NaOH precipitated (Rum Jungle B)
N.D. = Not Detectable.
DISCUSSION

Microorganisms are important agents in solubilization, precipitation, and accumulation of heavy metals in environments associated with leaching processes, acid mine-drainages and industrial waste effluents. Such microbial processes may be harmful or beneficial to man. There is worldwide interest in exploring the microbial extraction of U, Cu, Au, and other heavy metals from ores (Tuovinen et al. 1991). During the current metals-market slump, the mineral biotechnology accounts for a greater share of total metals production. Advancements in microbial metals extraction processes requires interdisciplinary research and communication between microbiologists, chemists, and engineers so that the most effective bioprocessing systems could be designed, operated and monitored. Attention has been mainly directed toward acid leaching of sulfide ores as catalyzed by iron- and sulfur-oxidizing bacteria (T. ferrooxidans). Bacterial leaching is especially valuable for leaching of uranium from low-grade ores (≤0.03% U₃O₈) which are uneconomical to extract uranium by conventional hydrometallurgical processes (Bhatti et al. 1991a).

Tyuyamunite [Ca(VO₄)₂(UO₂)₂·5·8 H₂O] was the main uranium mineral present in the sandstone ore sample, which presented the essential radioactivity of the sandstone ore (Moghal 1974; Rehman 1972). It is a uranium-vanadium complex in which uranium is present in hexavalent state [U⁶⁺], which is readily soluble in dilute acid or carbonate-bicarbonate solution as lixiviant (Zaman et al. 1980). In sedimentary-type ore deposits, uranium minerals occur as tyuyamunite [Ca(VO₄)₂(UO₂)₂·5·8H₂O], carnotite [K₂(VO₄)₂(UO₂)₂·1·3H₂O] and uranophane [Ca(SiO₃)₂(UO₂)₂(OH)₂·5·6H₂O] in the oxidized zones, whereas uraninite [UO₂], pitchblende [(U⁴⁺,U⁶⁺)O₂] and coffinite [U(SiO₄)₁·ₓ(OH)₄ₓ] exist in the un-oxidized zones. The oxidized ore is bright greenish yellow amorphous powder, existing above the water table while the unoxidized ore is grey to black in color and is found usually below water table. Uranium minerals are irregularly mixed with ore matrix and occur mainly as a thin coating on pebbles, sand grains and clay balls (Moghal 1974).
About 40% of the world’s reasonably assured deposits of uranium exist in the sandstone-type ore deposits, where uranium minerals occur usually within the grain-binding material. Calcite (CaCO₃) exists up to 5.0% while pyrite (FeS₂) as minor amount in the sedimentary-type ore deposits (Gow 1985). Baghalchur sandstone uranium ore contained 3.5% calcite as the main acid consuming component of the ore matrix. Pyrite was present as 0.1% FeS₂ the only iron-sulfide mineral (Table 7). The predominant mineral constituent of the ore was quartz (Table 7). Magnetite (Fe₃O₄) mineral constituted the major portion of the heavy minerals (Figure 6). Magnetite and pyrrhotite (Fe₁₋ₓS) minerals are considered to be the highly magnetic minerals (Muller 1977). Clay and calcite were found to be the major acid consuming materials of the ore (Zaman et al. 1980). The distribution of uranium was higher (0.021% U₃O₈) in the clay fraction of the ore corresponding to 91.3% U₃O₈ of the total uranium present in the ore sample. The sand fraction of the ore contained only 8.7% U₃O₈ of the total uranium content of the ore (Figure 7). The ore fraction with a particle size of ≥595µm was found as major particle size fraction and the weight of ore fraction was decreased concomittantly with particle size (Table 5).

Efforts were made to isolate indigenous microflora of acidophilic iron- and sulfur-oxidizing thiobacilli from the uranium mill tailings liquid sample. Microbial populations of *T. ferrooxidans*, *T. thiooxidans* and glucose-oxidizing heterotrophs were detected in the tailings liquid samples. Similar results have been reported from a full-scale uranium leaching operation (Tuovinen et al. 1981). The indigenous strains of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTh-1) were isolated and morphologically and physiologically characterized (Table 16). *T. ferrooxidans* (TFe-2) were acidophilic, motile, rod-shaped, gram-negative which morphologically resembled to *T. ferrooxidans*. The isolate oxidized Fe²⁺, pyrite, sulfur and reduced sulfur compounds to sulfuric acid and acidic ferric/metal sulfate (Figures 14, 15, 16 and 17).
Different solid media have been reported for the isolation and enumeration of *T. ferrooxidans*. Amongst these are the membrane filter-Ferrous iron-agar medium (Tuovinen and Kelly 1972), ISP medium (Manning 1975), and the double-layer solid medium (Harrison 1984). FeTSB medium (Johnson et al. 1987) has been reported as a solid medium for simultaneous growth of *T. ferrooxidans* and acidophilic heterotrophs. A new efficient "Gelrite-FeSO₄" solid medium was developed and successfully employed for isolation and enumeration of *T. ferrooxidans* in mine water, tailings liquid, microbially leached solutions and solid samples (Khalid et al. 1993). Dark reddish-brown and circular colonies which were robust and well differentiated, developed on Gelrite-FeSO₄ medium within 72-96 h (Figures 9, 10 and 11).

Sulfur-oxidizing strain designated as *T. thiooxidans* (TTh-1) was morphologically similar to iron- and sulfur-oxidizing bacterium (*T. ferrooxidans*) except that it did not oxidize Fe²⁺ (Table 16). The cells of *T. thiooxidans* (TTh-1) were motile, rod-shaped and closely resembled to *T. thiooxidans*. Off-white and very tiny colonies developed on Agar-S₂O₃⁻ medium, with a characteristics smell of elemental sulfur (Figures 12 and 13). The growth of *T. ferrooxidans* was indexed by the drop in initial pH of the liquid media of sulfur, slag and pyrite, and by the acid production and/or oxidation of ferrous to ferric (Figures 14, 15, 16 and 17). Both strains of *T. ferrooxidans* and *T. thiooxidans* were found in abundance in tailings liquid samples (Table 12).

The microbial population of iron-oxidizing bacteria (*T. ferrooxidans*) was found much higher in the tailings residues which were collected from one and two month old-dried tailings pile (Table 13). In fact, tailings liquid contained a significant amount of FeSO₄ (Figure 8) at the time when solid tailings residue was being dumped in the tailings pond of the mill after acid leaching of sandstone ore. FeSO₄ was oxidized to Fe₂(SO₄)₃ by iron-oxidizing bacteria and produced H₂SO₄ with the formation of a secondary iron(III) mineral called jarosite [KFe₃(SO₄)₂(OH)₆] at pH of ≥2.0. This jarosite formation changed the physical appearance of tailings residue.
Bacterial and chemical oxidation of Fe$^{2+}$:

$$4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ \rightarrow 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$$  \[19\]

Ferric iron hydrolysis:

$$\text{Fe}^{3+} + 2\text{H}_2\text{O} \rightarrow \text{FeOOH} + 3\text{H}^+$$  \[20\]

(Jeolinite)

Jarosite formation:

$$\text{K}^+ + 3\text{Fe}^{3+} + 2\text{SO}_4^{2-} + 6\text{H}_2\text{O} \rightarrow \text{KFe}_3(\text{SO}_4)_2(\text{OH})_6 + 6\text{H}^+$$  \[21\]

(K-jarosite)

Some sulfate-reducing bacteria like Desulfovibrio vulgaris have been reported to produce H$_2$S from SO$_4^{2-}$ under anaerobic environments. The oxidation of hydrogen sulfide resulted to elemental sulfur by Chromatium vinosum in reduced environment (Imai 1986).

$$\text{SO}_4^{2-} \xrightarrow{\text{Desulfovibrio}} \text{H}_2\text{S} \xrightarrow{\text{Chromatium}} \text{S}^0$$  \[22\]

Elemental sulfur can be utilized as energy source by iron- and sulfur-oxidizing bacteria to produce H$_2$SO$_4$ resulting in a pH drop of the tailings residue. Thiobacillus ferrooxidans can also oxidize elemental sulfur in the presence of Fe$_2$(SO$_4$)$_3$ at low pH (Pronk et al. 1992). Sulfuric acid thus produced can change the physical and chemical characteristics of tailings residue. U$_3$O$_8$ is solubilized from this residue by the action of sulfuric acid and seepage pass down into the underground water, thus causing an environmental pollution problem. The presence of glucose-oxidizing heterotrophs was also noted in tailings liquid, microbial leach liquors and solid samples obtained from columns and heap (Tables 12, 29 and 36).

Microbial leaching is a biochemical process involving enzymes as catalyst by which insoluble inorganic substrate is oxidized to a soluble form (Torma 1977). Metals are released from sulfide minerals directly through oxidative metabolism of
microorganisms or solubilized indirectly by chemical oxidants such as ferric sulfate and/or sulfuric acid produced as metabolic products of microorganisms (Lundgren et al. 1986). Ferric ions (Fe$^{3+}$) resulting from this reaction can chemically degrade sulfides, including pyrite (FeS$_2$), according to the following reaction:

$$\text{FeS}_2 + 2\text{Fe}^{3+} + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 3\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 4\text{H}^+ \quad [23]$$

There is some evidence that the reaction described by equation [23] proceeds via two steps: one of which, reaction [25] is catalyzed by acidophilic thiobacilli:

$$\text{FeS}_2 + 2\text{Fe}^{3+} \rightarrow 3\text{Fe}^{2+} + 2\text{S}^0 \quad [24]$$

$$2\text{S}^0 + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + 2\text{SO}_4^{2-} \quad [25]$$

Although the rate of abiotic ferrous iron oxidation at low pH-values is very slow, it can be accelerated to $10^5$-$10^6$ times in the presence of iron-oxidizing bacteria (Tuovinen and Kelly 1973). In the present studies on bacterial oxidation of ferrous iron, it was observed that ferrous iron was completely oxidized to ferric iron by *T. ferrooxidans* during 60-hrs of incubation (Figure 14). Bacterial oxidation of pyrite by *T. ferrooxidans* resulted in a sulfuric acid production followed by a drop in the initial pH of the leaching system (Figure 15). *T. ferrooxidans* oxidizes ferrous iron at much faster rates as compared to a solely chemicals system. The indigenous strain of *T. thiooxidans* (TTh-1) produced higher amount of sulfuric acid by oxidation of sulfur and sulfur slag as compared with *T. ferrooxidans* (TFe-2) (Figure 16). Sulfuric acid thus produced during the bacterial oxidation of sulfides, thereby accelerates the rate of metal solubilization. Sulfuric acid also neutralizes carbonate materials like calcite (CaCO$_3$) and dolomite [(Ca,Mg)CO$_3$] present in the ore matrix (Bhatti et al 1991a). Incomplete oxidation of the sulfide entity commonly occurs in the acid leaching process which results in the formation of polythionates and the precipitation of elemental sulfur. The latter effectively coats the metal sulfides and prevents their further oxidation until the sulfur is removed by bacterial oxidation.
The microbial degradation of silicate minerals requires the availability of external energy substrates (Ralph 1985). Therefore, in the present studies on bioleaching of sandstone uranium ore, external energy substrates in the form of sulfur, sulfur slag and pyrite were employed (Figure 25). The present work is believed to be the first in reporting uranium extraction from sandstone type ore deposits which has been up-scaled to a microbial heap leaching process. Baghalchur sandstone uranium ore contained a minor amount of pyrite content which is the main energy source for bacterial leaching process. The relationship between the characteristics of the uranium ore and the necessary amount of elemental sulfur and/or sulfur slag required for uranium bioleaching process was also determined. In general, the addition of 4.0-7.0% (wt/wt) elemental sulfur and/or sulfur slag was sufficient to induce bacterial leaching of uranium from indigenous sandstone-type ore deposit (Figure 29a).

*T. ferrooxidans* (TFe-2) solubilized much uranium from low-grade sandstone uranium ore as compared to *T. thiooxidans* (TTh-1) (Figure 21a). When the ore was added into the medium, first the calcite content of ore was neutralized by sulfuric acid which caused an increase in the pH of the ore suspension. The pH again dropped as the bacteria produced acid by bacterial oxidation of sulfur (Figure 22a). This drop in pH resulted in solubilization of uranium from ore (Figure 21a). Many laboratory studies have demonstrated that these acidophilic bacteria can accelerate leaching rates and increase the uranium recovery under controlled conditions (Lundgren and Silver 1980; Tuovinen 1986). A mixed culture of *T. ferrooxidans* and *T. thiooxidans* enhanced the uranium recovery from sandstone ore. Similar results have been reported by Bosecker and Wirth (1980).

\[
2S^0 + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \quad [26]
\]
\[
2Fe^{2+} + \frac{1}{2}O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad [27]
\]
\[
UO_2 + 2Fe^{3+} \rightarrow UO_2^{2+} + 2Fe^{2+} \quad [28]
\]
\[
UO_2 + 2H^+ + \frac{1}{2}O_2 \rightarrow UO_2^{2+} + H_2O \quad [30]
\]
Reactions [26] and [27] are catalyzed by the action of acidophilic thiobacilli. The solubilization of uranium from ores involves its oxidation from \( U^{4+} \) to \( U^{6+} \) which is generally affected through indirect action of bacteria which produce acidic ferric sulfate solutions (Guay et al. 1977; DiSpirito and Tuovinen 1982a, 1982b).

During microbial leaching of low-grade sandstone uranium ore, the elemental sulfur and sulfur slag supplied as external energy sources, were oxidized to sulfuric acid and ferric sulfate solution by the action of iron and sulfur oxidizing acidophilic thiobacilli. Both metabolites, sulfuric acid as well as ferric sulfate solution act as lixiviant during leaching processes. The physical appearance of bacterial leach liquor samples was light green indicating the presence of soluble iron salts and was very similar to acid leach liquor of uranium ore-processing plant. The pH of bacterial leach liquor was observed within the range of 1.5-1.9 after 30-days of incubation (Figure 21b).

In the present studies, bioleaching of sandstone uranium ore seemed to be more feasible after previous neutralization of carbonate or with simultaneous addition of sulfuric acid. Partial neutralization of the leaching system sufficiently provided the pH range of the leach suspension that was suitable for bacterial growth. Over 85% \( U_3O_8 \) was leached out microbi ally from sandstone ore when the pH-value of the ore-suspension was below 2.0 (Figure 22a). These results indicated that the microorganisms do not directly attack the uranium mineral, they rather set up chemical conditions suitable for the dissolution of uranium from ore. \( T. \) *thiooxidans* also play an effective role in the extraction of uranium from ores (Khalid et al. 1990).

*T. ferrooxidans*, which is more sensitive to pH fluctuations than *T. thiooxidans*, and is most active in the pH range of 1.8 to 2.5 (Bosecker and Wirth 1980; Torma 1986). Strains of *T. ferrooxidans* that had developed resistance to high levels of uranium oxidized \( U^{4+} \) faster than strains that were not previosuly exposed to uranium. Sub-culturing of the uranium-resistant strains in the absence of uranium results in a loss of resistance and a decreased rate of oxidation (DiSpirito and Tuovinen 1982b). These relationships are indicative of the biological activity which does not exclude the
Higher amount of uranium solubilization was obtained from ore amended with sulfur slag as compared with sulfur and pyrite (Figure 25). This might be due to high amount of sulfuric acid production and the presence of Fe$^{2+}$ and Fe$^{3+}$ in the slag (Table 10). Sandstone ore amended with sulfur (4.0-6.0% wt/wt) and slag (5.0-6.0% wt/wt) yielded maximum uranium recovery by mixed culture of *T. ferrooxidans* (TFe-2) and *T. thiooxidans* (TTTh-1) (Figure 29a).

An increase in ore pulp density may result in an inhibitory or even toxic effect on the growth of thiobacilli. Uranium extraction was found to be inversely proportional to the pulp density (Figure 30), which is in agreement with the findings of Torma et al. (1985). In practice, the optimum particle size has to be determined for each kind of ore to be leached. The particle size is dictated by the relative economics which is based on two alternatives: the gains due to increased rate of metal extraction by decreasing the particle size and the grinding cost (Torma and Rozgonyi 1980). The higher uranium recovery at smaller particle size can be explained by the fact that uranium recovery is a function of exposed surface area (Torma 1977). Uranium recovery of 90% $\text{U}_3\text{O}_8$ was obtained by *T. ferrooxidans* from an ore of particle size of about 4.0 mm in the presence of iron concentration varying from 0-1.0 g/L (Harrison et al. 1966). Similarly, a uranium recovery 80-90% $\text{U}_3\text{O}_8$ was solubilized by *T. ferrooxidans* from the Ningyo-Toge ore, which was ground to a particle size of about 12.5 mm (Tomizuka and Takahara 1972). Results of the present studies indicated that maximum uranium recovery of 89.12% $\text{U}_3\text{O}_8$ was obtained from an ore fraction with particle size of $\leq 53\mu$m (Table 20). A decrease in the grain size means an increase in the particle-specific surface and in the total surface, so that higher yields of metal can be obtained with no change in the total mass of the particles. A decrease in grain size leads not only to an enlargement of the sulfide minerals but also to an enlargement of the surface of gangue material. A grain size of about 42$\mu$m has been reported for optimum metal recovery (Torma 1977).
Bioreactor leaching of sandstone uranium ore proceeded at higher rates for uranium extraction than achieved in the shake flask leaching experiments (Table 21). This might be due to better stirring and aeration of the leach suspension in tank leaching experiments. This is in agreement with Bosecker and Wirth (1980), who reported for bioleaching of sedimentary-type uranium ore from Fortsua, Austria.

Uranium recovery from Baghalchur sandstone ore was correlated to the sulfuric acid consumed by high CaCO₃ content which was present as limestone and dolomite in the ore matrix. Sulfuric acid produced by the biochemical oxidation of sulfur and/or sulfur slag reacted with these minerals according to the following equations:

$$\text{CaCO}_3 + \text{H}_2\text{SO}_4 \rightarrow \text{CaSO}_4 + \text{CO}_2 + \text{H}_2\text{O}$$  \hspace{2cm} [31]

(Calcite)

$$\text{(Ca,Mg)CO}_3 + \text{H}_2\text{SO}_4 \rightarrow \text{(Ca,Mg)SO}_4 + \text{CO}_2 + \text{H}_2\text{O}$$  \hspace{2cm} [32]

(Dolomite)

$$\text{(Ca,Mg)O,Al}_2\text{O}_3,5\text{SiO}_2,\text{nH}_2\text{O} + \text{H}_2\text{SO}_4 \rightarrow \text{Al}_2(\text{SO}_4)_3 + \text{(Ca,Mg)SO}_4 + 5\text{H}_3\text{SiO}_3 + (\text{n-1})\text{H}_2\text{O}$$  \hspace{2cm} [33]

(Clay)

Calcium carbonate (CaCO₃), the major acid consuming component of sandstone uranium ore, is responsible for generation of CO₂ as well as the formation of very fine precipitate of CaSO₄ [Equations 31 and 32]. Therefore, the pH of column effluents increased continuously and CO₂ produced was utilized by chemolithoautotrophs. Calcium sulfate thus formed blocked to some extent the capillaries for liquid percolation. Similarly, sulfuric acid also reacts with the clay minerals present in the ore matrix [Equation 33]. The water soluble metal sulfate(s) were leached out with the formation of colloidal silica (SiO₂) and alumina (Al₂O₃). Their behaviour was similar to that of the calcium sulfate.
The iron oxides present as hematite (Fe₂O₃) and magnetite (Fe₃O₄) minerals in the ore also consumed H₂SO₄. Iron that was present both in ferrous and ferric states in the ore minerals and sulfur slag, also reacted with sulfuric acid and produced ferrous and ferric sulfate solutions as described in the reactions:

\[
\text{FeO} + \text{H}_2\text{SO}_4 \rightarrow \text{FeSO}_4 + \text{H}_2\text{O} \\
\text{(Oxide)}
\]

[34]

\[
\text{Fe}_2\text{O}_3 + 3 \text{H}_2\text{SO}_4 \rightarrow \text{Fe}_2(\text{SO}_4)_3 + 3 \text{H}_2\text{O} \\
\text{(Haematite)}
\]

[35]

\[
\text{FeO(OH)} + \text{H}_2\text{SO}_4 \rightarrow \text{FeSO}_4 + \text{H}_2\text{O} \\
\text{(Hydroxides, Geothite)}
\]

[36]

FeSO₄ produced in equations [34] and [36] is oxidized to Fe₂(SO₄)₃ at low pH by *T. ferrooxidans* which acted as lixiviant for uranium solubilization from ore. However, when iron bearing solution was recycled and percolated through columns, colloidal iron was precipitated as ferrous or ferric hydrated oxides, and CaCO₃ content of the ore matrix was not completely neutralized. These iron hydrated oxides are gelatinous in nature and responsible for rendering the sections of the ore bed totally impervious.

\[
\text{FeSO}_4 + \text{CaCO}_3 + \text{H}_2\text{O} \rightarrow \text{Fe(OH)}_2 + \text{CaSO}_4 + \text{CO}_2 \\
\]

[37]

\[
\text{Fe}_2(\text{SO}_4)_3 + 3 \text{CaCO}_3 + 4 \text{H}_2\text{O} \rightarrow 2 \text{Fe(OH)}_3 + 3 \text{CaSO}_4 + \text{H}_2\text{O} + 3 \text{CO}_2 \\
\text{[38]}
\]

\[
\text{UO}_2 + \text{Fe}^{3+} \rightarrow \text{UO}_2^{2+} + \text{Fe}^{2+} \\
\text{[39]}
\]

\[
\text{UO}_2^{2+} + \text{SO}_4^{2-} \rightarrow \text{UO}_2\text{SO}_4 \\
\text{[40]}
\]

\[
\text{UO}_2 + 2 \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{UO}_2^{2+} + \text{H}_2\text{O} \\
\text{[41]}
\]

\[
\text{Fe}_2(\text{SO}_4)_3 + \text{FeSO}_4 + \text{CaCO}_3 + \text{UO}_2\text{SO}_4 + \text{H}_2\text{O} \rightarrow \\
\text{Co-precipitate hydrated oxides} \\
\text{[42]}
\]
The most important constituent of the standard 9K medium (Silverman and Lundgren 1959) is ammonium sulfate. For this purpose, a fertilizer grade ammonium sulfate was used as a nitrogen nutrient during microbial column and heap leaching studies. The addition of ammonium sulfate (3.0 g/L) in mine water has influenced the uranium recovery (Figure 33). Tomizuka and Takahara (1972) reported higher recovery of uranium from the Ningyo-Toge ores with the addition of 0.30% ammonium sulfate as nitrogen source for \textit{T. ferrooxidans}. Results also demonstrated higher uranium leachability by using acidified mine water. The column containing ore amended with sulfur slag and irrigated with mine water of pH 3.5, exhibited 90% U\textsubscript{3}O\textsubscript{8} recovery, which was the highest during the present studies.

The pH-values of ore slurry samples taken from columns at the depth of 00-30 cm (at the top) was decreased to pH 3.4 from an initial pH-value of 7.8. However, the coloration of the leached core was changed from gray to reddish brown in columns C and D only. This change might be attributed to the formation of jarosite. The pH of the core samples taken from the top (00-30 cm) of the all columns drastically decreased to pH 2.7, probably due to the availability of much oxygen (Table 26). Thus, the bacterial activity in the top surface of columns enhanced the rate of oxidation of elemental sulfur and/or sulfur slag to produce H\textsubscript{2}SO\textsubscript{4}. However, as the depth of the ore bed increased, the availability of air and/or oxygen became limited. The color of ore in columns A and B was similar to the ore feed (grey). It was light yellow in column C, whereas dark brown/brick red in the column D. In all the columns, the pH-values of the core samples were found to increase with the increase of depths. Therefore, the pH of the columns effluents was found in the pH range of 2.4-4.4 (Table 26). It was probably due to the formations of microsites in the leaching system in which bacteria produced sulfuric acid, and thereby resulting in the solubilization of uranium. However, the pH of the column effluent raised as it passed through the un-neutralized portion of the calcite present in the ore matrix.

Physical appearance of the bacterial leach liquors was also found to be turbid and dark yellow in column D (Figure 35). In addition, soluble as well as precipitated
Fe$^{3+}$ was also found in the column effluents. There was a visual evidence of the formation of jarosite, particularly towards the base of the columns C and D, where there were areas of higher alkalinity. This jarosite is reported to hinder the leaching process by coating the ore particles (Madgwick and Ralph 1980).

Microbial population of acidophilic iron- and sulfur-oxidizing bacteria (*T. ferrooxidans*) were significantly higher in column D as compared to other columns (Table 27). However, column A, in which the ore was amended with elemental sulfur was predominantly loaded with sulfur-oxidizers (*T. thiooxidans*) (Table 28). Besides these two major groups of acidophilic thiobacilli, other microbial groups of acodophilic heterotrophs and S-oxidizers growing at pH 6.6 were also isolated and enumerated in the PVC columns (Tables 29 and 30).

In microbial heap leaching process, initially the uranium recovery from ore was encouraging but later the dissolved uranium was precipitated/entrapped into off-white sludge (Table 37). The formation of sludge during the microbial heap leaching operation was due to high calcium content of the NIBGE subsoil water that was used for inocula preparation (Table 38). Calcium ions are reported as the best host for uranium mineralization (Barthel 1980).

Uranium loading capacity of Amberlite IRA-400 was reported to be pH dependent and the maximum loading capacity was reported between 4.0-5.0 lbs U$_3$O$_8$/ft$^3$ at pH of 1.8-2.0 (Gow et al. 1971). In present studies, Amberlite IRA-400 adsorbed more than 97.6% U$_3$O$_8$ of the uranium present in the bacterial leach liquor of pH 2.0±0.1 (Table 39). Ion-exchange has two inherent advantages over the solvent extraction process. This process can treat solutions with a high content of suspended solids and does not introduce environmental contaminants into the discharge solutions. Moreover, it can have a cost advantage in the treatment of high flow rates of low uranium tenor solution (Merritt 1971).
Bicarbonate \((\text{HCO}_3^-)\) is the preferred additive in operations using \(\text{NaCl}\) solution for stripping the loaded uranium from resins and a 99.0\% \(\text{U}_3\text{O}_8\) elution efficiency has been reported with the addition of bicarbonate in the eluent (Merritt 1971). The stripping efficiency of 2.0 M \(\text{NH}_4\text{HCO}_3\) solution showed 99.0\% \(\text{U}_3\text{O}_8\) (Table 40). The ICP analyses of the yellow cake sample revealed that it contained 89.6\% \(\text{U}_3\text{O}_8\) (75.01\% \(\text{U}\)) with minor impurities of other metal ions (Table 43). The purity of this uranium concentrate meets the specification of Canadian \(\text{UO}_2\) to some extent (Table 44). The high purity of the yellow cake produced from the bacterial leach liquor might be due to selective leaching of uranium from ore.

The results shown above clearly demonstrate that the indigenous microflora of \(\text{T. ferrooxidans}\) and \(\text{T. thiooxidans}\) were efficient starting and effectively leaching the low-grade sandstone uranium ores. To the best of my knowledge, such experiments on microbial heap leaching of sedimentary-type ore have not been yet reported. As a consequence of these studies, a heap of 5,000 tons of low-grade sandstone ore is planned at the mine site, Baghalchur, D.G. Khan, for extraction of uranium on commercial scale. Apparently, the economic basis for the installation and running of the heap leaching process is sound. There is no doubt that mineral biotechnology will paly an increasingly important role in hydrometallurgy and indeed its development represents the low-cost and environmentally safe process.
REFERENCES


Choi PC and W Hopkin (1988) Percolator leach test on copper ore from Cerro Colorado, Panama. In: Biohydrometallurgy (PR Norris and DP Kelly eds.,) Science


CNA (1989) How important is nuclear energy in the world?; Nuclear Facts No. 16, Canadian Nuclear Association, Toronto.


Darnall DW (1990) Removal and recovery of heavy metal ions from waste-waters


Ehrlich HL and SI Fox (1967) Environmental effects on bacterial copper extraction

Elevatroski EA (1978) Uranium ores and minerals. MINOBRAS, Dana Point, California, USA.


Gow WA (1985) Recent advances in uranium ore processing. In: Advances in uranium ore processing and recovery from non-conventional resources. IAEA-TC-491/1, pp 3-13.


Hardwick TJ (1979) Acid leach of uranium ore: In-situ conditions. SPE-AIME Fall Mett, Las Vegas.


Karavaiko GI and SN Groudev (1985) Biogeotechnology of Metals. UNEP Centre of International Projects, GKNT, Moscow.


King DJ and PM Blithe (1979) The impact of the design of uranium extraction plants due to the use of a fluidized-bed solid ion-exchange loading system. Can Min Metall Bull 72(805): 135.


Manchee RJ (1977) Laboratory scale bacterially assisted leaching of Canadian uranium ores. Trans Inst Min Metall C80: 126-133.


Taylor JH and PF Whelan (1943) The leaching of cuprous pyrites and the precipitation of copper at Rio Tinto, Spain. Trans Inst Min Metall 52: 36-96.


Tuovinen OH and DP Kelly (1973) Studies on the growth of *Thiobacillus ferrooxidans*. I. Use of membrane filters and ferrous iron agar to determine viable numbers and comparison with $^{14}$CO$_2$ fixation and iron oxidation as measure of growth. Arch


