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Oxidation and Control of Iron in Bioleaching Solutions

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ERRATA AND UPDATES

Page i, 5th paragraph, last line, "retention time of 1 h" should read "retention time of 1–2 h"
Page v, 1st paragraph, line 5, "alle yhden tunnin" should read "alle 1–2 tunnin"
Page x, Paper VI, "Hydrometallurgy, *submitted*" now "Hydrometallurgy, *in press*"
Page 4, 2nd paragraph, line 1, "section 1.4" should read "section 1.5"
Page 6, Table 1, caption, should read also "(modified after Kinnunen (2004))"
Page 9, Table 2, caption, should read also "(modified after Kinnunen (2004))"
Page 19, Table 4, caption, should read also "(extended from Kinnunen (2004))"
Page 49, 1st paragraph, line 2, "pH 0.85±0.15" should read "pH 1.0±0.3"
Page 56, 1st paragraph, lines 1–2, "As in the KOH… with increasing pH." should be removed
Page 58, 1st paragraph, line 8, "similarity of 100%. Occasionally, also sequences matching "*Ferrimicrobium acidiphilum*" were seen. "*Ferrimicrobium acidiphilum*" has not been formally characterized but has been found in several mine drainage environments (Bacelar-Nicolau and Johnson 1999, Bond *et al*. 2000, Brofft *et al*. 2002)." should read "similarity of 98–100%. Occasionally, also sequences matching *Ferrimicrobium acidiphilum* (Johnson *et al*. 2009) were seen."
Page 72, References, should read also "Johnson, D. B., Bacelar-Nicolau, P., Okibe, N., Thomas, A., Hallberg, K. B. 2009. *Ferrimicrobium acidiphilum* gen. nov., sp. nov. and *Ferrithrix thermotolerans* gen. nov., sp. nov.: heterotrophic, iron-oxidizing, extremely acidophilic actinobacteria. International Journal of Systematic and Evolutionary Microbiology 59:1082–1089.
Page 73, References, should read also "Kinnunen, P. 2004. High-rate ferric sulphate generation and chalcopyrite concentrate leaching by acidophilic microorganisms. Thesis for the degree of Doctor of Technology. Tampere University of Technology. TTY-Paino, Tampere, Finland, 56 p."

Paper I:
Page 1123, under equation 3, "*S* is Fe^{2+} concentration" should read "*S* is initial Fe^{2+} concentration" and "inhibition concentration" should read "inhibition constant"
Page 1123, under equation 7, "*I* is inhibitor concentration" should read "*I* is initial inhibitor concentration" and "*I* value can be calculated… in the batch bottles." should be removed
Page 1126, 1st conclusion, "in the presence of 2 g/L of Fe^{2+}" should read "in the presence of 4 g/L of Fe^{2+}"

Paper II:
Page 141, 5th paragraph, lines 7 and 9, "within 70 days" and "within 45 days" should read "within 70 hours" and "within 45 hours"
Page 141, 5th paragraph, line 13, "Zn^{2+}" should read "Ni^{2+} and Zn^{2+}"
Page 143, 3rd paragraph, line 6, "40–60 g/L Fe^{3+}" should read "50–60 g/L Fe^{3+}"

Paper IV:
Page 115, 3rd paragraph, last line, "for the set" should read "for the test set"
Page 115, 4th paragraph, line 6, "(Fig. 4)" should be removed

Paper V:
Page 1316, Table 1, should read also "(extended from Kinnunen (2004))"
Page 1318, 2nd paragraph, line 9, "4–0.7 mg O_2/L" should read "4 to 0.7 mg O_2/L"

Paper VII:
Section "Experimental data", 5th paragraph, line 8, "1.5 g O_2/L h" should read "1.2 g O_2/L h"

ABSTRACT

In bioleaching processes biological oxidation of iron and sulfur is exploited to solubilize and recover metals from low-grade sulfide minerals. Ferric iron produced by the microorganisms acts as an oxidizing agent in this process. Iron control, i.e., partial removal, is needed to prevent the excessive accumulation of dissolved iron in bioleaching circuits. The final effluents from these processes have to be treated effectively to neutralize the streams and to decrease the high concentrations of dissolved iron and sulfate.

The objective of this study was to improve the effectiveness of bioleaching and iron removal from hydrometallurgical effluents by developing bioprocesses for the oxidation and control of iron to be used in these applications. The Fe^{2+} oxidation kinetics by a *Leptospirillum ferriphilum* dominated fluidized-bed culture at pH 1 was determined and models describing the individual and combined effects of inhibitors on the kinetics developed for predicting oxidation rates and thus determining the required retention times of Fe^{2+} oxidation bioreactors. For ferric iron regeneration and iron control during heap bioleaching and for iron and sulfate removal from bioleaching and other hydrometallurgical effluents an integrated laboratory-scale bioprocess was developed. The process involved a low-pH fluidized-bed reactor (FBR) with activated carbon matrix for continuous biological iron oxidation coupled with a pH adjustment unit and a gravity settler for precipitative removal of iron and sulfate. The objective in the development of the integrated bioprocess was to demonstrate the technical feasibility to couple biological Fe^{2+} oxidation with precipitative iron and sulfate removal, and to determine some of the key operational limits and optimal conditions for such a process on a laboratory scale. The performance of the system was modeled in order to recognize and verify the associations between operational and performance variables.

The *L. ferriphilum* dominated culture tolerated high concentrations of the test metals. Fe^{2+} oxidation proceeded even at the maximum Fe^{2+} (20 g/L) or Fe^{3+} (60 g/L) concentrations tested although specific Fe^{2+} oxidation rate decreased at above 4 g Fe^{2+}/L and 5 g Fe^{3+}/L indicating substrate and product inhibition, respectively. Fe^{2+} oxidation also proceeded in the presence of binary combinations of 40 g Fe^{3+}/L + 10 g Ni^{2+}/L, 30 g Fe^{3+}/L + 40 g Zn^{2+}/L, 60 g Zn^{2+}/L + 10 g Ni^{2+}/L and 60 g Ni^{2+}/L + 10 g Zn^{2+}/L. The results show that it is possible to use high concentrations of ferric sulfate in two-stage tank bioleaching applications for zinc and nickel concentrates.

The model accounting for substrate inhibition, i.e., Haldane equation, described well the Fe^{2+} oxidation kinetics in the presence of only Fe^{2+}. Fe^{3+} was found to competitively inhibit Fe^{2+} oxidation, and Zn^{2+} and Ni^{2+} toxicity conformed to the non-competitive inhibition model. The order of increasing toxicity was Fe^{3+}>Zn^{2+}>Ni^{2+}. For describing the combined effects of Fe^{3+} (a competitive inhibitor) and Zn^{2+} or Ni^{2+} (non-competitive inhibitors) or two non-competitive inhibitors a combined model was developed which fairly accurately described the experimental data. The developed model can be used as basis for developing kinetic models for various bioleaching processes provided that the kinetic constants are determined for process-specific metals and microorganisms.

With the integrated laboratory-scale bioprocess, experiments were conducted with simulated and real barren heap leaching solution and excellent performance was demonstrated. The highest Fe^{2+} oxidation rates in the FBR were 10 g Fe^{2+}/L·h (conversion efficiency of 96%) and 8.2 g Fe^{2+}/L·h (conversion efficiency of 99%) for the simulated and real heap leaching solution, respectively. Below the retention time of 1 h oxygen mass transfer from gas to liquid

limited iron oxidation rate. For < 30% Fe^{3+} precipitation, the optimum pH range was between 1.5 and 2.0. At pH 2, the concurrent Fe^{2+} oxidation and partial precipitative iron removal was optimized at the retention time of the FBR of 1.5 h, with iron oxidation rate of 5.1 g Fe^{2+}/L·h and 37% iron removal.

For the treatment of effluents from bioleaching processes, the process was further developed by using KOH and $CaCO_3$ to neutralize the pH in the range of 2.5–3.5. Upon increasing the pH, the Fe^{2+} oxidation rate remained high and ferric iron removal was significantly enhanced as the precipitation increased to optimally 96–99%. During the optimal process conditions also averagely 66% of the sulfate precipitated. Iron and sulfate precipitated mainly as jarosites.

It can be concluded that the process described above has potential for high-rate and high-efficiency ferric iron regeneration and control and for high-rate and high-efficiency precipitative iron and sulfate removal from solutions in bioleaching operations and possibly also other hydrometallurgical effluents and acid mine drainage. Scientific studies have not previously been published on combined ferric iron regeneration and control aiming at improving the efficiency of heap bioleaching. Compared to the most commonly used chemical iron oxidation and removal techniques, the developed bioprocess has the advantages of operating at ambient temperatures and pressures, not needing chemical reagents for iron oxidation and avoiding the formation of voluminous ferric hydroxide precipitates. The few other iron removal methods utilizing biological iron oxidation reported in scientific journals have not been developed or tested for bioleaching solutions. Compared to the few related patented bioprocesses the bioprocess developed in this study removes also sulfate in addition iron, and achieves comparable or better iron and sulfate removal performances with simpler reactor configuration. Further research is needed on the performance of the process on a larger scale to determine the detailed design criteria and scale-up principles for the individual unit operations and to optimize the performance of the system. Especially the optimization of the precipitation and gravity settling needs attention because the hydraulic conditions are extremely important in terms of determining the properties of the Fe(III) precipitates produced and the required downstream processing and disposal measures.

Effective management of complex bioleaching systems and hydrometallurgical effluent treatment processes requires models that accurately describe these systems. For the data from the experiments with the simulated heap leaching solution an artificial neural network (ANN) based model was applied to model the Fe^{3+} production in the integrated reactor system. The proposed model reliably (R^2 value of 0.90) predicted the effluent Fe^{3+} concentration based on the selected operational parameters (influent and effluent pH, redox and dissolved oxygen concentration in the FBR, retention time of the FBR, and Fe^{2+} loading rate). For the data from the experiments with the barren heap leaching solution, three modeling approaches were applied. Self-organizing maps provided a useful visualization tool which also enabled the recognition of the relationships between process parameters. An ANN based model reliably predicted the amounts of ferric iron precipitated based on the selected operational parameters (feed pH and Fe^{2+} concentration, dissolved oxygen concentration, pH and redox in the FBR, and retention time of the FBR). The best-fitting regression model also gave a good fit (R^2 value of 0.87) with the experimental data used but the ANN model was found to perform most accurately (R^2 value of 0.97). This study demonstrated that ANN type models can be used to determine the effects of changes in process parameters on the performance of complex processes once they have been calibrated for the process in question with experimental data.

ANN modeling successfully applied to the laboratory-scale processes suggests its applicability also in modeling and managing similar kind of larger scale processes.

In summary, this study enables further development of bioleaching and hydrometallurgical effluent treatment processes by providing new insights into the Fe^{2+} oxidation kinetics of *L. ferriphilum* at high dissolved metal concentrations at pH 1 and into the combined effects of multiple metals on Fe^{2+} oxidation kinetics, that have not been previously modeled. This is also the first published work to systematically report and model concurrent high-rate biological ferric iron regeneration and control in bioleach liquors, and iron and sulfate removal from solutions associated with bioleaching processes.

TIIVISTELMÄ

Bioliuotuksessa hyödynnetään mikrobiologista raudan ja rikin hapettumista arvometallien liuottamiseksi ja talteenottamiseksi köyhistä sulfidimineraaleista. Tässä prosessissa hapettimena toimii mikro-organismien tuottama ferrirauta. Bioliuotussovelluksissa liuenneen raudan liiallinen kertyminen kierrätysliuoksiin on estettävä raudan pitoisuutta kontrolloimalla eli raudan osittaisella poistolla. Bioliuotusprosessien lopulliset jätevedet on käsiteltävä vesien neutraloimiseksi ja liiallisen raudan ja sulfaatin poistamiseksi.

Tämän tutkimuksen tavoitteena oli parantaa bioliuotuksen ja hydrometallurgisten prosessivesien raudanpoiston tehokkuutta kehittämällä näissä sovelluskohteissa raudan hapettamiseen ja poistoon käytettäviä bioprosesseja. Työssä määritettiin pääasiassa *Leptospirillum ferriphilum* -bakteereja sisältävän leijupetiviljelmän raudan hapetuskinetiikka pH:ssa 1. Lisäksi kehitettiin malleja kuvaamaan raudan hapetusta inhiboivien aineiden yksittäisiä ja yhteisvaikutuksia hapetuskinetiikkaan raudan hapetusnopeuksien ennustamiseksi ja näin ollen raudanhapetukseen käytettävien bioreaktoreiden edellyttämien viipymäaikojen määrittämiseksi. Työssä kehitettiin ja testattiin integroitua laboratoriomittakaavan bioprosessia ferriraudan uudelleentuottamiseksi ja raudan pitoisuuden kontrolloimiseksi biokasaliuotuksen aikana, sekä raudan ja sulfaatin poistamiseksi bioliuotuksen ja muista hydrometallurgisista prosessivesistä. Prosessi koostui jatkuvatoimiseen biologiseen raudanhapetukseen käytetystä alhaisen pH:n leijupetireaktorista (FBR), sekä saostamiseen ja selkeytykseen perustuvaa raudan- ja sulfaatinpoistoa varten tähän yhdistetyistä pH:n säätöyksiköstä ja laskeutusaltaasta. Leijupetireaktorissa käytettiin kantaja-aineena aktiivihiiltä. Integroidun bioprosessin kehittämisen tavoitteena oli osoittaa laboratoriomittakaavassa, että biologinen raudan hapetus on teknisesti mahdollista yhdistää saostukseen ja laskeutukseen perustuvaan raudan- ja sulfaatinpoistoon, sekä määrittää joitakin keskeisiä toimintarajoja ja optimaalisia olosuhteita kyseiselle prosessille. Prosessin suorituskykyä mallinnettiin toiminnallisten ja suorituskykymuuttujien välisten yhteyksien tunnistamiseksi ja varmistamiseksi.

L. ferriphilumin hallitsema viljelmä sieti korkeita pitoisuuksia tutkittuja metalleja. Rautaa hapettui jopa suurimmissa tutkituissa pitoisuuksissa (20 Fe^{2+} g/L tai 60 Fe^{3+} g/L), vaikka raudan ominaishapetusnopeus pieneni yli 4 g Fe^{2+}/L ja 5 g Fe^{3+}/L pitoisuuksissa, mikä viittasi substraatti- ja lopputuoteinhibitioon. Viljelmä hapetti rautaa myös seuraavissa yhdistelmäpitoisuuksissa: 40 g Fe^{3+}/L + 10 g Ni^{2+}/L, 30 g Fe^{3+}/L + 40 g Zn^{2+}/L, 60 g Zn^{2+}/L + 10 g Ni^{2+}/L ja 60 g Ni^{2+}/L + 10 g Zn^{2+}/L. Tulokset osoittavat, että kaksivaiheisessa sinkki- ja nikkelirikasteiden bioreaktoriliuotuksessa on mahdollista käyttää suuria ferrisulfaattipitoisuuksia.

Substraatti-inhibition huomioiva Haldane-yhtälö kuvasi hyvin raudan hapetuskinetiikkaa ferroraudan läsnä ollessa. Ferriraudan inhibitiovaikutus raudanhapetukseen oli luonteeltaan kilpailevaa, ja kilpailemattoman inhibition malli kuvasi Zn^{2+}:n ja Ni^{2+}:n vaikutusta. Metallien toksisuus kasvoi järjestyksessä Fe^{3+}>Zn^{2+}>Ni^{2+}. Työssä kehitettiin myös malli kuvaamaan ferriraudan (kilpaileva inhibiittori) ja sinkin tai nikkelin (kilpailemattomia inhibiittoreita) tai kahden kilpailemattoman inhibiittorin yhteisvaikutuksia. Kehitetty malli vastasi kokeellista aineistoa melko tarkasti. Kyseistä mallia voidaan käyttää lähtökohtana, kun kehitetään kineettisiä malleja erilaisiin bioliuotussovelluksiin edellyttäen, että kineettiset vakiot on määritetty prosessikohtaisille metalleille ja mikro-organismeille.

Integroidulla laboratoriomittakaavan bioprosessilla suoritettiin kokeita käyttäen simuloitua ja todellista biokasaliuotuksen prosessivettä, ja tulokset olivat erinomaisia. Suurin leijupetireaktorissa saavutettu raudan hapetusnopeus oli simuloidulla liuoksella 10 g Fe^{2+}/L·h (hapetustehokkuus 96%) ja todellisella prosessivedellä 8,2 g Fe^{2+}/L·h (hapetustehokkuus 99%). Alle yhden tunnin viipymäajoilla hapen aineensiirto kaasusta liuokseen rajoitti hapetusnopeutta. Optimaalinen pH-alue alle 30% raudan saostumiselle oli 1,5–2,0. Yhdistetty raudan hapettuminen ja osittainen poisto olivat pH:ssa 2 suurimmillaan leijupedin viipymäajalla 1,5 tuntia, jolloin raudan hapetusnopeus oli 5.1 g Fe^{2+}/L·h ja 37% raudasta saostui.

Bioliuotusprosessien jätevesien käsittelyä varten laboratoriomittakaavan prosessia kehitettiin edelleen käyttämällä KOH:ia tai $CaCO_3$:ia neutraloimaan pH arvoihin 2,5–3,5. pH:n nostaminen ei alentanut raudan hapetusnopeutta, ja raudanpoisto tehostui merkittävästi. Enimmillään raudasta saostui 96–99%. Optimaalisissa prosessiolosuhteissa saostui lisäksi keskimäärin 66% sulfaatista. Rauta ja sulfaatti saostuivat pääosin jarosiitteina.

Tutkimus osoitti, että edellä kuvatulla prosessilla voidaan saavuttaa nopea ja tehokas ferriraudan uudelleentuotto ja pitoisuuden kontrollointi, sekä nopea ja tehokas saostukseen perustuva raudan- ja sulfaatinpoisto bioliuotuksen prosessivesistä ja mahdollisesti myös muista hydrometallurgisista vesistä ja happamista kaivosvesistä. Biokasaliuotuksen tehostamiseen pyrkivästä yhdistetystä ferriraudan uudelleentuotosta ja osittaisesta raudanpoistosta ei aikaisemmin ole julkaistu tieteellisiä tutkimuksia. Verrattuna tällä hetkellä laajimmin käytettäviin kemiallisiin raudan hapetus- ja poistotekniikoihin tässä työssä kehitetyn bioprosessien etuja ovat toimiminen ympäristön lämpötila- ja painealueella sekä se, ettei raudan hapettamiseen tarvita kemiallisia reagensseja, eikä prosessissa synny paljon tilaa vieviä ferrihydroksidisakkoja. Harvoja muita tieteellisissä julkaisuissa kuvattuja mikrobiologista raudan hapetusta hyödyntäviä raudanpoistomenetelmiä ei ole kehitetty tai testattu bioliuotuksen prosessivesillä. Vastaavia biologiaa hyödyntäviä prosesseja on patentoitu muutamia, ja näihin prosesseihin verrattuna tässä työssä kehitetty bioprosessi poistaa raudan lisäksi myös sulfaattia, ja sillä saavutetaan yhtä hyviä tai parempia raudan- ja sulfaatinpoistotehokkuuksia yksinkertaisemmalla prosessikokonaisuudella. Prosessin edelleen kehittäminen edellyttää sen suorituskyvyn määrittämistä suuremmassa mittakaavassa, jotta yksikköoperaatioiden yksityiskohtaiset suunnittelukriteerit ja mittakaavanmuunnosperiaatteet voidaan määrittää ja prosessin toiminta optimoida. Erityisesti saostuksen ja laskeutuksen optimointiin on kiinnitettävä huomiota, koska hydrauliset olosuhteet ovat erittäin tärkeitä ferrirautasakkojen ominaisuuksien ja tarvittavien sakkojen jatkokäsittely- ja loppusijoitustoimenpiteiden määräytymisessä.

Monimutkaisten bioliuotus- ja hydrometallurgisten vesienkäsittelyprosessien tehokas hallinta edellyttää malleja, jotka kuvaavat täsmällisesti näitä prosesseja. Simuloidulla biokasaliuotuksen prosessivedellä suoritetuista kokeista saatuun aineistoon sovellettiin neuroverkkomallinnusta ennustamaan ferriraudan tuotantoa integroidussa bioprosessissa. Ehdotettu malli ennusti luotettavasti (R^2-arvo 0,90) käsitellyn liuoksen ferrirautapitoisuutta valittujen operatiivisten muuttujien (syöttöliuoksen ja käsitellyn liuoksen pH, redox ja liuenneen hapen pitoisuus leijupetireaktorissa, leijupetireaktorin viipymäaika ja ferroraudan syöttönopeus) perusteella. Todellisella biokasaliuotuksen prosessivedellä suoritettujen kokeista saatuun aineistoon sovellettiin kolmea mallinnusmenetelmää. Itseorganisoituvat kartat osoittautui hyödylliseksi havainnollistamisvälineeksi, jonka avulla oli myös mahdollista tunnistaa muuttujien välisiä yhteyksiä. Neuroverkkopohjainen malli ennusti luotettavasti saostuneen ferriraudan määriä valittujen operatiivisten muuttujien (syöttöliuoksen pH ja

ferrorautapitoisuus, liuenneen hapen pitoisuus, pH ja redox leijupetireaktorissa ja leijupetireaktorin viipymäaika) perusteella. Parhaiten soveltuva regressiomalli vastasi myös melko hyvin (R^2-arvo 0,87) kokeellisia tuloksia, mutta neuroverkkomalli ennusti saostumamääriä tarkimmin (R^2-arvo 0,97). Tämä tutkimus osoitti, että neuroverkkomallien avulla voidaan määrittää prosessimuuttujissa tapahtuvien muutosten vaikutuksia monimutkaisten prosessien suorituskykyyn, kun mallit on kokeellisen aineiston avulla kalibroitu kyseisille prosesseille. Neuroverkkomallinnuksen erinomainen soveltuvuus laboratoriomittakaavan prosessien mallintamiseen viittaa siihen, että kyseinen mallinnusmenetelmä on sovellettavissa myös vastaavantyyppisten suuremman mittakaavan prosessien mallintamiseen ja hallintaan.

Yhteenvetona voidaan todeta, että tässä tutkimuksessa kehitettiin bioliuotus- ja hydrometallurgisia prosessivesienkäsittelyprosesseja tuottamalla uutta tietoa *L. ferriphilumin* raudan hapetuskinetiikasta korkeissa liukoisten metallien pitoisuuksissa pH:ssa 1 sekä useiden metallien yhteisvaikutuksista raudan hapetuskinetiikkaan, mitä ei aikaisemmin ole mallinnettu. Tämä on myös ensimmäinen julkaistu systemaattinen tutkimus yhdistetystä, tehokkaasta biologisesta ferriraudan uudelleentuotannosta ja rautapitoisuuden kontrolloinnista, sekä raudan- ja sulfaatinpoistosta bioliuotusliuoksen prosessivesistä.

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I am indebted to several people for support and guidance throughout this research. Above all, I would like to express my sincere gratitude to my supervisor Dr. Jaakko A. Puhakka for having faith in me from the beginning, and for his excellent guidance and continuous enthusiasm and support. I am grateful to Dr. Bestamin Özkaya for his patience, excellent guidance and encouragement throughout my study. I was also privileged to be supervised by Dr. Olli H. Tuovinen, who I sincerely thank especially for his excellent guidance and valuable comments during manuscript preparations. My warmest thanks to Dr. Anna H. Kaksonen for her kind help, guidance and friendship. I also want to thank Lic. Marja Riekkola-Vanhanen, Talvivaara Mining Company Plc., for the excellent advice and support. I am thankful to Dr. Åke Sandström and Dr. Jochen Petersen for pre-reviewing this thesis and for their valuable comments and suggestions. I would also want to thank my co-workers at the department for the pleasant working atmosphere, practical help in the lab and valuable lunchtime company.

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TABLE OF CONTENTS

LIST OF ORIGINAL PAPERS

This thesis is based on the following original papers, in this thesis referred to by to the roman numerals:

I Özkaya, B., Sahinkaya, E., Nurmi, P., Kaksonen, A. H., Puhakka, J. A. 2007. Kinetics of iron oxidation by *Leptospirillum ferriphilum* dominated culture at pH below one. Biotechnology and Bioengineering 97:1121–1127.

II Nurmi, P., Özkaya, B., Kaksonen, A. H., Tuovinen, O. H., Puhakka, J. A. 2009. Inhibition kinetics of iron oxidation by *Leptospirillum ferriphilum* in the presence of ferric iron, nickel and zinc ions. Hydrometallurgy 97:137–145.

III Ozkaya, B., Sahinkaya, E., Nurmi, P., Kaksonen, A. H., Puhakka, J. A. 2007. Iron oxidation and precipitation in a simulated heap leaching solution in a *Leptospirillum ferriphilum* dominated biofilm reactor. Hydrometallurgy 88:67–74.

IV Ozkaya, B., Sahinkaya, E., Nurmi, P., Kaksonen, A. H., Puhakka, J. A. 2008. Biologically Fe^{2+} oxidizing fluidized bed reactor performance and controlling of Fe^{3+} recycle during heap bioleaching: an artificial neural network-based model. Bioprocess and Biosystems Engineering 31:111–117.

V Nurmi, P., Özkaya, B., Kaksonen, A. H., Tuovinen, O. H., Riekkola-Vanhanen, M.-L., Puhakka, J. A. 2009. Process for biological oxidation and control of dissolved iron in bioleach liquors. Process Biochemistry 44:1315–1322.

VI Nurmi, P., Özkaya, B., Sasaki, K., Kaksonen, A. H., Tuovinen, O. H., Riekkola-Vanhanen, M., Puhakka, J. A. Biooxidation and precipitation for iron and sulfate removal from heap bioleaching effluent streams. Hydrometallurgy, *submitted.*

VII Nurmi, P., Özkaya, B., Kaksonen, A. H., Tuovinen, O. H., Puhakka, J. A. 2009. Predictive modelling of Fe(III) precipitation in iron removal process for bioleaching circuits. Bioprocess and Biosystems Engineering, *in press,* DOI 10.1007/s00449-009-0346-5.

THE AUTHOR'S CONTRIBUTION

Paper I:
Pauliina Nurmi participated in performing the experimental work, interpreting the results and writing the manuscript.

Paper II:
Pauliina Nurmi drafted the paper and is the corresponding author. She performed the experimental work and modeling and interpreted the results. Modeling was supervised by Dr. Bestamin Özkaya.

Paper III:
Pauliina Nurmi participated in performing the experimental work, interpreting the results and writing the manuscript.

Paper IV:
Pauliina Nurmi participated in performing the experimental work and modeling, interpreting the results and writing the manuscript.

Paper V:
Pauliina Nurmi drafted the paper and is the corresponding author. She performed the experimental work and interpreted the results.

Paper VI:
Pauliina Nurmi drafted the paper and is the corresponding author. She performed most of the experimental work and interpreted the results. Precipitate analyses were performed by Dr. Keiko Sasaki and interpreted by Dr. Keiko Sasaki and Dr. Olli H. Tuovinen.

Paper VII:
Pauliina Nurmi drafted the paper and is the corresponding author. She performed most of the experimental work and modeling and interpreted the results. Multiple regression modeling was performed by Dr. Bestamin Özkaya.

ABBREVIATIONS

A	Frequency factor
A_S	Surface area of settling tank
AAS	Atomic absorption spectrometer
AMD	Acid mine drainage
ANN	Artificial neural network
BMU	Best matching unit
c	Actual concentration of the substance to precipitate
c^*	Equilibrium solubility of the substance to precipitate
DGGE	Denaturing gel gradient electrophoresis
E_a	Activation energy
EPS	Extracellular polymeric substances
FBR	Fluidized-bed reactor
H	Depth of the clarification zone
I_c	Initial competitive inhibitor concentration
I_{nc}	Initial non-competitive inhibitor concentration
I_{uc}	Initial uncompetitive inhibitor concentration
ICP	Inductively coupled plasma atomic emission spectroscopy
k	Rate constant
K_i	Self-inhibition constant
K_{iic}	Competitive inhibition constant
K_{iinc}	Non-competitive inhibitor constant
K_{iiuc}	Uncompetitive inhibition constant
K_s	Half saturation constant
MLP	Multi-layer perceptrons
PCR	Polymerase chain reaction
Q_e	Flow rate of the final system effluent
Q_f	Feed flow rate
Q_r	Recycling rate
q_m	Maximum specific substrate conversion rate, i.e., oxidation rate
R	Gas constant
R_{FBR}	Fe^{2+} oxidation rate with fluidized-bed volume as the reference volume
R_{tot}	Fe^{2+} oxidation rate with total volume of the reactor as the reference volume
S	Substrate concentration
dS/dt	Specific substrate conversion rate, i.e., specific oxidation rate
SOM	Self-organizing map
S_r	Relative supersaturation
SVI	Sludge volume index
T	Temperature
τ_{FBR}	Retention time of the FBR
τ_S	Retention time of the gravity settler
TSS	Total suspended solids
v	Particle settling velocity
V_C	Volume of the clarification zone
VS	Volatile solids
X	Biomass concentration
dX/dt	Bacterial growth rate
Y	Yield coefficient
XRD	X-ray diffraction

1 INTRODUCTION TO BIOHYDROMETALLURGY AND BIOLEACHING

This thesis focuses on exploring the reactions involving iron in bioleaching. In order to understand these reactions and the related bioprocesses basic information on bioleaching and the wider context of biohydrometallurgy is provided in this chapter. The history of bioleaching and the principles, techniques, applications and microorganisms used in bioleaching are briefly discussed.

1.1 Biohydrometallurgy

In hydrometallurgy aqueous solution chemistry is used for the extraction of metals. The field further concerns itself with solution concentration and purification and metal recovery. In biohydrometallurgy, which is a subfield of hydrometallurgy, the interactions between microorganisms and minerals are utilized by combining biotechnology with these processes. Biohydrometallurgy aims at exploiting mineral resources, i.e., achieving metal extraction with the help of bioleaching, or protecting and remediating the environment by treating effluents from mining and metallurgical industries and other metal containing wastewaters. Biohydrometallurgical applications typically exploit the activities of iron- and sulfur-oxidizing or sulfate-reducing microorganisms to achieve these aims. The focus of this thesis is on the biological iron oxidation and the related biohydrometallurgical processes which are discussed in detail in the following chapters.

1.2 Bioleaching - principle and historical perspective

Bioleaching is one of the alternative methods to conventional smelting for metal recovery from low-grade ores. In bioleaching, metals are dissolved from sulfidic minerals by microorganisms through oxidation to water soluble metal sulfates. In industrial applications, this phenomenon is accelerated by increasing the amount of microbes and making the conditions favorable for their growth (Kinnunen and Puhakka 2004).

Bioleaching has been used for the extraction of metals for thousands of years. From as early as the 1000's B.C. there are records of recovering copper from mine drainage waters in the Mediterranean basin. Large-scale leaching of copper was applied at least at the Tharsis and Rio Tinto mines in Spain in the 18th century (Brandl 2001, Rawlings 2002, Rawlings *et al*. 2003). The miners were not, however, aware of the role of microorganisms and leaching was considered to be natural decomposition of minerals. Microbial involvement was discovered for the first time in 1947 when bacteria belonging to the *Thiobacillus* (later *Acidithiobacillus*) genus were found in acid mine waters (Colmer and Hinkle 1947, Temple and Colmer 1951). In the 1950s, bioleaching applications used dump leaching techniques and the designs were not optimized for microbial activity. In these applications the reactions were limited especially by the poor oxygen availability. Heaps were later in the 1980s designed to facilitate the activity of microorganisms. The first actual commercial applications of biotechnology in mining were designed for the bioleaching of refractory gold-bearing sulfide concentrates using mesophilic microorganisms. In these applications bioleaching was used as pretreatment (often referred as biooxidation) to open the structure of the mineral prior to gold dissolution by cyanidation (Brierley 2008, Brierley and Brierley 2001, Olson *et al*. 2003). Later, copper was the first base metal to be extracted by solubilizing it from the mineral with the help of

microorganisms and then using solvent extraction and electrowinning to treat the resulting solution (Clark *et al*. 2006).

The importance of bioleaching is continuously increasing partly because high-grade ores are becoming scarce resulting in a growing need to exploit lower-grade ores (Rawlings *et al*. 2003, Watling 2006). The lower capital and operational costs make bioleaching a good alternative particularly for ores containing less than 0.5 % (w/w) of valuable metals (Bosecker 1997, Brierley and Brierley 2001). Bioleaching has also been tested for recovering metals from mining wastes (Bosecker 1997, Krebs *et al*. 1997, Rawlings *et al*. 2003) other waste material such as electronic scrap (Brandl 2001, Brandl *et al*. 2001, Cui and Zhang 2008, Krebs *et al*. 1997, Ilyas *et al*. 2007), municipal and industrial sludge from waste water treatment (Blais *et al*. 1992, Solisio *et al*. 2002), municipal solid waste incineration fly ash (Wu and Ting 2006, Yang *et al*. 2009) and contaminated sites (Bosecker 2001, Gadd 2000). Industrial-scale bioleaching applications are currently in use primarily for copper (Rawlings 2002, Watling 2006) but also for gold, nickel and cobalt (Brandl 2001, Clark *et al*. 2006). Previously bioleaching was also used for leaching of uranium (Olson *et al*. 2003).

The most important advantages of bioleaching of sulfidic minerals – when compared to conventional smelting or pressure leaching – are its suitability for relatively low-grade ores and small operations, short start-up times and lower required operational pressures and temperatures. In addition, smelting plants have relatively high capital costs, require larger amounts of energy compared to bioleaching plants and produce air emissions that can cause severe negative environmental effects. The costs associated with the maintenance of bioleaching microorganisms are low as they gain energy from redox reactions, utilize carbon dioxide from the air as carbon source and obtain their phosphorus, nitrogen, potassium and other nutrients from the bioleaching environment (Watling 2006). Plants applying bioleaching for recovering copper are typically small to medium-size on-site processes, production ranging from 10 000 to over 100 000 tons of copper/year (Olson *et al*. 2003). Simplicity and low capital and operational costs make bioleaching a good alternative also for developing countries, where significant mineral deposits exist (Acevedo 2000, 2002).

Among the major challenges of bioleaching compared to conventional smelting are the long leaching times, risk for acidic metal containing leakages to the environment and in heap leaching the dependency on climatic conditions (Krebs *et al*. 1997, Watling 2006).

1.3 Techniques used in bioleaching

The main bioleaching techniques may be divided into irrigation-based techniques (*in situ*, dump and heap bioleaching) and continuous stirred tank bioleaching. *In situ* leaching does not require the metal-containing material to be removed from the ground. Acidic leaching solutions are injected directly to the subsurface ore body and the pregnant leaching solution percolated through the ore is collected with deep drill-holes for further processing on the surface. This technique is used for low-grade ores that are uneconomical to treat by conventional open-pit and underground methods. *In situ* leaching requires relatively long contact times and strict control of the metal-bearing acid solutions but the costs are typically lower than in the other bioleaching techniques and it may reduce the visual environmental impact of the mining operations. Because of the potential risk of environmental damage, extensive knowledge on the hydrology and geology of the area in question is needed, making it difficult to obtain a permit to apply the *in situ* method. This technology is not generally

applicable for high productions rates and the importance of the technique has thus declined during the last few decades (Plessis *et al*. 2007, Rawlings 2002).

Heap bioleaching is economically the most important bioleaching method. The general principle of heap bioleaching is shown in Fig. 1. Leaching takes place in 6–10 m high heaps which are piled on an impermeable ground. Prior to piling the ore is first crushed and agglomerated with sulfuric acid. Agglomeration of the ore attaches the fine particles to the surfaces of large particles, which improves the permeability of the heap and preconditions the ore for oxidation. In some cases the heap is inoculated with microorganisms and the microbial activity enhanced by addition of nutrients, but in general microbial growth is bioheaps proceeds naturally with little external intervention. Ore heap is irrigated with acidic, possibly nutrient containing leaching solution, the solution percolated through the heap is collected at the bottom of the heap and recycled back to the heap until required amounts of metals have been dissolved to it. After the recovery of metals the solution is used again for the irrigation of the heap. Aeration is provided to the heap to provide oxygen for the aerobic microorganisms. The heap can be covered in order to prevent evaporation in warm conditions and/or freezing in cold conditions (Rawlings 2002, Rawlings *et al*. 2003, Watling 2006). In heaps leaching takes a long time, typically months or even years. Conditions in the heap are usually very heterogeneous and the heaps are more difficult to inoculate and aerate than tanks (Acevedo 2000, Rawlings *et al*. 2003).

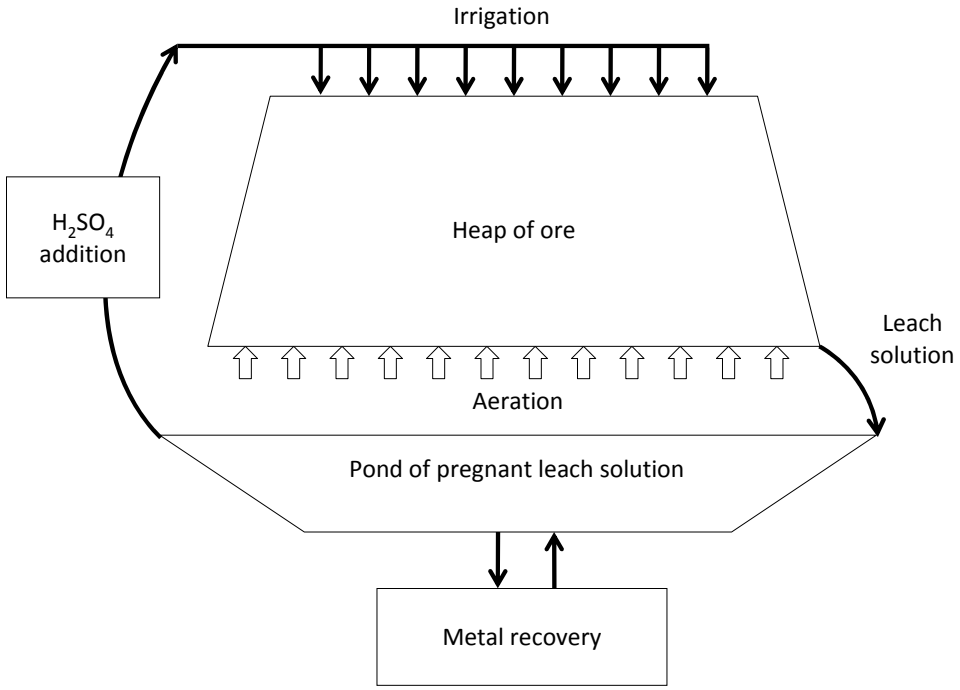


Figure 1. Schematic diagram of heap bioleaching.

Dump bioleaching is similar to heap bioleaching but it is less efficient and controlled and used for lower grade ores as compared to heap bioleaching. Dump bioleaching can be regarded as a subclass of heap leaching in that solutions percolate through a bed of ore and the dissolved metals are recovered from the leachate. Dump leaching includes minimal ore preparation, and is thus less efficient than heap leaching. Dump leaching continues to be an important processing option for extracting copper from very low-grade (0.1–0.5% w/w Cu) ores because of the low production costs (Brierley 2008).

Tank bioleaching has the highest capital and operating costs of the alternative bioleaching techniques and it is thus used for higher value ores and concentrates. Constraints of the technique include the restriction to low pulp densities. At pulp densities higher than 20%, physical and microbiological problems occur. The high viscosity of the slurry hinders efficient gas transfer, and the increased shear forces may cause physical damage to the microbial cells. However, aerated and mixed tanks operating in a continuous mode provide good control and efficient leaching because of homogenous conditions. In tanks adequate leaching of metals can typically be achieved in 4–6 days (Olson *et al*. 2003, Rawlings 2002, Rawlings *et al*. 2003).

Microorganisms used in heap bioleaching (section 1.4) can occur naturally at the mine site or alternatively the microbes may be introduced to the heap with the leaching solution or during the agglomeration (Leahy *et al*. 2007). Heap bioleaching processes typically contain indigenous iron- and sulfur-oxidizing mesophilic and thermophilic microorganisms (Olson *et al*. 2003). Indigenous bacteria, being adapted to high levels of metals, are often the most effective ones as bioleaching catalysts (Watling 2006). Heterogeneous conditions in the heap make it possible for different microorganisms to coexist in the heap (Brierley 2001).

1.4 Current commercial bioleaching applications and their importance

Heap and tank bioleaching are currently well-established processes especially for recovering copper and refractory gold concentrates around the world. Industrial scale tank bioleaching is relevant to high-value minerals or mineral concentrates, due to the high capital and operational costs. Most of the existing commercial processes applying tank bioleaching are used in the biooxidation of gold ores (Rawlings *et al*. 2003). Tank bioleaching for the recovery of cobalt has also been successfully conducted at industrial scale (Morin and d'Hugues 2007). Application for the recovery of copper, nickel and zinc are also at a pilot or demonstration scale (Morin 2007, Viera *et al*. 2007).

Copper is the metal recovered in the largest quantity with the help of heap bioleaching (Olson *et al*. 2003, Rawlings *et al*. 2003). The largest copper leaching plants produce typically around 200 000 tonnes of Cu per year. Gold ore is also pretreated by heap bioleaching (Rawlings 2005). In her review Watling (2006) lists 23 historical and current copper heap bioleaching plants. Secondary copper sulfide chalcocite (Cu_2S) is the most important mineral leached with bioleaching. The full commercialization of heap bioleaching for low-grade primary sulfide ores has been delayed by the long leaching times and the low recoveries from primary copper sulfides, particularly chalcopyrite ($CuFeS_2$), the most abundant and the most refractory copper sulfide (Clark *et al*. 2006). Both sulfur and iron containing insoluble reaction products formed on the chalcopyrite surface during leaching have been invoked as the cause of slow dissolution (Pradhan *et al.* 2008, Watling 2006). New technologies are being developed using thermophilic microorganisms for economically feasible heap bioleaching of copper from chalcopyrite, and also for using heap leaching for the recovery of nickel, cobalt and zinc (Rawlings *et al*. 2003, Watling 2006). Heap bioleaching of multi-metal ore containing nickel, cobalt, zinc and copper has been expanded into full-scale production in Talvivaara, Finland (Riekkola-Vanhanen 2007).

In Fig. 2 a simplified schematic illustration of the role and importance of bioleaching in copper industry is shown. Heap bioleaching is the economically most important technology used for low-grade copper sulfide ores. For higher-grade copper sulfide ores, tank bioleaching

is applied, and for the richest ores, smelting is used. Chemical leaching is used for copper oxides. According to Clark *et al*. (2006), in copper industry, some of the largest opportunities lie in heap bioleaching. This refers to the possibilities of applying the technology more widely, also to primary copper sulfides.

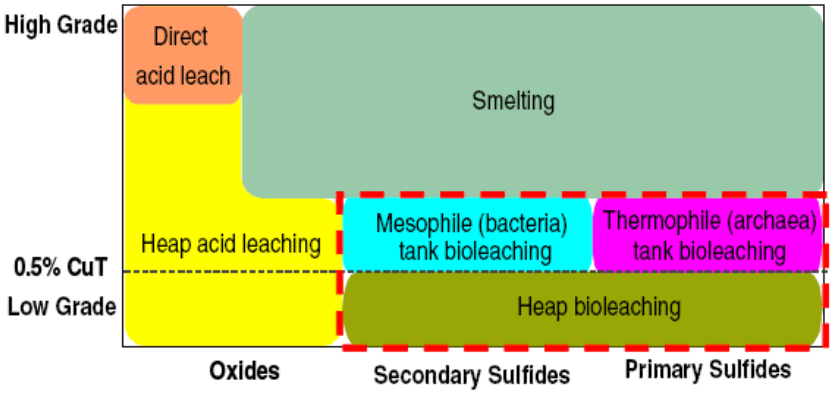


Figure 2. Shares of copper extraction technologies (Clark *et al*. 2006).
Dashed line (0.5% CuT) represents the Cu content of 0.5 % (w/w) in the ore.

1.5 Microorganisms used in bioleaching

The microorganisms used in bioleaching for producing the ferric iron and sulfuric acid (see section 2.1) are iron- and sulfur-oxidizing bacteria and archaea (Rawlings 2002). Irrespective of the type or process or operating temperature, these microbes share several common features making them suitable for bioleaching. The most important of these characteristics include a) autotrophic growth by fixing CO_2 from the atmosphere; b) obtaining energy by using either ferrous iron or reduced inorganic sulfur compounds or both as the electron donor, and generally using oxygen as the electron acceptor; c) being acidophiles that grow in low pH, typically pH 1.4 to 1.6, and d) being remarkably tolerant to a wide range of metal ions, although there is considerable variations within and between species (Rawlings 2005, Schippers 2007). Bioleaching microorganisms include mesophiles (organisms that grow at temperatures up to 45°C), moderate thermophiles (organisms that grow at temperatures up to 60°C) and also thermophiles (organism with their optimal temperature for growth above 60°C) (Brierley 2001, Franzmann *et al*. 2005). Some of the most studied bioleaching organisms and their characteristics are presented in Table 1.

Table 1. Characteristics of some of the most studied bioleaching microorganisms.

Microbe		Oxidation of iron/sulfur	Temperature range (°C)	pH range	References
Bacteria					
Acidithiobacillus (previously *Thiobacillus*) *ferrooxidans*	M	Iron, sulfur	30–35* 10–37	1.8–2.5* 1.3–6.0	Brandl (2001), Krebs *et al*. (1997), Rawlings (2002), Schippers (2007)
Acidithiobacillus thiooxidans	M	Sulfur	28–30* 10–37	2.0–3.5* 0.5–6.0	Brandl (2001), Krebs *et al*. (1997), Rawlings (2002), Schippers (2007)
Acidithiobacillus caldus	MT	Sulfur	45* 32–52	2.0–2.5* 1.0–3.5	Brandl (2001), Rawlings (2002), Schippers (2007), Watling (2006)
Leptospirillum ferrooxidans	M	Iron	28–30* <10–45	1.5–3.0* 0.7–4.0	Baker and Banfield (2003), Brandl (2001), Rawlings (2002), Schippers (2007)
Leptospirillum ferriphilum	M	Iron	30–37* <45	1.3–1.8* 0.5– >3.5	Baker and Banfield (2003), Kinnunen and Puhakka (2005), Rawlings (2002), Schippers (2007), this study
Acidimicrobium ferrooxidans	MT	Iron	45–50* <30–55	2*	Brandl (2001), Clark and Norris (1996), Schippers (2007)
Sulfobacillus thermosulfidooxidans	MT	Iron, sulfur	45–48* 20–60	2* 1.5–5.5	Brandl (2001), Rawlings (2002), Schippers (2007)
Archaea					
Ferroplasma acidiphilum	M	Iron	35* 15–45	1.7* 1.3–2.2	Brandl (2001), Golyshina *et al*. (2000), Schippers (2007)
Sulfolobus metallicus	T	Iron, sulfur	65* 50–75	2.0–3.0* 1.0–4.5	Brandl (2001), Huber and Stetter (2001a), Rawlings (2002), Schippers (2007)
Metallosphaera sedula	T	Iron, sulfur	75* 50–80	2–3* 1.0–4.5	Brandl (2001), Huber and Stetter (2001b), Rawlings (2002), Schippers (2007)
Acidianus brierleyi	T	Iron, sulfur	70* 45–75	1.5–2.0* 1–6	Brandl (2001), Huber and Stetter (2001c), Rawlings (2002), Schippers (2007)
Acidianus infernus	T	Iron, sulfur	90* 65–96	2.0* 1.0–5.5	Brandl (2001), Huber and Stetter (2001c), Rawlings (2002), Schippers (2007)

M = mesophile, MT = moderate thermophile, T = thermophile
* = optimum

There have been relatively few studies on the microbial consortia of bioleaching heaps, and most of these have focused on analyzing the microorganisms present in the liquid phases, but based on the current knowledge, similar types of microorganisms seem to be found in heapleaching processes and in stirred tank processes. The proportions of the microbes vary, however, depending on the mineral and operational conditions. In addition to iron-oxidizers, also acidophilic sulfur-oxidizing microorganisms such as *A. thiooxidans* and *A. caldus* are

significant in bioleaching applications. Sulfur-oxidizing microorganisms provide sulfuric acid (Eq. 4, section 2.1) for proton attack (Rawlings 2005) and for maintaining the low pH required (Johnson 2008). Sulfur-oxidizers also reduce the accumulation of sulfur, which may form leaching rate decreasing sulfur layers on the surfaces of mineral particles, by transforming intermediary sulfur compounds to sulfuric acid and may thus improve bioleaching efficiency of certain minerals (Dopson and Lindström 1999, Fowler and Crundwell 1999, Rohwerder *et al*. 2003). Although some microorganisms are capable of using both energy sources, a combination of iron-oxidizing and sulfur-oxidizing microbes has often been found to be very effective (Rawlings 2005). Heterotrophic microorganisms such as bacteria belonging to the genus *Acidiphilium* or *Ferroplasma*-like archaea are believed to assist the growth of iron-oxidizing bacteria like *A. ferrooxidans* and the leptospirilli. This is thought to be due to their ability to provide essential nutrients or to remove toxic organic compounds or other inhibitory substances. However, it is not fully known how much heterotrophs contribute to the overall efficiency of bioleaching (Johnson 2008, Rawlings 2005, Schippers 2007).

The first found and most studied bioleaching bacteria belong to the genus *Acidithiobacillus* (Rohwerder *et al*. 2003). Due to its ease of culturing on plates in the laboratory, it was originally considered that bioleaching processes were dominated by *A. ferrooxidans*. Later molecular phylogenetic techniques have shown that other species are likely to be more important in natural and commercial bioleaching sites (Dopson *et al*. 2003, Rawlings *et al*. 1999). In recent years (e.g., Kinnunen and Puhakka 2004, 2005, Watling 2006) more attention has been paid to the use of *Leptospirillum ferriphilum* in bioleaching. *Leptospirillum* like species are interesting alternatives and dominant species in some conditions as they tolerate much higher Fe^{3+} concentrations, higher temperatures and lower pH than e.g. *A. ferrooxidans* (Rawlings *et al*. 1999).

There is a growing interest to find microbes with higher optimal temperatures in order to increase the rates of bioleaching processes (Franzmann *et al*. 2005, Leahy *et al*. 2007, Norris *et al*. 2000, Stott *et al*. 2003). Thermophiles may allow bioleaching processes to be operated at high temperatures, even up to 100°C with hyper-thermophiles (Leahy *et al*. 2007). Thermophiles generally have greater growth rates, simpler nutritional requirements and faster substrate utilization rates than mesophiles (Brandl 2001). The yield of bioleaching can also increase at higher temperature because the passivation through the formation of sulfur layers and other oxidation products on the mineral surfaces is reduced (Rawlings *et al*. 2003). However, there are fewer reports on types of microbes that occur in mineral treatment processes that operate at temperatures above 70°C than at lower temperatures. Thermophilic bioleaching applications are believed to be dominated by archaea rather than bacteria, with species of *Sulfolobus* and *Metallosphaera* being most prominent. Since leaching reactions are highly exothermic, the temperature of the heap rises, favoring thermophilic microorganisms (Rawlings 2005). In recent studies (e.g., Hawkes *et al*. 2006) it has been suggested that the conditions within a heap environment would favor the growth of moderate thermophiles.

In heap bioleaching applications, it is very likely to be possible to rely on the natural development of mesophilic and possibly moderately thermophilic microorganisms, but unless extreme thermophiles are naturally present at the region in question, i.e., the heaps are constructed for example in geothermally heated regions, it is unlikely that these microorganisms would populate the heap since the heaps are constructed at temperatures below the temperature operating window of thermophiles. Also, a new heap is likely to take some time before regions with temperatures as high as 80°C develop, and extreme

thermophiles might not persist although they would originally be present. If high temperature heap bioleaching is targeted, heaps need most likely to be inoculated with extreme thermophiles after the heaps reach temperatures of 50°C or greater (Franzmann *et al*. 2005, Rawlings and Johnson 2007). Because of the temperature variations inside a heap and with time, microorganisms able to leach sulfide minerals over a wide range of temperatures with rapid leaching rates at high temperatures would be ideal for heap bioleaching applications (Plumb *et al*. 2002).

There is also a growing interest to develop bioleaching, especially heap bioleaching, applications working at low temperatures with psychrophilic or psychrotolerant microorganisms, since low temperatures prevail at many potential mining sites such as underground mines and mining sites in the mid-high latitudes. The fact that precipitation of ferric iron compounds is significantly reduced at low temperatures has increased the interest to study bioleaching at lower temperatures (Dopson *et al*. 2007, Johnson 1998, Langdahl and Ingvorsen 1997, Nemati *et al*. 1998). However, especially heaps containing large quantities of highly reactive pyrite or pyrrhotite have been shown to reach elevated temperatures even during the boreal winter conditions (Riekkola-Vanhanen 2007).

The limited number of bacteria that have been discovered may partly be a consequence of the selective methods by which bacteria are enriched and isolated in the laboratory (Watling 2006). Large portion of the microbial diversity of the acidic metal-rich environments is most likely yet to be cultured (Dopson *et al*. 2003).

There has been great amount of research efforts to increase our knowledge and understanding of the microbiology of bioleaching environments and to find the most suitable and effective microbial consortia for different bioleaching applications. The search for finding the "ideal" consortia might be more feasible in tank bioleaching applications where the conditions can be more effectively controlled. In heap bioleaching applications it seems to be more important to ensure sufficient biodiversity of bioleaching related microorganisms (Johnson 2008).

1.6 Factors affecting bioleaching

Various physicochemical, microbiological and mineralogical factors affect bioleaching. The most important factors are summarized in Table 2. In chapter 3 the key factors affecting the reactions of iron and the growth iron-oxidizing microorganisms are discussed in more detail.

Table 2, p. 1/2. Main factors affecting bioleaching.

Factor	Effect or role	References
Physicochemical		
Temperature	- affects microbial activity and community composition - higher rates of chemical kinetics at higher temperatures - affects the formation of precipitates	Bosecker (1997), Brandl (2001), Haddadin *et al*. (1995), Ojumu *et al*. (2006), Rawlings *et al*. (2003), Watling (2006)
pH	- affects microbial growth - affects the formation of precipitates	Ahonen and Tuovinen (1995), Bosecker (1997), Brandl (2001), Ojumu *et al*. (2006), Watling (2006)
Redox potential	- has to be high to obtain the fastest leaching rates, but in some studies low redox potential enhanced chalcopyrite leaching and delayed passivation	Ahonen and Tuovinen (1995), Córdoba *et al*. (2008a–d), Olson *et al*. (2003), Watling (2006)
O_2	- electron acceptor for chemical and biological oxidations - influence on microbial activity - may become limiting factor because of the low solubility in water especially at elevated temperatures	Bosecker (1997), Brandl (2001), Haddadin *et al*. (1995), Ojumu *et al*. (2006), Rawlings *et al*. (2003), Watling (2006)
CO_2	- carbon source for microbial growth - affects microbial activity - may become limiting because of the low solubility in water	Bosecker (1997), Brandl (2001), Haddadin *et al*. (1995), Nemati *et al*. (1998), Rawlings (2002)
Nutrients (e.g. N, P and K)	- necessary nutrients for the bacteria and archaea	Bosecker (1997), Brandl (2001), Watling (2006)
Fe^{2+}	- at high concentrations begins to inhibit biological iron oxidation	Nemati *et al*. (1998)
Fe^{3+}	- chemical solvent of sulfide minerals - may precipitate, typically as jarosites - at high concentrations begins to inhibit biological iron oxidation	Brandl (2001), Haddadin *et al*. (1995), Nemati *et al*. (1998), Rawlings *et al*. (2003)
Heavy metals	- at high concentrations, inhibition of bacterial activity	Bosecker (1997), Haddadin *et al*. (1995), Ojumu *et al*. (2006)
Microbiological		
Strain	- the system selects for the optimal microbial strains	Rawlings (2005), Rawlings and Johnson (2007)
Microbial diversity	- mixed cultures more robust and efficient than pure cultures	Brandl (2001), Rawlings (2005), Rawlings and Johnson (2007)
Population density and activity	- high density and microbial activity increase the leaching rate	Brandl (2001)
Spatial distribution of microorganisms	- in heaps heterogeneous distribution - in tanks homogenous distribution - microorganisms attached to the mineral surfaces and in the liquid phase	Brandl (2001)
Adaptation ability	- adapted strains exhibit generally better leaching efficiencies - indigenous bacteria growing in a particular environment are likely to be those best adapted to that environment	Brandl (2001), Rawlings (2005), Rawlings and Johnson (2007), Watling (2006)

Table 2, p. 2/2. Continued.

Factor	Effect or role	References
Mineralogical		
Type and composition	- affects the need of acid and pH - provides electron donors and trace elements	Bosecker (1997), Watling (2006)
Particle size	- affects the available mineral/liquid contact area - leaching more efficient with smaller particle size (leaching proportional to the increase in mineral surface area), but below a critical point of particle size can impose attrition on the cells and reduce leaching rates, fine particles in a heap can decrease the permeability of the heap to both air and solution.	Ahonen and Tuovinen (1995), Bosecker (1997), Brandl (2001), Watling (2006)
Porosity	- cracks and pores in the particles increase the internal area - microbes may attach to the scratches on the mineral surface - structural imperfections on mineral surface serve as highly reactive sites in mineral oxidation	Brandl (2001), Rohwerder *et al*. (2003)
Solids concentration	- in tank bioleaching, high solids concentration cause physical damage to the cells, can cause toxicity effects to microorganisms and decrease mass transfer	Bosecker (1997), Brandl (2001), Haddadin *et al*. (1995), Rawlings *et al*. (2003)
Presence of different metal sulfides	- mineral having the lowest potential is generally oxidized first	Olson *et al*. (2003)

2 IRON IN BIOLEACHING

Iron is the key element in bioleaching and biooxidation processes affecting microbial growth, leaching efficiency and precipitate formation. Due to its natural abundance, iron is closely associated with valuable metals in mineral deposits and it is invariably extracted during hydrometallurgical processing and the production of acid mine drainage. In this chapter the key roles and reactions of iron in bioleaching and the corresponding chemical, physical and biological unit operations are discussed. This chapter focuses on the reactions of iron during two-stage tank bioleaching and heap bioleaching.

2.1 Bioleaching mechanisms and oxidation pathways of sulfide minerals

The mechanisms for microbiological sulfide mineral leaching can be divided into direct and indirect mechanisms although the indirect mechanism is considered to be the only relevant mechanism (Rohwerder and Sand 2007, Watling 2006). The direct and indirect mechanisms can be summarized by the following reactions (M=metal, Bosecker 1997, Rohwerder *et al*. 2003, Rohwerder and Sand 2007, Sand *et al*. 2001):

Direct: $MS + 2O_2 \rightarrow M^{2+} + SO_4^{2-}$ (1)

Indirect: $MS + 2Fe^{3+} \rightarrow M^{2+} + S^0 + 2Fe^{2+}$ (2)

$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$ (3)

$S^0 + 1.5O_2 + H_2O \rightarrow 2H^+ + SO_4^{2-}$ (4)

In the direct mechanism, the microorganisms attached to the mineral surface are believed to directly oxidize the sulfur enzymatically. Leaching of copper was shown to occur via the direct mechanism in a study made in the absence of iron ions at pH 2.5, whereas indirect mechanism is assumed to be the prevalent one preferred when soluble iron exists (Lilova and Karamanev 2005). In the indirect mechanism, the microorganisms oxidize iron, which in turn oxidize metal sulfides. Indirect mechanism has been further divided into contact, non-contact and cooperative leaching mechanisms. These leaching mechanisms are presented in Fig. 3. In the non-contact mechanism, the planktonic microorganisms produce ferric iron and protons, which chemically leach minerals. For the contact mechanism, it is assumed that these bioleaching reactions take place within a layer of extracellular polymeric substances (EPS) interface between the bacterial cell and the sulfide mineral. In cooperative leaching the microorganisms attached to the surface of the mineral release sulfur particles and mineral fragments, which feed other iron- and sulfur-oxidizers (Rawlings 2002, Rohwerder *et al*. 2003, Rohwerder and Sand 2007).

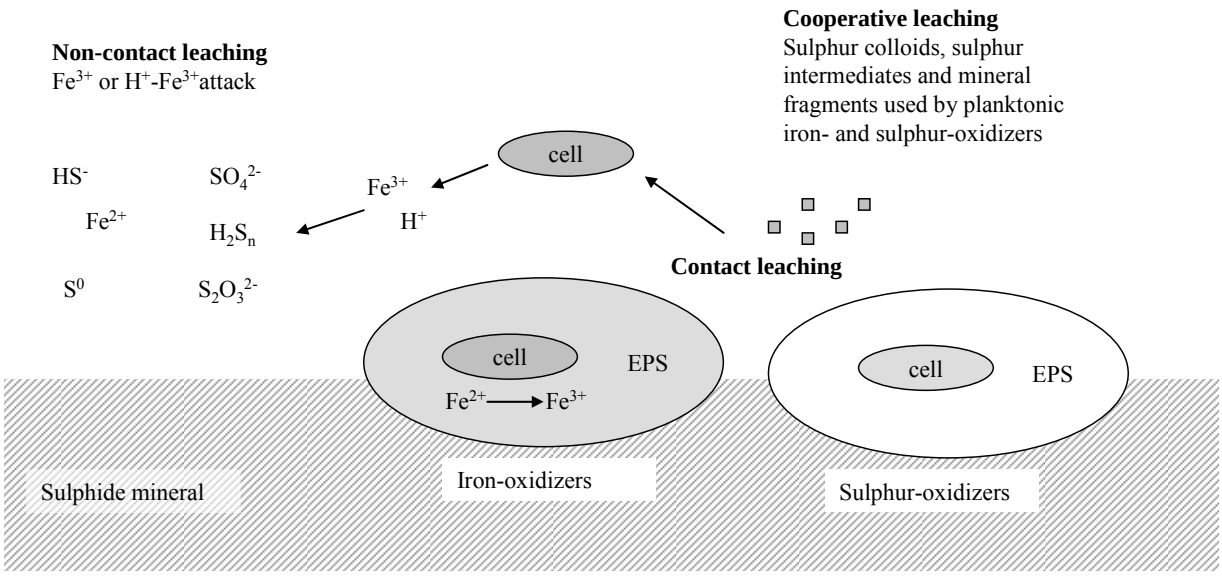
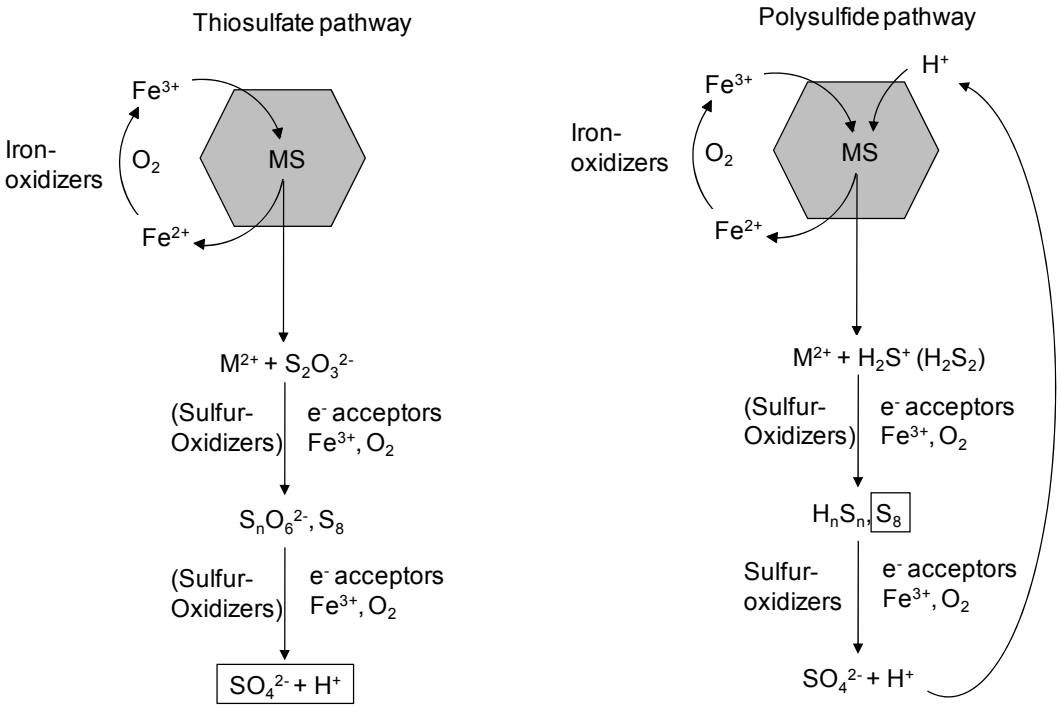


Figure 3. A simplified schematic diagram illustrating the proposed indirect leaching mechanisms of sulfide minerals (adapted from Rawlings 2002).

According to the current understanding the oxidation of the metals from sulfidic minerals to soluble metal sulfates occurs mainly via the indirect contact mechanism, i.e., chemically with the help of ferric ions (Fe^{3+}) and protons which act as oxidizing agents (Watling 2006). The role of microorganisms is to create the environment in which the leaching reactions take place (bioleaching reactions occur most efficiently in the EPS layer mentioned above) and to produce the leaching chemicals. This involves the generation of protons by sulfur oxidation (Eq. 4), and the oxidation of ferrous iron (Eq. 3), generated in dissolution, back to ferric iron (Salo-Zieman *et al*. 2006, Rawlings 2005). Microbial oxidation of metal sulfides at low pH takes place at a high rate. It may be about 10^5–10^6 times faster than the chemical oxidation of ferrous iron (Bosecker 1997).

Metal sulfide oxidation can proceed via two different pathways: the thiosulfate or the polysulfide pathway (Fig. 4).



Overall reactions:

$MS_2 + 6Fe^{3+} + 3H_2O \rightarrow M^{2+} + S_2O_3^{2-} + 6Fe^{2+} + 6H^+$

$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+$

$MS + Fe^{3+} + H^+ \rightarrow M^{2+} + 0.5H_2S_2 + Fe^{2+}$

$0.5H_2S_2 + Fe^{3+} \rightarrow 0.125\,S_8 + Fe^{2+} + H^+$

$0.125\,S_8 + 1.5\,O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$

Figure 4. Schematic diagram of the thiosulfate and polysulfide leaching pathways of metal sulfide oxidation (adapted from Rohwerder and Sand 2007). The sulfur-oxidizers are in parentheses, when the sulfur oxidation reactions are mainly abiotic. The main sulfur containing reaction products that accumulate in the absence of sulfur-oxidizers are boxed.

Acid-nonsoluble metal sulfides (Table 3) are oxidized via electron extraction by Fe^{3+} ions. This mechanism is called after its first free sulfur compound as the thiosulfate pathway. Free thiosulfate is further oxidized via tetrathionate and other polythionates finally to sulfate. In thiosulfate mechanism also significant amounts of elemental sulfur (10–20%) may be produced in the absence of sulfur-oxidizing bacteria or chemical oxidation. Acid-soluble metal sulfides are, in turn, dissolved by the combined action of electron extraction by Fe^{3+} ions and proton attack. In this polysulfide pathway the first free sulfur compound is most likely a sulfide cation, which can spontaneously dimerize to free disulfide and is further oxidized via higher polysulfides and polysulfide radicals to elemental sulfur. In both pathways, the main role of the leaching bacteria is the regeneration of ferric iron and protons (Rohwerder and Sand 2007, Schippers and Sand 1999, Steudel 1996).

Table 3. Examples of dissolution pathways of metal sulfides

Metal sulfide	Dissolution pathway	
	Thiosulfate pathway	Polysulfide pathway
Acid-nonsoluble metal sulfides:		
Molybdenite (MoS_2)	x	
Pyrite (FeS_2)	x	
Tungstenite (WS_2)	x	
Acid-soluble metal sulfides		
Arsenopyrite (FeAsS)		x
Chalcopyrite ($CuFeS_2$)		x
Galena (PbS)		x
Hauerite (MnS_2)		x
Sphalerite ((Zn,Fe)S)		x

2.2 Oxidation of iron during two-stage tank bioleaching

Biologically generated Fe^{3+} chemically oxidizes sulfide minerals and is reduced in this redox reaction to Fe^{2+}. Iron re-oxidation is essential in leaching since Fe^{3+} is an important electron shuttle and a chemical oxidant in the process. Biological ferric iron regeneration can occur in the same stage as the chemical dissolution of the sulfide mineral (direct or one-stage bioleaching) or in a separate bio-oxidation stage (indirect or two-stage bioleaching).

Current tank bioleaching applications utilize the simpler one-stage approach but the two-stage process has been demonstrated at least in laboratory and pilot scale. The two-stage approach separates the chemical process (oxidation of minerals) from the biological process (regeneration of ferric iron), and allows the separate optimization of the conditions for both stages (Morin *et al*. 2008, Rawlings and Johnson 2007, Rowe and Johnson 2008). For example, efficient leaching of minerals such as chalcopyrite, may require elevated temperatures (>80°C) that exceed the possible growth temperature of iron-oxidizing microorganisms (Rawlings and Johnson 2007). In addition, iron oxidation rates with mesophiles can be significantly higher than with thermophiles (Kinnunen *et al.* 2003, Nemati and Harrison 2000). In the two-stage approach it is possible to heat the biologically generated ferric iron solution, oxidize the target mineral with this heated solution and then cool the ferrous iron-rich leach solution generated to be re-oxidized in the biological process (Rawlings and Johnson 2007). Increasing temperature increases the leaching rates of sulfide minerals (see section 3.1), whereas the increased rate of ferric iron precipitation may be of concern at higher temperatures (Kinnunen *et al*. 2006). Separating the biological ferric iron regeneration step alleviates the potential precipitation challenges induced by the elevated temperature as the ferric iron concentrations in the heated chemical stage are decreased.

The pH of the two stages can be adjusted to stage specific levels. Low pH is favorable in the ferric iron regeneration step as the extent of ferric iron precipitation is greatly decreased at low pH values (see section 3.2). Two-stage leaching has also the advantages of enabling the use of catalysts in the chemical step, typically silver ions (Carranza *et al.* 1997, Palencia *et al.* 2002), which may be inhibitory to the iron-oxidizing microorganisms (Carranza *et al*. 1997), and avoiding the shear effects exerted by the mineral particles and impellers on the microorganisms which may cause physical damage to the microbial cells at high pulp densities (Rawlings *et al*. 2003). Contrary to the one-stage leaching, in the two-stage approach the sulfur moiety of sulfide minerals is oxidized to polysulfides and elemental sulfur (Brierley and Brierley 2001). This difference has both advantages and disadvantages. Downstream neutralization costs are reduced as no sulfuric acid is produced. However, elemental sulfur has

been suggested to be one of the agents inducing the passivation of certain sulfide minerals, such as chalcopyrite (Bevilaqua *et al.* 2002, Córdoba *et al.* 2008a, Fowler and Crundwell 1999, Tshilombo and Dixon 2003).

Depending on the application-specific characteristics of two-stage tank bioleaching processes such as the properties of the mineral concentrate, need for several leaching cycles, and options for metal recovery, the metal concentrations also in the biological iron oxidation stage may reach extreme values: for example up to 5 g/L Co, 20 g/L Cu, 24 g/L Ni and 50 g/L Zn (Heinzle *et al*. 1999, Morin and d'Hugues 2007, Morin *et al.* 2008, Viera *et al.* 2007). High resistance to metals is thus required from the iron-oxidizing microorganisms (see more on metal tolerance in section 3.5).

Two-stage bioleaching has been applied in laboratory and pilot scale to copper-zinc sulfide concentrates (Barriga *et al.* 1993, Carranza *et al*. 1990, Carranza *et al*. 1997, Palencia *et al.* 1990, Palencia *et al*. 1998, Romero *et al.* 1998), primary and secondary copper sulfide concentrates and ores (Carranza *et al.* 2004, Palencia *et al.* 2002, Romero *et al.* 2003), and zinc sulfide concentrates (Morin *et al*. 2008).

2.3 Oxidation and precipitation of iron during heap bioleaching

Heap bioleaching is a complex process involving various interacting reactions and subprocesses at various scales. The spatially and temporally varying heterogeneous conditions inside a heap make the process even more complex as different reactions may occur in different parts of the heap, the reactions may proceed at different rates and different subreactions may be rate-controlling depending on, for example, pH, temperature, composition and structure of the ore particles and solution chemistry. Petersen and Dixon (2007a) describe the complex nature of heap leaching by identifying the nature of the relevant processes at different scales. These sub-processes include:

1) at the scale of the entire heap: solution, heat and gas flows,
2) at the scale of cluster of ore particles: gas transfer from gas phase to the liquid phase, bacterial attachment, growth and activity, and intra- and inter-particle diffusion,
3) at the scale of particles: intra-particle diffusion, effects induced by the composition, structure, and the size distribution of ore particles, and
4) at the scale of grains: oxidation and reduction reactions, other chemical and electrochemical interactions at the grain surface.

In heap bioleaching the biological iron oxidation occurs in the heaps simultaneously with the dissolution of the mineral and in oxidation ponds, where the pregnant leach solution is collected from the heap (Fig. 1). However, the heterogeneous conditions within the heap may reduce the effectiveness of biological oxidation of iron during heap bioleaching. As seen from Eq. 3 (section 2.1), the oxidation of ferrous iron to ferric iron requires acid (H^+ ions) and oxygen. Iron-oxidizing microorganisms also require CO_2 as their carbon source. Heaps are usually aerated from the bottom of the heap and the O_2 and CO_2 are provided with the air. Uneven distribution of the gas flow within the heap and possible mass transfer (O_2 and CO_2 uptake form the gas phase to the liquid phase) challenges may limit the availability of O_2 and CO_2 (Casas *et al.* 1998, Leahy *et al.* 2003, Petersen and Dixon 2007a). Air may have to be blown into the oxidation ponds to enhance the oxidation of remaining ferrous iron to ferric iron before recycling the solution back to the heap (Logan *et al*. 2007).

The low pH required for biological iron oxidation is maintained with the irrigation solution which is supplemented with acid or through the acid-generating oxidation reactions of sulfur compounds and minerals. The distribution of acid may also be uneven because of the heterogeneous and uncontrolled nature of the solution flows and diffusion reactions inside the heap (Bouffard and Dixon 2001, Petersen and Dixon 2007a, b). Temperature variations may also control iron oxidation by affecting the growth and activity of iron-oxidizing microorganisms (Petersen and Dixon 2007a, Ojumu *et al*. 2006). Finally the ferric iron generated has to migrate by diffusion to mineral grains, which may be located deep inside the ore particles far away from the location of iron oxidation (Petersen and Dixon 2007b).

Especially the insufficient supply of CO_2 and acid have been suggested as the possible reasons for the slower leaching rates at commercial heap bioleaching applications compared to developed models and smaller scale experiments (Petersen and Dixon 2007a). Because of these limitations affecting the microbially-catalyzed iron oxidation the concentration of ferrous iron in the leaching solution is often higher than sought for (Logan *et al*. 2007). However, the sub-processes of heap bioleaching and their interactions are not yet fully understood and more research is needed on the iron oxidation kinetics under heap conditions (Petersen and Dixon 2007a, Ojumu *et al*. 2006, Watling 2006).

Recirculation of Fe^{3+} and Fe^{2+} containing leach solutions back to the process is common practice but leads to the accumulation of high concentrations of dissolved iron (Haddadin *et al*. 1995, Hansford and Vargas 2001, Kinnunen and Puhakka 2004) because sulfidic ores typically contain iron, which is solubilized during leaching. Accumulation of iron may result in adverse effects on bioleaching due to precipitation of iron and toxicity of high iron concentrations to microorganisms.

The solubility of Fe^{3+} is a function of the pH in leach solutions (see section 3.2). Fe^{3+} precipitates mainly as jarosites (Fe(III)-hydroxysulfates, $XFe_3(OH)_6(SO_4)_2$ where X is usually Na^+, K^+, NH_4^+, H_3O^+ or a divalent metal ion at ambient temperatures and pressures) at pH values < 4 and as hydroxides at higher pH values according to the following equations:

Jarosite formation $$3Fe^{3+} + X^+ + 2HSO^-_4 + 6H_2O \rightarrow XFe_3(SO_4)_2(OH)_6 + 8H^+ \quad (5)$$

Ferric hydroxide formation $$Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+ \quad (6)$$

Goethite, an Fe(III)-oxyhydroxide (α-FeOOH), or schwertmannite, an Fe(III)-hydroxysulfate ($Fe_8O_8(OH)_6(SO_4)$) can also form at similar pH ranges according to the following equations:

Goethite formation $$Fe^{3+} + 2H_2O \rightarrow FeOOH + 3H^+ \quad (7)$$

Schwertmannite formation $$8Fe^{3+} + SO_4^{2-} + 14H_2O \rightarrow Fe_8O_8(OH)_6(SO_4) + 22H^+ \quad (8)$$

The precipitation of ferric iron in heaps is one of the most important challenges connected to bioleaching (Nemati *et al*. 1998, Watling 2006). Ferric iron precipitates accumulate on mineral surfaces, pipelines and orifices, precipitation creates diffusion barriers on mineral surfaces and thus decreases the leaching rates, decreases the amount of Fe^{3+} acting as an oxidizing agent, occupies space within the heap and blocks the heap and the pumps and valves. Precipitates may prevent the contact between leaching solution and the ore by covering the ore particles (Logan *et al*. 2007, Nemati *et al.* 1998, Petersen and Dixon 2007b, Pradhan *et al*. 2008, Stott *et al.* 2000). Ferric iron precipitation naturally diminishes the

concentration of ferric iron available for further sulfide oxidation. This is unlikely to be a limiting factor for a low-grade sulfide ore (Watling 2006). Iron precipitates on the surfaces of the target sulfide may, however, have a significant impact on leaching rates.

Modeling of heap bioleaching is complex and has to incorporate a wide range of phenomena, as described above. Heap bioleaching processes offer limited control over the operating conditions, and parameters such as temperature and pH can vary widely over time and locations within the heap. Several models have over the years been developed for heap bioleaching, but still these bioreactors are rather poorly understood (Watling 2006). According to Watling (2006) the difficulties in understanding heap bioleaching processes have partly arisen from the fact that many studies have focused on specific sub processes in isolation and have failed to account for the interactions between those processes. Heap leaching models have been reviewed by Dixon (2003) and Watling (2006). Of the reviewed models, the HeapSim model (Petersen and Dixon 2007b) and the CSIRO heap model (Leahy *et al*. 2005, 2007) are the most comprehensive ones accounting for the highest number of sub processes. Also the Phelps Dodge copper stockpile model (Bennet *et al.* 2003) and the model developed by Neuburg *et al*. (1991) are relatively comprehensive. Dixon (2003) sees that one of the most serious shortcomings in most of the heap modeling developments is the insufficient model validation and testing at the heap scale. Development of appropriate alternative smaller scale ore testing methods would facilitate the development of heap leaching models. Dixon (2003) suggests the use of a combination of batch and short column leaching tests, tracer tests, permeability tests, and dynamic QEM-SEM (quantitative evaluation of materials using scanning electron microscopy) analysis as an alternative to large column and pilot heap tests in order to more quickly demonstrate the relationships between model parameters and leaching outcomes.

Specific subprocesses of heap bioleaching can be studied and optimized separately to a degree, but in order to realize the full potential of the technique the interactions between as many of the subprocesses as possible have to be taken into account and a holistic model is required (Watling 2006). Significant amount of research has been done to understand the complex process of heap bioleaching, but further knowledge is still required. According to Petersen and Dixon (2007b) it is unlikely that there will ever be a generic heap leaching process and thus a holistic model applicable to all or even several heap leaching applications. Existing and new applications have to be assessed, modeled and optimized, at least in part, individually.

2.4 Processes for iron oxidation and control during bioleaching

As described above, two-stage tank bioleaching applications require an iron oxidation process as an inherent part of the overall process. Considering also heap bioleaching, because of potential challenges in achieving adequate iron oxidation within the heap and in the oxidation pond, an additional iron oxidation step may be required before recycling the leaching solution back to the heap. For example at the Talvivaara bioleaching plant in Finland (Riekkola-Vanhanen 2007) the majority of the iron in the pregnant leaching solution is in the form of ferrous iron (Riekkola-Vanhanen 2009). A separate iron oxidation process within the heap bioleaching process circuit could be combined to iron control, i.e., to the precipitation of the excess iron in the leaching solution to alleviate the above described adverse effects of ferric iron precipitates within the heap.

A separate iron oxidation step is required also for the barren heap leaching solution coming from the recovery of valuable metals if the recovery stage involves reduction of ferric iron to ferrous iron (as for example in the Talvivaara mine in Finland, Riekkola-Vanhanen 2009) regardless whether the barren solution is recycled back to the heap or further treated for discharge.

Iron oxidation can be achieved chemically. However, the rate of chemical iron oxidation is considerably lower than that of microbial iron oxidation, especially at low pH values (pH < 4). Chemical oxidation of ferrous iron may be about 10^4–10^6 times slower than biological oxidation (Bosecker 1997, Meruane and Vargas 2003, Rao *et al*. 1995). Chemical oxidation of ferrous iron by molecular oxygen or air can be catalyzed e.g., by Cu(II), SO_2, H_2O_2 and iron oxides (Ferron 2006, Krause 2006, Tamura *et al.* 1980, Le Truong *et al.* 2004, Zhang *et al.* 2000a–c) - SO_2 being the most widely studied catalyst. However, the commercial exploitation of SO_2 as catalyst is limited, because it has an important side reaction with water and oxygen, i.e., the formation of sulfuric acid (Krause 2006). The use of SO_2 may also involve environmental and safety risks associated with gaseous SO_2 emissions, especially considering heap leaching (Ferron 2006). The rate of chemical oxidation of ferrous iron can also be increased by conducting it above atmospheric pressure, requiring the use of autoclave equipment (Ferron 2006, Lahtinen *et al.* 2006). However, in the oxidation of bioleach liquors, autoclaving cannot be applied as the microorganisms essential for the process do not survive the elevated pressures.

Biological iron oxidation options have also been studied widely. Microbially catalyzed oxidation processes may possess considerable advantages over chemical options. Bioprocesses typically have simpler configurations, reduced costs and reduced risks of environmental damage as they do not require additional chemical or physical catalysts to achieve adequate oxidation rates (Nemati and Webb 1996). Various types of bioreactors, including fluidized-beds, packed-beds, trickle-beds, circulating-beds, agitated reactors and rotating biological contactors, have been tested for their potential for high-rate ferrous iron oxidation by acidophilic microorganisms. These reactor types and their performances are compared in Table 4. The rate comparison between literature data is, however, somewhat arbitrary as the volume bases in the calculations often remain unspecified. The tendency of iron-oxidizing microorganisms to grow on surfaces has been exploited in reactors with various cell immobilization matrices, thereby effectively ensuring high and stable biomass retention. The highest iron oxidation rates have been achieved in packed-bed and fluidized-bed bioreactors with powdered activated carbon as the best support material (van der Meer *et al*. 2007, Nemati *et al*. 1998). Fluidized-bed bioreactors are discussed in more detail in section 4.2.

Biological iron oxidation has been studied in detail. There is, however, a very limited amount of published information on simultaneous iron biooxidation and precipitation or partial removal processes and they are rarely reported in sufficient detail. Integration of these processes is essential for the treatment of leach liquors since the concentration of dissolved iron must be controlled for successful leaching. A key process is the oxidation of ferrous iron in leach liquor for subsequent precipitation of Fe(III).

Table 4, p. 1/2. Biological iron oxidation rates achieved in various bioreactor configurations.

Reactor type	**Main microbial species present**	**Support matrix**	**pH**	**Temp. (°C)**	**Aeration using**	**Max. iron oxidation rate (g Fe^{2+}/L·h)**	**Nature of precipitates (if any), other comments concerning precipitation**	**Reference**
Fluidized-bed	*L. ferriphilum, "Ferri-microbium acidiphilum"*	Activated carbon	2.1±0.2	37±1	Air	8.2	Jarosites*)	this study
Fluidized-bed	*A. ferrooxidans*	Activated carbon	1.35-1.5	23	Air	0.9	Jarosite, amount of precipitation was negligible, no problems of clogging	Grishin and Tuovinen (1988)
Fluidized-bed	*L. ferriphilum*	Activated carbon and jarosites	1.4±0.1	37±1	Air	6.9	Jarosites	Kinnunen and Puhakka (2004)
Fluidized-bed	*L. ferriphilum*	Activated carbon and jarosites	1.4±0.1	37±1	0.5% CO_2 / 99.5% O_2	26.4	Jarosites	Kinnunen and Puhakka (2004)
Inverse fluidized-bed	*Leptospirillum* sp.	Activated carbon felt	1.05	40	Air	0.9	Not mentioned	Ginsburg *et al*. (2009)
Flooded packed-bed	1) *A. ferrooxidans* 2) *L. ferrooxidans* 3) *Ferrimicrobium* like isolate 4) Novel Betaproteobacterium isolate	Glass beads	2.4-2.5	18-20	Air	1) 1.4 2) 1.5 3) 1.6 4) 1.6	Not mentioned	Rowe and Johnson (2008)
Flooded packed-bed	*A. ferrooxidans*, *L. ferrooxidans*	Siliceous stone	1.25	31	Air	11.25	Not mentioned	Mazuelos *et al*. (2000)
Packed-bed	*A. ferrooxidans*	Quartz sand	2	20	Not mentioned	0.33	Not mentioned	Wood *et al*. (2001)
Packed-bed	N.d.	Expanded polystyrene	2.3	25	Air	0.72	Not mentioned	Diz and Novak (1999)
Packed-bed	*A. ferrooxidans*	Poly(vinyl alcohol) gryogel	1.7	31	Air	3.1	N.d., above 95% of the initial iron was found to be in solution	Long *et al*. (2003)
Packed-bed	*A. ferrooxidans*	Glass beads	1.35-1.5	23	Air	8.1	Jarosite, amount of precipitation was negligible, no problems of clogging	Grishin and Tuovinen (1988)
Packed-bed	*A. ferrooxidans*	Nickel alloy fiber	1.8	30	Air	20	N.d., extensive precipitation had a detrimental effect on iron oxidation rate and oxygen mass transfer rate to the medium	Gómez and Cantero (2003)
Packed-bed	*A. ferrooxidans*	Resin beads	1.35-1.5	23	Air	29.3	Jarosite, amount of precipitation was negligible, no problems of clogging	Grishin and Tuovinen (1988)
Packed-bed	*A. ferrooxidans*	Polyurethane foam	1.7	30	Air	34.3	Not mentioned	Nemati and Webb (1996)
Packed-bed	*A. ferrooxidans*	Activated carbon	1.35-1.5	23	Air	52	Jarosite, amount of precipitation was negligible, no problems of clogging	Grishin and Tuovinen (1988)

N.d. not determined

*) Precipitates characterized by x-ray diffraction analysis.

**) Solid phase analysis not performed.

Table 4, p. 2/2. Continued

Reactor type	Main microbial species present	Support matrix	pH	Temp. (°C)	Aeration using	Max. iron oxidation rate (g Fe^{2+}/L·h)	Nature of precipitates (if any), other comments concerning precipitation	Reference
Trickle-bed	*A. ferrooxidans*	Polyurethane foam	2.3	28-30	Air	4.4	Jarosites[**]), extensive precipitation had a detrimental effect on iron oxidation rate	Jensen and Webb (1994)
Airlift	*A. ferrooxidans, L. ferrooxidans*	Basalt, ferric iron precipitates	0-1.8	30	Air	8.1	N.d., initially used basalt was slowly removed and the ferric iron precipitates formed served as a biofilm carrier, these precipitates had highly suitable characteristics as a carrier material	Ebrahimi *et al*. (2005)
Rotating biological contactor	N.d.	Polyvinyl chloride	1.5-2.6	10-40	-	0.80	N.d., solid films of ferric iron developed on disk surfaces, max. iron precipitation was around 9 g /day	Nakamura *et al*. (1986)
Rotating biological contactor	*A. ferrooxidans*	Polyvinyl chloride	2.0-2.5	18	-	1.4	Not mentioned	Nikolov *et al*. (1986)
Circulating-bed	*A. ferrooxidans*	Polyurethane foam	2.3	28	Air	1.56	N.d., 64% of initial total iron precipitated and mainly accumulated inside the support particles	Armentia and Webb (1992)

N.d. not determined

[*]) Precipitates characterized by x-ray diffraction analysis.

[**]) Solid phase analysis not performed.

In addition, there are not many published studies exploring the potential for iron control during bioleaching. At least one related patent has been published. Kohr *et al.* (1997) recognized the importance of iron control and patented a method for precipitating part of the iron from bioleaching solutions prior to recycling the solutions back to the heap.

2.5 Iron removal from bioleaching effluents

Effluents from bioleaching processes have very low pH and contain relatively high concentrations of iron (up to 10–20 g/L, Qin *et al.* 2009, Watkin *et al*. 2009) and sulfate (up to over 100 g/L, Ojumu *et al.* 2006), which must be treated before recirculation or discharge. Dissolved iron may otherwise interfere with the subsequent metal recovery (Cunha *et al*. 2008, Dutrizac and Riveros 2006), and the effluents cause severe adverse effects on vegetation and fauna in receiving streams if dispersed in the environment (Johnson 2003, 2006). The chemical composition of these effluents varies with the source. In addition to high concentrations of iron and sulfate, other metals may also be present. Effluents from bioleaching operations have similar characteristics as acid mine drainage (AMD). AMD is the result of exposure of sulfidic minerals at abandoned and active mine sites to water, oxygen and naturally occurring microorganisms, which oxidize iron and sulfur compounds in the solution and solid phases. The adverse environmental effects of these streams extend beyond the immediate vicinity of the source. Acidity can reach levels more than 10 000 times higher than neutral waters, presenting a strong leaching agent that can dissolve significant amounts of metal compounds and leach additional acid from rocks. The combination of low pH and high concentrations of especially iron (up to few grams per liter, Banks *et al.* 1997, Gray 1998, Groudev 1997, Nieto *et al*. 2007) and sulfate (up to 5–10 g/L, Banks *et al.* 1997, Gray 1998, Groudev 1997, Nieto *et al*. 2007) can have severe toxicological effects on aquatic ecosystems. Acute exposure to the high metal concentrations can kill organisms directly, while long-term exposure even to lower concentrations can cause mortality or other effects such as lower reproduction rates and deformities. Sediments in affected areas may become enriched with metals that are toxic to plants, fish, and sediment fauna. Associated with these changes is the precipitation of various ferric compounds which can travel downstream in receiving waters. Contamination can extend to surface waters as well as groundwater aquifers affecting potable water resources. Acid streams also corrode metal pipes and structures and damage concrete. The problems are of global scale because they are associated with all mine sites with exposed sulfide minerals (Gaikwas and Gupta 2008, Kaksonen *et al*. 2008).

2.5.1 Chemical effluent treatment processes

One of the most commonly used methods for iron removal from hydrometallurgical solutions is hydroxide precipitation. Hydroxide precipitation is typically used for iron removal prior to solvent extraction by adding lime ($CaOH_2$) or limestone ($CaCO_3$) to increase the pH approximately to 3, forming initially amorphous Fe(III)-precipitate (mainly ferrihydrites and schwertmannite). Iron oxidation is required if the iron in the solution to be treated is in the form of ferrous iron. Gypsum ($CaSO_4 \cdot 2H_2O$) is a by-product from the neutralization step (Chaudhury 2002, Cunha *et al*. 2008, Maree *et al.* 2004, White *et al.* 2006). Hydroxide precipitation results in a non-crystalline, fluffy precipitate containing loosely bound or entrapped water (White *et al*. 2006). The ferric hydroxide precipitate formed is rarely one pure phase but often contains two or more precipitate types (Jönsson *et al*. 2006), e.g., different kinds of ferrihydrites (Jambor and Dutrizac 1998) and schwertmannite (Bigham *et al*. 1996), depending on the process conditions. Most of the ferric hydroxide precipitates are

metastable and transform to other more crystalline precipitates over time, depending on the process or environmental conditions. Ferrihydrite transforms slowly to goethite and hematite, depending primarily on changes in pH, temperature and water activity (Loan *et al*. 2006), and schwertmannite transforms to goethite (Bigham *et al.* 1996, Jönsson *et al.* 2005) or jarosite (Wang *et al.* 2006).

Hydroxide precipitation creates major disposal problem due to the generation of voluminous sludge (Chaudhury 2002, Cunha *et al*. 2008). Hydroxide precipitates are also often contaminated with toxic metals as they tend to readily sorb trace elements from the liquid due to their high specific surface area (Carlson *et al.* 2002, Regenspurg and Peiffer 2005). Co-precipitation can be also a favorable reaction when resulting in the removal of small amounts of impurities. Hydroxide precipitation itself is a relatively simple process, but the downstream physical solid-liquid separation (e.g. thickening and filtration) is difficult because of the properties and water content of the precipitates (White *et al.* 2006).

Alternative chemical iron removal methods to hydroxide precipitation include jarosite (Eq. 5 in section 2.3), goethite (Eq. 7 in section 2.3) and hematite (Fe_2O_3) precipitation. Jarosite precipitation is the most widely used technology in zinc industry (Beualieu *et al*. 2006, Claassen *et al*. 2002, Lahtinen *et al.* 2006). The jarosite process involves the addition of a cation source (usually NH_3, Na_2CO_3, or Na_2SO_4) and a neutralizing chemical to the solution to be treated at 95°C and pH 1–1.5. Jarosite precipitation includes the advantages of the production of iron precipitates with relatively good settling, filtering and washing characteristics, simultaneous precipitation of sulfate and alkali ions, and a simple, inexpensive and easy to control process (Beaulieu *et al*. 2006, Claassen *et al*. 2002, Dutrizac 1999, Ismael and Carvalho 2003). However, jarosite precipitates have low iron content (25–30%), elevated temperatures are preferred for the process, and the co-precipitation of various other metal ions can become a challenge (Beaulieu *et al*. 2006, Ismael and Carvalho 2003, Latva-Kokko 2006). So far the residue produced does not have any known use, and the controlled landfilling greatly increases the costs of the process (Ismael and Carvalho 2003, Lahtinen *et al*. 2006).

Goethite process is also in use in the zinc industry. Goethite precipitation is carried out at a temperature of 70–90°C and at pH 2.8–3. The acid generated in the precipitation has to be neutralized, but other reagents are not required (Beaulieu *et al*. 2006, Fujikawa *et al.* 2006). The iron content of goethite (40–50%) is higher than the iron content of jarosite and thus the volume of the precipitates produced is lower than in the jarosite process (Beaulieu *et al*. 2006, Ismael and Carvalho 2003, Pelino *et al.* 1996). With further treatment, goethite may be used in the construction industry (Pelino *et al.* 1997). Goethite residues may be contaminated with significant amounts of zinc ferrite (Ismael and Carvalho 2003). Both jarosite and goethite residues are classified as hazardous waste because of the content of leachable elements such as Cd, Pb and As (European Commission 2001).

The hematite process aims at producing a clean marketable iron product that can be used for example in the cement industry. Currently, the only commercial hematite process utilizes high temperatures (around 200°C) and an oxygen atmosphere in an autoclave to precipitate hematite. Neutralization (with calcium carbonate) is also required (Arima *et al.* 2006, Beaulieu *et al*. 2006). Outokumpu Research Centre, Finland, has developed and patented a hematite process which operates at atmospheric pressures at pH range of 2.5–3 and temperature of around 95°C (Lahtinen *et al.* 2006). Careful control of pH and temperature are needed to produce pure hematite. Hematite has higher iron content (50–60%) and lower

moisture content (10%) than jarosite and goethite. Until recently the need for autoclave equipment has limited the wider use of hematite process (Lahtinen *et al.* 2006).

Typical compositions of iron based solids from chemical effluent treatment processes are compared in Table 5.

Table 5. Example compositions of different type of iron residues (European Commission 2001, Pelino *et al.* 1996).

Process	Fe (%, w/w)	S (%, w/w)	Zn (%, w/w)	Pb (%, w/w)	Cu (%, w/w)	Cd (%, w/w)
Hematite	50–60	2–3	< 1	< 0.01	< 0.02	< 0.02
Goethite	40–45	2.5–5	5–10	< 2	< 0.3	< 0.1
Jarosite	20–30	10–12	2–6	0.2–6	< 0.2	0.05–0.2

The stability areas of ferric precipitates as a function of pH and temperature are presented in Fig. 5.

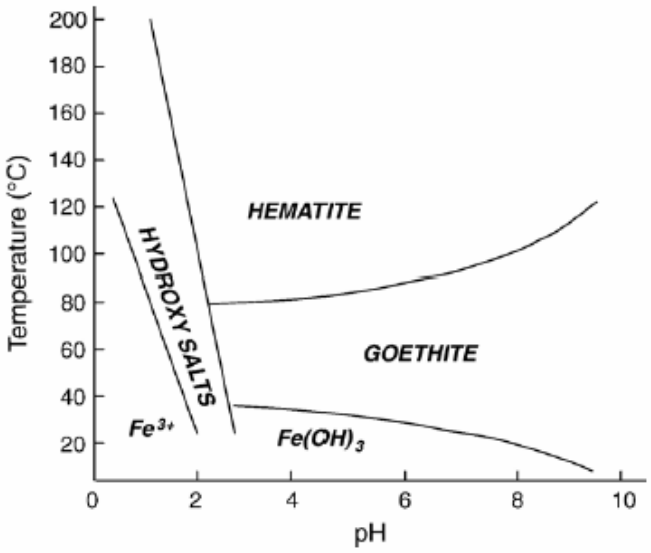


Figure 5. Stability areas of ferric phases from 0.5 M (~ 28 g/L) ferric sulfate solutions as a function of pH and temperature, hydroxy salts = Fe(III)-hydroxysulfates, i.e., jarosites (Das *et al*. 1996).

In addition to iron, bioleaching effluents also contain elevated levels of sulfate which have to be partially removed before discharge. Of the above described typical iron removal methods, hydroxide precipitation can involve the simultaneous precipitation of sulfate, e.g., in the form of schwertmannite, but especially jarosite precipitation is favorable in terms of effective sulfate removal.

Solvent extraction can also be used for iron control. However, the higher costs involved in solvent extraction have prevented the use of this method as the primary method for iron removal (Beaulieu *et al.* 2006, Santos *et al.* 2006). Other possible but thus far less often used iron removal processes include the paragoethite process (Binks *et al.* 2006, Loan *et al.* 2006), Zincor process (Claassen *et al.* 2002), and ion exhange (Shaw *et al*. 2006).

2.5.2 Biological effluent treatment processes

As alternatives to chemical effluent treatment processed, biological and combined biological and chemical iron removal processes have been examined in an effort to decrease the

chemical costs of effluent treatment and improve the handling characteristics of the produced sludge (Chaudhury 2002). The biological or combined biological and chemical treatment of AMD has been studied in detail (for reviews, see e.g. Gaikwas and Gupta 2008, Johnson and Hallberg 2005, Kaksonen *et al*. 2008, Kaksonen and Puhakka 2007).

There are two broad classes of methodologies that exploit biological iron oxidation or biological sulfate reduction used to treat AMD. The treatment can be passive or active. Passive treatment systems include constructed wetlands and *in situ* remediation. A constructed wetland can be aerobic, anaerobic or include both aerobic and anaerobic zones. An anaerobic wetland is also called a compost bioreactor. A typical constructed wetland system involves the flow of AMD through a constructed wetland where iron oxidation takes place in the surface zone, resulting in the precipitation of Fe(III) hydroxysulfates. Underlying the surface zone is an anaerobic organic layer, which promotes the activity of sulfate reducing bacteria leading to the formation of Fe- and other metal sulfides. *In situ* treatment processes are carried out by enhancing the activity of sulfate reducing bacteria directly in the contaminated water body in water-filled underground workings and open pits. This can be conducted, for example, by injecting carbon and energy sources below the surface or with the help of permeable reactive barriers constructed in the flow path of contaminated groundwater or infiltration beds constructed in ditches or trenches (Johnson and Hallberg 2005, Kaksonen *et al.* 2008, Kaksonen and Puhakka 2007). Passive treatment alternatives are relatively low cost and low maintenance processes compared to chemical or active biological treatment methods (Kaksonen and Puhakka 2007).

Active treatment options have, however, the advantages of being more predictable and readily controlled, and allowing valuable heavy metals such as copper and zinc present in the AMD to be selectively recovered and reused. Active treatment methods for AMD can also utilize sulfate reduction, i.e., the biogenic production of sulfide to generate alkalinity and to remove metals as insoluble sulfides. Numerous reactor designs for enhancing biological sulfate reduction have been developed for high rate treatment of AMD and other waste water streams (Lens *et al*. 2002). Sulfate reducing bacteria are, in general, heterotrophic bacteria which, unlike iron-oxidizing acidophiles, require provision of organic material as electron donor. High cost of the electron donor usually limits the feasibility of sulfate reduction in the treatment of bioleaching effluents with high concentrations of iron and sulfate and large volumes, especially in heap bioleaching applications (Buisman *et al*. 2007).

Examples of reported active treatment technologies for iron removal utilizing iron-oxidizing microorganisms are listed in Table 6. In these treatment options, iron-oxidizing bacteria are utilized to oxidize Fe^{2+} to Fe^{3+} which can be more easily precipitated from the solution. Examples of commercial or pilot-scale processes employing iron-oxidizing microorganisms include aerated lagoons and stirred tanks (Umita 1996), packed-bed bioreactors (Diz and Novak 1999) and rotating biological contactors (Nakamura *et al*. 1986, Olem and Unz 1980). High-rate iron oxidation has been achieved with bioreactors using immobilized iron oxidizing microorganisms, e.g. packed-bed bioreactors (Diz and Novak 1999) and rotating biological contactors (Nakamura *et al*. 1986, Olem and Unz 1980). In the laboratory-scale, a treatment process has been developed consisting of a packed-bed bioreactor for iron oxidation and a fluidized-bed reactor for chemical precipitation of iron onto the surface of seed particles (Diz 1998, Diz and Novak 1998). These applications have been developed for treating AMD and a few patents (Dietz 2003, Sasamoto *et al*. 1987, Shiomi and Fukuda 1988) have also been granted for processes designed primarily for the treatment of AMD. In contrast, very limited information has been published on the treatment of process waters generated in leaching

Table 6. Teatment technologies for iron removal utilizing iron-oxidizing microorganisms.

Process type	**Main microbial species present**	**pH**	**Temp. (°C)**	**pH adj. with**	**Feed solution**	**Max. iron oxidation rate (g Fe^{2+}/L·h)**	**Max. iron removal efficiency (%)**	**Max. iron prec. rate (g Fe^{3+}/ day)**	**Nature of precipitates (if any), other comments concerning precipitation**	**Reference**
Lab-scale fluidized-bed with a gravity settler	*L. ferriphilum, "Ferri-microbium acidiphilum"*	2.5–3.5	37±1	KOH and $CaCO_3$	Barren heap leaching solution, 6-14 g Fe^{2+}/L	8.2	99	55	Jarosites, goethite and gypsum	this study
Lab-scale packed-bed with an FBR for chemical prec.	N.d.	2.3–5.3	25	$CaCO_3$ gravel bed	Simulated AMD, with 0.25-0.70 g Fe^{2+}/L	0.72[*])	99	N.d.	Schwertmannite	Diz (1998), Diz and Novak (1998), [*])Diz and Novak (1999)
Pilot-scale rotating biological contactor	N.d.	2.2–5.5	5–24	$CaCO_3$	AMD with 0.03-0.36 g Fe^{2+}/L	N.d	N.d	N.d	N.d., dark brown inner layer and orange-brown outer layer	Olem and Unz (1980)
Full-scale biol. oxidation tank with neutralization tank and gravity settler	*A. ferroxidans*	4	N.d.	$CaCO_3$	AMD with 0.3-0.5 g Fe^{2+}/L	N.d.	N.d., effluent total iron conc. < 3 mg/L	N.d.	N.d.	Umita (1996)

N.d. not determined

operations. A few related patents have been published, e.g. for a process including simultaneous oxidation and precipitation of iron in hydrometallurgical leach liquors in the presence of *A. ferrooxidans* (Das and Das 2004) and a bioprocess scheme for the treatment of acidic raw water containing high concentrations of dissolved ferrous ions (Maree and Johnson 1999).

2.6 Downstream processing and disposal of iron precipitates

The quality of the precipitates formed greatly influences the economics and thus the viability of the iron removal process. Sludge properties such as the chemical composition, water content, particle size, impurity content, crystallinity, filterability, settleability and stability are directly related to the costs of downstream processing, loss of valuable target metals in the residue streams, final disposal options for the sludge or the possibilities to produce marketable side products. The precipitates must be carefully characterized to determine the appropriate treatment, end use or disposal methods. Waste disposal poses economic, environmental and logistical challenges, including land requirements, long term potential for the migration of contaminants from disposal sites, and public concerns over environmental degradation, all within an increasingly restrictive legislative framework (Mitsopoulos and Belanger 2006).

Precipitation reaction can be divided into two distinct stages:
1. Nucleation, where tiny particles (nuclei) are formed by aggregation of small groups of ions or molecules,
2. Particle growth, where nuclei become relatively large macro-particles by growing in three dimensions. The rate of precipitation depends on the rates of these two stages. Nucleation is typically the rate determining step as growing into macrosized particles is easier than nuclei formation (Fifield and Kealey 2000).

Driving force behind the precipitation reaction is the supersaturation of liquid. The particle size of the precipitate has been found to be inversely proportional to the relative supersaturation of the solution during precipitation. Relative supersaturation, S_r, can be expressed as:

$$S_r = \frac{c - c^*}{c^*} \qquad (9)$$

where c = actual concentration of the substance to precipitate, c^* = equilibrium solubility of the substance (Fifield and Kealey 2000).

A high relative supersaturation induces a high rate of nucleation and precipitate of small particle size is likely to be produced, forming colloidal and disordered precipitates. When nucleation rate is very high precipitation may occur practically without particle growth. Formation of a well crystalline coarse precipitate requires small relative supersaturation. Small relative supersaturation results in slow rate of nucleation and encourages subsequent precipitation to take place on the existing nuclei with the consequent formation of a highly crystalline precipitate (Fifield and Kealey 2000, Loan *et al*. 2002). Relatively large and well crystalline particles usually settle better than colloidal, disordered precipitates (Muir and Jamieson 2006). Particle growth, i.e., agglomeration, can be enhanced by adding chemical flocculants or by seed addition, i.e., adding or recycling precipitates in the process. Mixing

also promotes agglomeration to a certain point after which disruption occurs (Claassen and Sandenbergh 2006).

In downstream processing, various precipitate purification and dewatering (solid/liquid separation) methods are in use. For removing soluble heavy metals that may be problematic in final disposal, various combinations of washing, precipitation as sulfides or hydroxides or fixation as other insoluble compounds with cement or lime may be used (Fugleberg 1999). Pyrometallurgical treatment methods for residues are also currently in use. Pyrometallurgical methods are considered to be complex, costly and may be subject to atmospheric emission problems, but the slag produced is relatively compact, stable and possibly saleable (Lahtinen *et al*. 2006). For solid/liquid separation and dewatering of iron precipitates, i.e., increasing the dry matter content of the residues and thus decreasing their volume, gravity settling is the most common method, often in combination with some type of filtering. For filtration, e.g., filter presses (Foged *et al*. 2006, Mehta and Ram 2006, Talonen *et al*. 2006) and belt filters (Allen and Strand 2006, Haavanlammi *et al*. 2006, Roux *et al*. 2006) are used. For gravity settling, circular, conical thickeners are commonly used in industrial scale hydrometallurgical applications (Allen and Strand 2006, Arima *et al*. 2006, Binks *et al*. 2006, Coffin *et al*. 2006, Fujikawa *et al*. 2006, Haavanlammi *et al*. 2006, Jankola and Salomon-de-Friedberg 2006, Latva-Kokko 2006, Moctezuma *et al*. 2006, Nagle *et al*. 2006, Rocha and Ayla 2006, Shaipr *et al*. 2006, Talonen *et al*. 2006). In circular thickeners (Fig. 6) the feed is directed typically to the middle of the settler (*a* in Fig. 6), settled solids are collected to a central underflow discharge point (*b* in Fig. 6) typically with the help of a rotating rake mechanism (*c* in Fig. 6), and the clarified liquid discharges at the periphery of the basin (*d* in Fig. 6). Gravity settlers can also be rectangular or square shaped in plan area (European Commission 2001).

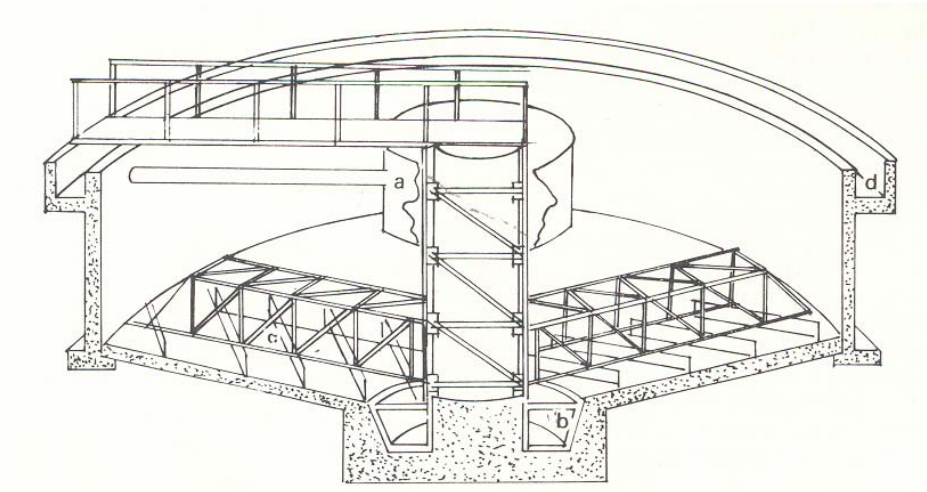


Figure 6. Circular thickener (Fitch 1986).

In gravity settling, four different types of particle settling may be identified: (a) particulate clarification (also called discrete particle settling), (b) flocculating clarification, (c) zone settling (also called hindered settling), and (d) compression. Distinguishing between these sedimentation types is important as they make different demands on the size and shape of the settling tank in which they occur. Particulate clarification describes the sedimentation of particles with little tendency to cohere at low solids concentration. Particles are, on average, far apart and free to settle individually. At low solids concentration particles may also settle by flocculating clarification. When the interparticle cohesion is strong, collisions between particles can result in the formation of flocs which settle at an increasing rate as they grow.

As the concentration of the particles increases they eventually reach a point where each is in contact with neighbors. Particles with tendency to cohere form a plastic structure and settle all at the same rate. This manner of settling is called zone settling, and it is characterized by a sharp interface between pulp and supernatant, which moves downward. At even higher concentration the pulp structure becomes so firm that it develops compressive strength. Each layer of pulp solids is able to provide mechanical support for layers above. This is characteristic for compression settling. Thickening is a term used for describing both zone settling and compression (Fitch 1986, Metcalf & Eddy 2003). Typically the upper section of a settler functions as a clarifier and flocs removed from the clarification zone enter the thickening zone in the lower section of the settler (Dixon 1979).

In clarification, the basic design criteria for gravity settlers is that the size of the settling tank and the time a unit volume of solution is in the settling tank (retention time, τ) should be such that all particles with the design velocity, v, will settle to the bottom of the tank. The rate at which clarified water is produced can be calculated as follows:

$$Q = A_S v \qquad (10)$$

where Q = flow rate = volume of the overflow per unit time (m^3h^{-1}), A_S = surface area of settling tank (m^2), v = particle settling velocity, i.e., design velocity (mh^{-1})

Rearranging Eq. 10 yields:

$$v = \frac{Q}{A_S} \qquad (11)$$

Thus, settling velocity can also be expressed as surface loading rate ($m^3m^{-2}h^{-1}$). The design velocity, retention time, and depth of the clarification zone are related as follows:

$$\tau = \frac{V_C}{Q} = \frac{A_S H}{Q} = \frac{H}{v} \qquad (12)$$

where τ = retention time (h), V_C = volume of the clarification zone (m^3), and H = depth of the clarification zone, measured in the direction of gravity force vector (m).

In actual practice, the design factors must be adjusted to account for the possible effects of inlet and outlet turbulence, short circuiting, sludge storage, and velocity gradients due to the operation of sludge-removal equipment (Fitch 1986, Metcalf & Eddy 2003).

Because of the variability in iron precipitates to be settled, settling column tests are usually required to determine particle settling velocities, and settling characteristics of suspensions of flocculent particles and suspensions where zone or compression thickening are important considerations. On the basis of data derived from column settling tests, the required volumes for settling facilities and the rate of sludge withdrawal can be determined (Metcalf & Eddy 2003).

According to the Reference Document on the Best Available Techniques in the Non Ferrous Metals Industries (European Commission 2001) solid residues have one of three possible destinations: (i) recycling in or upstream of the process, (ii) downstream treatment to recover

other metals, (iii) final disposal, if necessary after treatment to ensure safe disposal. The most common final disposal method for iron residues from hydrometallurgical processes is the use of a well-managed disposal site at or near the production plant. The disposal site may be an artificial pond, cavern or mine pit. Polymer linings and clay and soil layers are used in the pond structure to prevent any possible contamination of the soil or ground water (Lahtinen *et al.* 2006). The disposal of the solid residues can be a considerable cost as specially constructed, lined ponds or isolated areas are used to contain the material (European Commission 2001).

Ideally the effluent treatment process for bioleaching effluents is economically competitive, energy efficient, has high iron, sulfate and acidity removal efficiency, generates as little solid residue as possible with suitable physical and chemical characteristics for easy solid-liquid separation and stability for solids disposal without any direct or potential damage to the ecosystems (Kalin *et* al. 2006, Perales Perez *et al*. 1998).

3 KEY FACTORS AFFECTING THE REACTIONS INVOLVING IRON AND THE GROWTH OF IRON-OXIDIZING MICROORGANISMS

In bioleaching and effluent treatment bioprocesses, the rate of the reactions involving iron and the growth of iron-oxidizing microorganisms depends on several factors. In this chapter the most important of these factors are described.

3.1 Temperature

Temperature affects living microorganisms in two opposing ways. As the temperature increases, the reaction rates increase up to an optimum temperature and chemical and enzymatic reactions in the cell proceed at more rapid rates, and growth becomes faster. However, above the maximum temperature, particular proteins may be irreversibly denatured. Thus, as the temperature is increased, metabolic functions increase up to a point where denaturation reactions begin and cell functions fall sharply to zero. The Arrhenius equation (Fig. 7a) can be used to describe the relationship between reaction rate and temperature:

$$\ln k = -\frac{E_a}{RT} + \ln A \qquad (13)$$

where ln k = natural log of the 0th-order rate constant (k) for any temperature (T, in Kelvin), E_a = activation energy (J/mol), R = gas constant (8.3145 J/K·mol), A = frequency factor (Franzmann *et al*. 2005).

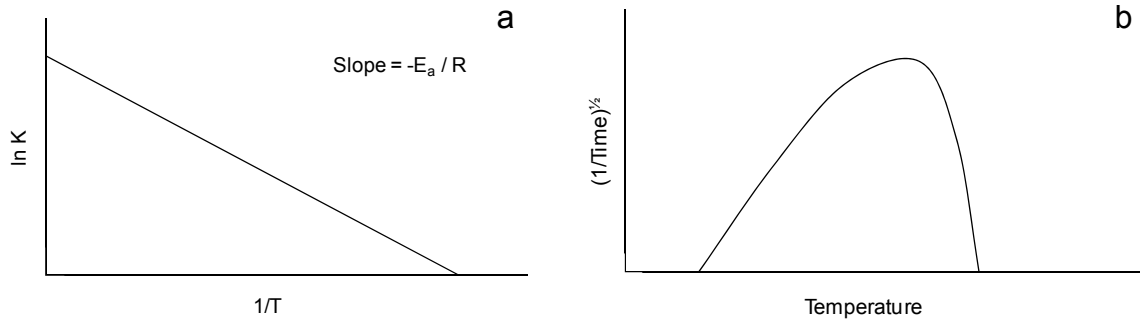


Figure 7. Illustrations of **a:** Arrhenius equation and **b:** Ratkowsky equation.

The relationship between microbial growth and temperature is also described by the Ratkowsky equation (Fig. 7b):

$$\sqrt{\frac{1}{Time}} = b \cdot \left(T - T_{\min}\right) \cdot \left(1 - e^{(c \cdot (T - T_{\max}))}\right) \qquad (14)$$

where T is temperature (^{o}C), T_{min} and T_{max} are the minimum and maximum temperatures, respectively, and b and c are the fitting parameters. The optimum temperature (T_{opt}), the point of inflection in the fitted curve, gives the greatest value for square root of *1/Time*. *Time* can be taken as the time (h) required for the oxidation of half of the initial ferrous iron concentration (Ratkowsky *et al*. 1983).

Temperature has a significant effect on the rates of bioleaching reactions. Almost all leaching reactions are highly exothermic increasing the temperature of the bioleaching process up to 80°C (Franzmann *et al*. 2005). The use of moderately and extremely thermophilic leaching organisms is being considered for bioleaching as a means to improve the mineral sulfide oxidation rates and yields especially from chalcopyrite, and to reduce the costs associated with the cooling of the process (Acevedo 2002, Ehrlich 2001, Olson *et al*. 2003, Rohwerder *et al*. 2003, Watling 2006). The chemical dissolution rate is also increased as the temperature increases (Rodríquez *et al*. 2001). In addition, thermophiles have generally high growth rates, resistance to stress and high metal tolerance (Brandl 2001, Olson *et al*. 2003). Ferric precipitation is also affected by temperature. The solubility of ferric iron decreases at high temperatures even at low pH (Deveci *et al*. 2004).

The amount of heat generated in the oxidation reactions depends on the sulfide and sulfur content of the given ore. The rate of oxidation and heat losses for example through evaporation can cause significant temperature gradients inside a bioheap. The mineral content of the heap is never homogenous, which in part causes temperature fluctuation inside the heap (Brierley 2001). Temperatures in a heap tend to be low near the surface, but higher in the center (Petersen and Dixon 2007b). While pyrite and pyrrhotite heaps are known to reach considerable temperatures, increasing often into the temperature range of thermophiles, heaps of copper sulfide minerals remain largely cold (Ojumu *et al*. 2006, Riekkola-Vanhanen 2007). With ore containing both chalcocite and pyrite, the heap has found to overheat in the lower and middle sections, which prevents the whole heap from leaching uniformly (Leahy *et al*. 2005). The temperature in the heaps can be affected by seasonal variation. The temperature of the heap can vary from below-freezing to even 80–90°C. In heaps containing large quantities of highly reactive pyrite and pyrrhotite, the elevated temperatures have been shown to maintain even over the boreal winter conditions (Leahy *et al*. 2005, Olson *et al*. 2003, Riekkola-Vanhanen 2007).

The effect of temperature on the growth and activity vary with bioleaching microorganisms. In bioleaching operations, a succession of mesophiles, moderate thermophiles and thermophiles can develop. The temperature variations within the heap make it also possible for both mesophilic and thermophilic microorganisms to be active in the same heap. In some bioleaching operations the temperatures can rise above the maximum temperature for growth of the predominant bioleaching organisms. The temperature has to be maintained within the physiological operating temperature range and preferably near to the optimal temperature of the microorganisms active in the processes (Brierley 2001, Franzmann *et al*. 2005).

The effect of temperature is crucial considering the bioleaching microorganisms of the processes. The variation in the range of microorganisms present appears to be more dependent on temperature than on the type of iron- and sulfur-containing mineral being oxidized (Rawlings 2005).

3.2 pH and redox potential

Bioleaching is usually carried out at a pH below 2. At low pH, ferric iron remains soluble and usable for the bioleaching reactions. However, in heaps the pH can vary considerably in micro and macro scale because of the heterogeneous conditions and differences in the characteristics of the ore. Oxidation of the sulfidic minerals by ferric iron is an acid producing reaction,

whereas most gangue materials accompanying the sulfide minerals (e.g., quartz, micas, chlorite, potassium- and calcium-feldspar) are acid-consuming (Rawlings *et al*. 2003).

Acid is also consumed in the oxidation of Fe^{2+} (Eq. 3). In contrast to the oxidation of ferrous iron to ferric iron, the hydrolysis of ferric iron produces acid (Eq. 6) thus reducing the pH and tends to stabilize the pH at approximately 2.4 (Nemati *et al*. 1998). Jarosite precipitation (Eq. 5) is also an acid-producing reaction. Elemental sulfur produced in bioleaching may also be oxidized by *A. ferrooxidans* (Nemati *et al*. 1998) and *Sulfobacillus*-like bacteria (Yahya and Johnson 2002), which is an acid producing reaction (Eq. 4). Simultaneous dissolution, precipitation, oxidation and reduction reactions contribute to the net acid consumption or production in bioleaching. It is thus difficult to maintain the pH with the desired range, especially in heaps (Rawlings *et al*. 2003).

According to the review by Ojumu *et al*. (2006), changes in the pH do not essentially affect either the growth or the iron oxidation kinetics of iron oxidizing microbes at a narrow range around their optimum pH. The literature reports on the effects of larger pH changes differ quite significantly. Some sources report significant inhibition below pH 1.5 and above 3.5 (Jensen and Webb 1995, Nemati *et al*. 1998). According to Breed and Hansford (1999) no simple relationship exists between the maximum bacterial specific ferrous iron and oxygen utilization rates by *L. ferrooxidans* in the pH range from 1.1 to 1.7.

The pH has been shown to affect solubilization differently depending on the metal. For example, Ahonen and Tuovinen (1995) reported that with a complex sulfide ore, the leaching rates of cobalt, copper, nickel and zinc each responded differently to pH changes. The rates of solubilization of cobalt and copper were highest at low pH, whereas nickel and zinc showed practically no pH dependency.

Controlling the pH in the heap is crucial in regard to the problematic precipitation of ferric iron. Solubility of iron is defined by the redox potential and pH of the solution (Nemati *et al*. 1998). Generally, Fe^{3+} has an extremely low solubility at pH above 2.5. In sulfate-rich environments Fe^{3+} precipitates mainly as jarosites (Eq. 5), hydroxides (Eq. 6), goethite (Eq. 7) or schwertmannite (Eq. 8). Schwermannite transforms to goethite or jarosite with time, depending on the pH conditions (Bevilaqua *et al*. 2002). Jarosite formation is enhanced at high concentrations of monovalent cations, Fe^{3+} and SO_4^{2-} (Nemati *et al*. 1998, Gramp et al. 2008). The threshold pH for jarosite formation is close to pH 1.5, but this depends on the ionic composition of the solution. Jarosite seeding has been shown to extend the pH range of precipitation to pH 0.6 (Dutrizac 1996).

Precipitation of ferric compounds is thus affected by temperature, pH and concentrations of the species present. The stability areas of ferric precipitates as a function of pH and temperature are presented in Fig. 5.

3.3 O_2 and CO_2

Oxygen is the electron acceptor of microbial ferrous iron oxidation. In high-rate bioleaching systems the availability of oxygen may be gas-liquid mass transfer limited and thus govern the overall rate rather than microbial oxidation kinetics (Kinnunen and Puhakka 2004, Ojumu *et al*. 2006). It is critical to maintain an adequate concentration of the electron acceptor in the process. The development of anaerobic conditions may favor the reduction of ferric iron through anaerobic respiration by *A. ferrooxidans* (Malki *et al*. 2006). Gleisner *et al*. (2006)

reported that increasing the concentration of dissolved oxygen resulted in increased pyrite oxidation rate.

Chemoautotrophic microorganisms such as *A. ferrooxidans* and most other bioleaching microorganisms have strict requirements for CO_2 as a carbon source for growth (Nemati *et al*. 1998). Barron and Lueking (1990) reported that sparging batch bottles with air containing 7 to 8% (vol/vol) of CO_2 supported the maximal rate of growth. An increase in the level of CO_2 beyond 8% resulted in the inhibition of the growth of *A. ferrooxidans* (Barron and Lueking 1990). Holuigue *et al*. (1987) reported that a culture sparged with air containing around 5% of CO_2 provided maximal simulation of culture growth, and that significantly higher cell yields, although not growth rates, were obtained as the level of available CO_2 was increased. From the viewpoint of the limited solubility of CO_2, at low pH the growth of iron-oxidizing bacteria could become limited by CO_2 availability. However, the maximum oxidation rate may require an optimum CO_2 concentration different from the one required for maximum growth rate or cell yield (Jensen and Webb 1995).

3.4 Ferrous iron and ferric iron

The growth of iron-oxidizing microorganisms and their ability to oxidize iron is significantly affected by the concentration of ferrous iron (Barron and Lueking 1990, Gómez and Cantero 2003, Nemati and Webb 1996, Okereke and Stevens 1991). Increasing the ferrous iron concentration to a certain critical value enhances the oxidation rate while higher ferrous iron concentrations results in lower oxidation rates. For *A. ferrooxidans*, which is the iron-oxidizing microorganisms used in most of the studies, the critical value has been reported to vary between 2 and 6 g/L (Barron and Lueking 1990, Gómez and Cantero 2003, Jones and Kelly 1983). Concentration of 20 g/L of ferrous iron has been found to completely inhibit the oxidation of ferrous iron (Barron and Lueking 1990).

Bacterial growth and Fe^{2+} oxidation are also subject to product inhibition by ferric iron (Curutchet *et al*. 1992, Jones and Kelly 1983, Shrihari and Gandhi 1990). Shrihari and Gandhi (1990) observed an extended lag phase and reduced oxidation rates with 5 g/L of initial ferric iron, whereas ferric iron at initial concentration of 20 g/L completely prohibited the oxidation of ferrous iron by *A. ferrooxidans*. Curutchet *et al*. (1992) reported partial and complete inhibition with 4 g/L and 16 g/L of ferric iron, respectively. *L. ferriphilum* has been reported to tolerate higher concentrations of both ferrous and ferric iron than *A. ferrooxidans* (Kinnunen and Puhakka 2005). The review of rate equations by Ojumu *et al*. (2006) indicates that the inhibition by ferric iron is perhaps not as significant as previously assumed, but that it is rather the lack of ferrous iron substrate that limits the growth and oxidation kinetics.

The tolerances of mesophilic iron oxidizers to Fe^{2+} and Fe^{3+} in different studies are shown in Table 7.

Table 7. Tolerance of iron oxidizers to Fe^{2+} and Fe^{3+}.

	Fe^{2+} (g/L)			Fe^{3+} (g/L)			
Microbe	**NE**	**PI**	**TI**	**NE**	**PI**	**TI**	**Reference**
A. ferrooxidans	2-3	-	20	-	-	-	Barron and Lueking (1990)
A. ferrooxidans	2.7	8.4	-	-	-	-	Gómez and Cantero (2003)
A. ferrooxidans	-	-	-	2	-	16	Curutchet *et al*. (1992)
A. ferrooxidans	-	-	-	-	5-10	-	Das *et al*. (1997)
A. ferrooxidans	-	-	-	-	5	20	Shrihari and Gandhi (1990)
L. ferriphilum	24	30	-	5	10	>20	Kinnunen and Puhakka (2005)
L. ferriphilum	4	-	>20	5	-	>60	this study

NE, no effect, PI, partial inhibition, TI, total inhibition, -, not reported.

3.5 Other metals

Microorganisms require the presence of several metals that have significant biochemical roles such as catalysts, enzyme co-factors and protein structure stabilizers (Bruins *et al*. 2000). However, above normal physiological concentrations, metals become toxic. In recent studies biological iron oxidation has been reported to be adversely affected by the presence of arsenic, copper, cobalt, mercury, nickel, lead, zinc, uranium, aluminum and silicon.

There are numerous studies in the literature on the toxicity of metals to acidophilic iron oxidizers (Table 8). Kinnunen and Puhakka (2005) studied the effects of pH, Fe^{2+}, Fe^{3+} and Cu^{2+} on Fe^{2+} oxidation by an enrichment culture dominated by *L. ferriphilum*, which was tolerant to relatively high metal concentrations. Cabrera *et al*. (2005b) also determined the tolerance limits of *A. ferrooxidans* in the presence of heavy metals. *A. ferrooxidans* was found to tolerate high levels of the metals tested. Malik *et al.* (2004), Leduc *et al*. (1997) and Sampson and Phillips (2001) have also reported on the resistance to heavy metals in different strains of *A. ferrooxidans* and *L. ferrooxidans.* Recently, Watkin *et al*. (2009) demonstrated the metal tolerances of isolates from a spent copper sulfide heap, closely related to *A. caldus*, *Acidimicrobium ferrooxidans* and *Sulfobacillus thermosulfidooxidans*, and found adaptation to Cu, Zn, Ni and Co. Acidophilic Fe^{2+} oxidizers are exposed to high concentrations of Fe^{3+} and other metals in bioleaching processes, yet there are only few articles presenting rate equations where an inhibition term for these metals would have been included (Ojumu *et al*. 2006), and the combined effects of multiple metals on oxidation kinetics have not been systematically examined.

The tolerances of mesophilic iron oxidizers to selected metals in different studies are shown in Table 8.

Table 8. Tolerance of iron oxidizers to Ni^{2+}, Zn^{2+} and Cu^{2+}.

	Ni^{2+} (g/L)		Zn^{2+} (g/L)		Cu^{2+} (g/L)		Co^{2+} (g/L)		
Microbe	**NE**	**TI**	**NE**	**TI**	**NE**	**TI**	**NE**	**TI**	**Reference**
A. ferrooxidans	-	31	-	31	-	11	-	-	Cabrera *et al.* (2005b)
A. ferrooxidans	-	1	-	>10	-	-	-	-	Malik *et al.* (2004)
A. ferrooxidans	-	9, >9	-	-	-	>10	-	-	Leduc *et al.* (1997)
Mix of *A. ferrooxidans* and *L. ferrooxidans*	5	>9	-	-	0.5	>10	2	>9	Sampson and Phillips (2001)
L. ferriphilum	-	-	-	-	2	-	-	-	Kinnunen and Puhakka (2005)
L. ferriphilum	-	>60	-	>60	-	-	-	-	this study

NE, no effect, TI, total inhibition, -, not reported.

There are large variations in the results on the toxicity of heavy metals to bioleaching microorganisms owing to strain variation and the abilities for adaptation (Nemati *et al*. 1998). Adaptation to high levels of metal through exposure to the metal is likely to be responsible for large part of the variation (Rawlings 2005). Although microorganisms do not react well to sudden and significant changes in metal ion concentrations, they can be adapted to incrementially-increased concentrations over a period of time (Watling 2008). Microorganisms inhabiting naturally occurring bioleaching environments have developed effective resistance mechanisms to metals ions, providing them with competitive advantage (Dopson *et al*. 2003). Metal resistance mechanisms in acidophilic microorganisms have been reviewed for example by Dopson *et al*. (2003). Information on metal toxicity is needed in operating bioleaching systems and in developing novel processes. A possible example for the optimization of the existing techniques is the transfer of arsenic resistance genes to thermophilic archaea. These microorganisms are naturally not as resistant to arsenic as moderately thermophilic bacteria but would enable the use of higher temperatures and thus increased reaction rates in bioleaching (Dopson *et al*. 2003).

3.6 Ore characteristics

Mineralogy of the ore, ore particle size and size distribution of the ore affect leaching. However, surprisingly limited quantitative research on the correlation between ore mineralogy, reaction chemistry and leach residue mineralogy has been reported so far (Watling 2006).

Non-sulfide minerals occurring with the sulfide ore significantly affect the growth and activity of metal sulfide-oxidizing microorganisms and the economics of the process. For example, if the carbonate content of the ore is relatively high the organisms may not be able to maintain the required acidic conditions. In heap bioleaching, acid consumption by gangue minerals is a key parameter and sulfuric acid usually a major cost factor in ore processing (Watling 2006). In addition, the relatively large quantity of gangue compared to valuable minerals within a typical heap, continuous recycle of the leaching solution, and the long times of exposure can result in the release of considerable concentrations of gangue cations into the heap leaching solution – to the point where they may exceed limits commonly considered toxic to iron-oxidizing microorganisms (Ojumu *et al*. 2006). Empirical evidence for this notion is lacking, however.

The presence of excessive fine material, especially clay minerals, may also be problematic, especially in heap bioleaching. Fines may migrate with the leaching solution to the bottom of the heap and restrict water flow and access of oxygen (Olson *et al*. 2003). In addition to the reactions connected to the oxidation of sulfide minerals, metals and other compounds dissolving during bioleaching may affect also the subsequent metal recovery.

4 MODELING OF THE REACTIONS OF IRON AND BIOPROCESSES RELATED TO BIOLEACHING

There is a growing interest among the research on bioleaching to develop models in order to improve heap and reactor design and management (Watling 2006). Developing models for complex bioleaching and effluent treatment systems requires profound understanding concerning the individual reactions occurring during the processes and the possibilities to model the sub processes. In this chapter a brief introduction to the basic modeling considerations of some of these reactions and processes is given.

4.1 Iron oxidation kinetics

Given the importance of iron oxidation by acidophilic bacteria in bioleaching processes, the oxidation kinetics has been addressed in numerous studies. A number of kinetic models for the bacterial Fe^{2+} oxidation have been proposed and they can be broadly classified as either empirical or based on Michaelis-Menten/Monod rate expressions (Boon *et al*. 1999a, Kelly and Jones 1978, Lacey and Lawson 1970, Ojumu *et al*. 2006). Generally, experimental data on Fe^{2+} oxidation kinetics with acidophilic microorganisms and the corresponding growth rates have been adjusted to the Monod equation (Ojumu *et al*. 2006), which is most frequently used to describe microbial growth rate:

$$\frac{dX}{dt} = Y\left(-\frac{dS}{dt}\right) - bX \qquad (15)$$

$$\frac{dS}{dt} = -\frac{q_m XS}{K_s + S} \qquad (16)$$

where dX/dt = bacterial growth rate (g VS/L·h), dS/dt = substrate conversion, i.e., oxidation rate (g Fe^{2+}/L·h), S = substrate concentration (g Fe^{2+}/L), X = biomass concentration (g VS /L), Y = yield coefficient (g VS/g Fe^{2+}), q_m = maximum specific substrate oxidation rate (g Fe^{2+}/gVS·h), and K_s = half saturation concentration (g Fe^{2+}/L).

The Monod-based data analysis is applicable only to initial rates and does not consider inhibitory effects. In the presence of toxicants, both rates in Equations 15 and 16 can become slower. The basic inhibition models are presented in Table 9. A common type of inhibition is the so-called self-inhibition (Haldane kinetics, Eq. 17), in which the rate of enzyme-catalyzed degradation of the substrate is slowed down because of high concentration of the substrate. When additional inhibition occurs with the presence of a competitive inhibitor at a concentration of I, inhibitor binds to the catalytic site of the enzyme, which excludes substrate binding in proportion to the degree to which the inhibitor is bound (Rittman and McCarty 2001). In the presence of a competitive inhibitor and considering self-inhibition, Eq. 17 converts to Eq. 18. Another type of inhibition is non-competitive inhibition, in which the inhibitor binds to the enzyme at a site other than the active site, altering the enzyme conformation (Rittman and McCarty 2001). In the case of non-competitive inhibition, Eq. 19 can be used to describe the rate of iron oxidation. Third type of inhibition is un-competitive, in which the inhibitor binds only to the complex formed between the enzyme and the substrate (Rittman and McCarty 2001, Eq. 20).

Table 9. Inhibition equations tested in this study and the definitions of the parameters used in the equations.

Number of the equation	Name of the model	Equation
17	Haldane equation (substrate inhibition model)	$\frac{dS}{dt} = \frac{q_m SX}{K_s + S + S^2/K_i}$
18	Competitive inhibition	$\frac{dS}{dt} = \frac{q_m SX}{(1 + I_c / K_{iic})K_s + S + S^2/K_i}$
19	Non-competitive inhibition	$\frac{dS}{dt} = -\frac{q_m SX}{(K_s + S + S^2 / K_i) \cdot (1 + I_{nc} / K_{iinc})}$
20	Uncompetitive inhibition	$\frac{dS}{dt} = -\frac{q_m SX}{K_s + (S + S^2 / K_i) \cdot (1 + I_{uc} / K_{iiuc})}$

Parameter	Definition
$\frac{dS}{dt}$	Fe^{2+} oxidation rate (g Fe^{2+}/L·h)
S	Substrate (Fe^{2+}) concentration (g/L)
X	Biomass concentration (g VS / L)
q_m	Maximum specific Fe^{2+} oxidation rate (g Fe^{2+}/ g VS·h)
K_s	Half saturation constant (g/L)
K_i	Self-inhibition constant (g/L)
K_{iic}	Competitive inhibition constant (g/L)
K_{iinc}	Non-competitive inhibition constant (g/L)
K_{iiuc}	Uncompetitive inhibition constant (g/L)
I_c	Initial competitive inhibitor (Fe^{3+}) concentration (g/L)
I_{nc}	Initial non-competitive inhibitor (Zn^{2+} or Ni^{2+}) concentration (g/L)
I_{uc}	Initial uncompetitive inhibitor concentration (g/L)

Ojumu *et al*. (2006) conducted an extensive review of the models developed for describing and predicting the kinetics of microbial ferrous iron oxidation. Most of the reviewed studies had been performed with *A. ferrooxidans* (Boon *et al.* 1999a, b, Braddock *et al.* 1984, Crundwell 1997, Jones and Kelly 1983, Lacey and Lawson 1970, Liu *et al.* 1988, Lizama and Suzuki 1989, Meruane *et al.* 2002, Nemati and Webb 1998, Nikolov and Karamanev 1992) but some also with *L. ferrooxidans* and *L. ferriphilum* (Hansford 1997). Most of the studies have focused on the effects of ferrous and ferric iron concentrations on microbial iron oxidation kinetics whereas most of the other factors have been much less extensively studied. Modified forms of Monod equation have been used in different studies to interpret the inhibition effect of substrate [Fe^{2+}] on the growth and activity of iron-oxidizing bacteria (Nemati *et al*. 1998, Ojumu *et al*. 2006). For describing the inhibitory effect of ferric iron on the rate of ferrous iron oxidation, both competitive and non-competitive models have been suggested. Non-competitive inhibition by ferric iron has been reported by Jones and Kelly (1983) and by Nemati and Webb (1998); however, most of the works published to date on *A. ferrooxidans* have reported competitive product inhibition rather than non-competitive (Boon *et al.* 1999a, b, Gómez *et al*. 1996, Kawabe *et al*. 2003, Nyavor *et al*. 1996). According to Kawabe *et al*. (2003) the inhibition of ferrous ion oxidation of *A. ferrooxidans* by ferric ions can be described as competitive inhibition in which the enzyme is coupled with about two unit of the inhibitor (Fe^{3+}).

The inhibitory effects of other metal ions on iron oxidation kinetics have also been studied widely (see section 3.5), but the models describing inhibition kinetics are applicable only to a single inhibitor. Kupka and Kupsáková (1999) studied the effects of Ni^{2+} and Cu^{2+} on the kinetics of Fe^{2+} oxidation by *A. ferrooxidans* and the experimental data could be described by the Monod equation and non-competitive inhibition. Cabrera *et al*. (2005a) developed a kinetic equation for Fe^{2+} oxidation by *A. ferrooxidans* in the presence of toxic metals (Cr^{3+}, Cu^{2+}, Cd^{2+}, Zn^{2+} and Ni^{2+}) and the results showed a non-competitive inhibition of Fe^{2+} oxidation caused by the test metals.

Studies exploring the effects of temperature and pH have largely been limited to conditions near the optimum or those used in tank bioleaching applications. Only Crundwell's (1997) equation includes a combination of rate terms accounting for three factors affecting microbial iron oxidation (ferrous ions, oxygen and acid). None of the models suggested for describing the kinetics of microbial iron oxidation seem to describe the kinetics well at high ferric to ferrous ratios, where maintenance effects dominate over growth related ferrous iron utilization (Ojumu *et al*. 2006). Ojumu *et al*. (2006) suggested that future research should be directed especially at expanding the ranges of applicability of the developed rate equations as well as incorporating the variation of all significant parameters within one comprehensive rate equation.

4.2 Fluidized-bed bioreactors for iron oxidation

The highest microbially-catalyzed iron oxidation rates have been achieved in packed-bed and fluidized-bed bioreactors (Table 4). Compared to packed-beds, fluidized-bed bioreactors can be operated with smaller sized support particles without the drawbacks of clogging, occurrence of preferential flow patterns, high liquid pressure drop, or particle compression due to bed weight. Fluidization provides large surface area for biofilm formation and growth, and fluidized-bed bioreactors with high rate liquid recycling approach completely mixed conditions. More efficient mixing enhances mass transfer from gas to liquid and provides homogenous conditions within the bed. In fluidized-bed bioreactors also the control of the overall activity of the reactor is easier as the biomass can be more easily replaced without disturbing the operation of the reactor. In some respects the fluidized-bed bioreactors are more complex than packed-bed bioreactors. Hydrodynamic characteristics of the fluidized-bed are challenging to determine and changing as the properties of the support particles are changing during the operation due to the dynamically evolving biofilm (Shieh and Keenan 1986, Gòdia and Sola 1995, Jördening and Buchholz 1999).

Most of the fluidized-bed reactors developed for biological processes include three phases: solid, liquid and gas. Basic fluidized-bed bioreactors can be classified as cocurrent up-flow and countercurrent flow reactors, and they can have liquid or gas phase as the continuous phase (Gòdia and Solà 1995). The following description of the main factors affecting the performance of fluidized-bed reactors focuses on the most commonly used bioreactor type for iron oxidation, the cocurrent up-flow reactor, with liquid as the continuous phase. The solid phase consists of the support matrix and the cells immobilized on the surfaces of the support particles. In some cases the solid phase can also be formed of cell aggregates created by self-immobilization. In the case of iron oxidation in fluidized-bed bioreactors, gas is present because of the aeration needs of the cells. Gas can also be present as a product of the metabolisms of the microorganisms (Shieh and Keenan 1986, Gòdia and Solà 1995).

If the flow rate of the solution fed to the reactor is not adequate to maintain fluidization of the solid particles, fluidization can be obtained by recycling the solution or by gas loaded or produced in the reactor. The liquid and gas flow rate required for bed fluidization is determined by the density, size and shape of the solid particles present, the density, viscosity, and surface tension of the liquid, and the dimensions of the reactor. Changes in these parameters have to also be considered. For example, the density of the solid particles may change due to cell growth and deaths, and also the density of the liquid may decrease because substrate is being consumed. These changes are, however, of particular relevance only at the beginning of the reactor operation before a dynamic pseudo-steady-state has been reached (Shieh and Keenan 1986, Gòdia and Solà 1995).

Microbiological aspects of fluidized-bed bioreactors such as biofilm characteristics and the factors affecting biofilm formation have been discussed for example by Shieh and Keenan (1986). The most important microbiological parameter affecting the overall performance of an FBR is the biofilm thickness. Thicker biofilm provides higher amount of biomass, but when determining the optimal biofilm thickness also the mass transfer resistance imposed by the gelatinous structure of the biofilm has to be considered (Shieh and Keenan 1986).

The development of models to describe the operation of fluidized-bed bioreactors is a complex task, as high number of interconnecting hydrodynamic aspects has to be considered. The flow patterns of the gas and liquid flows, degree of mixing and heat and mass transfer coefficients are the most critical aspects in this regard (Gòdia and Solà 1995). Modeling of fluidized-bed bioreactors is typically based the assumption of pseudo-steady-state conditions (Shieh and Keenan 1986, Gòdia and Solà 1995). The flow patterns are affected by the liquid and gas superficial velocities in the reactor, degree of mixing, internal gas generation, particle size and size distribution of the solid particles, and external liquid recirculation (Chen *et al.* 1995, Gòdia and Solà 1995). Depending on the hydrodynamic conditions within the reactor and thus the degree of mixing in the liquid phase, a fluidized-bed bioreactor will have a flux pattern between completely mixed and plug flow. If the recycle ratio is above 2, which is commonly the case, an FBR can be treated as a completely-mixed reactor (Shieh and Keenan 1986). The gas phase also affects the liquid and solid mixing in a fluidized-bed bioreactor (Gommers *et al.* 1986). In addition, dimensions of the bioreactor, especially the height to diameter ratio, have an effect on the mixing conditions and the operation of the reactor (Gòdia and Solà 1995).

Gòdia and Solà (1995) identify the key building blocks for developing a reliable model of the fluidized-bed bioreactor performance as follows:

1) reaction kinetics and diffusion within the biocatalyst particles,
2) flux model in the bioreactor,
3) mass balance equations for the species taking part in the reaction (substrates and products), and
4) transport phenomena between the different phases (gas–liquid, liquid–solid).

In a three-phase fluidized-bed bioreactor, reactions occur within the biofilms growing on the surfaces of the solid particles and same kind of substrate and product diffusion and transport processes at different levels have to take place as with biooxidation occurring in bioleaching heaps (see section 2.3) (Gòdia and Solà 1995). The gelatinous biofilm tends to hinder substrate transport and thus causes the substrate concentration surrounding the microorganisms to be less than that in the bulk solution. Thus the combined effects of microbial reaction kinetics and physical mass transport phenomena have to be considered

when modeling FBRs. Substrate conversion in an FBR can be described by the following steps:

1) transport of substrate from the bulk liquid to the liquid-biofilm interface (external mass transfer),
2) transport of substrate within the biofilm (internal mass transfer), and
3) substrate conversion reactions within the biofilm (Shieh and Keenan 1986).

However, the effects of external mass transfer are commonly considered negligible, due to the good mixing in the bioreactor (Shieh and Keenan 1986, Gòdia and Solà 1995).

4.3 Artificial neural networks (ANN) and self-organizing maps (SOM)

As an alternative to physical models, artificial neural networks (ANNs) also provide potential modeling and forecast tools. ANNs, originally developed to imitate the human brain, are composed of a number of interconnected simple processing elements called neurons or nodes. Each node receives one or more input signals from other nodes or external source and sums them to produce an output. Usually a node calculates the weighted sum of its inputs, and then the sum is passed through a function to produce a result. The transfer function is typically a S-shaped curve (a sigmoid curve), but they may also take the form of other non-linear functions, piecewise linear functions, or step functions, processes it through an activation or transfer function and produces a transformed output signal to other nodes or external outputs. ANNs learn from examples and capture functional relationships among the data even if the underlying relationships are unknown or hard to describe. ANNs are thus well applicable to many practical problems whose solutions are difficult to obtain but for which there is enough data or observations (Hammerstrom 1993, Zhang *et al*. 1998).

Many different types of ANN models have been developed. Those most commonly used are multi-layer perceptrons (MLP) models. An MLP is typically composed of several layers of nodes. The first layer is an input layer which is a passive layer where external information is entered. The last layer is an output layer where the solution is obtained. Between the input and the output layers there are one or more intermediate layers called hidden layers. The nodes in the neighboring layers are connected by arcs (Hammerstrom 1993, Zhang *et al*. 1998). A typical MLP network is presented in Fig. 8.

ANN modeling or forecasting always begins by training the ANN with a part of the total available data (input/output pairs), which typically includes experimental data on several parameters. First the input data are normalized. Training includes determining the arc weights so that when certain input data are entered to the input layer the desired output is obtained from the output layer. The values of the weights are initialized typically to random numbers between -0.3 and 0.3. The training algorithm is used to find the weights that minimize some overall error measure such as the sum of squared errors. The values of the weight are changed in response to errors. Hence the network training is actually an unconstrained nonlinear minimization problem. Most networks use back-propagation as the learning system. In back-propagation an output error signal is fed back through the network, altering connection weight as to minimize the error. After training the network is tested with data that was not used in training (Hammerstrom 1993, Zhang *et al*. 1998).

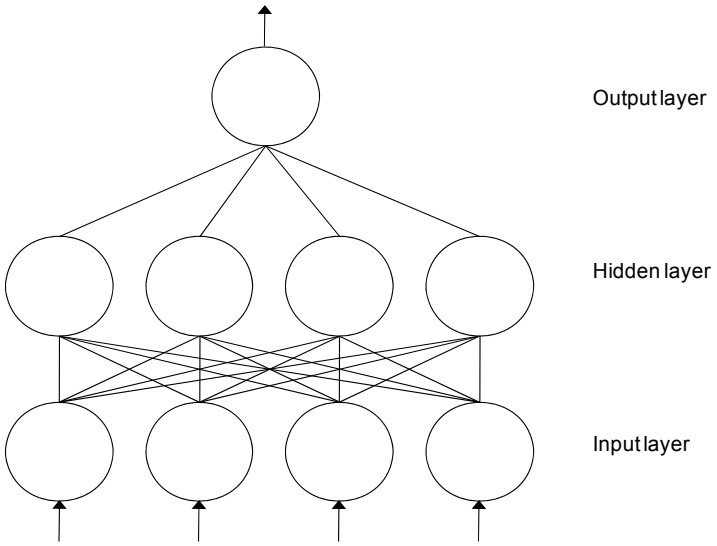


Figure 8. A typical MLP network structure.

So far, there are several applications of ANN models in the environmental engineering and bioengineering areas. Some of the recent applications are listed in Table 10.

Table 10. Applications of ANN models in the environmental engineering and bioengineering areas.

Area of application	**Reference**
Analyzing the system behavior and determining operational problems of a full-scale activated sludge wastewater treatment plant	Cinar (2005)
Evaluating the performance of a membrane bioreactor	Cinar *et al*. (2006)
Simulating the effect of climate change on discharge and the export of dissolved organic carbon and nitrogen from river basin	Clair and Ehrman (1996)
Predicting biogas production and composition in anaerobic digesters	Holubar *et al*. (2002)
Predicting the leachate quantity from a full-scale municipal solid waste landfill	Karaca and Özkaya (2006)
Predicting H_2 production rates in a sucrose-based bioreactor system	Nikhil *et al.* (2008)
Modeling SO_2 concentration of air pollution	Nunnari *et al*. (2004)
Estimating methane fraction in biogas from field-scale landfill	Ozkaya *et al*. (2007)
Determining the performance of high rate sulfidogenic fluidized-bed reactor treating acidic metal containing wastewater	Sahinkaya *et al*. (2007)
Modeling the H_2S and NH_3 components of biogas from anaerobic digestion	Strik *et al*. (2005)

One special type of artificial neural network model is the self-organizing map (SOM, also called the Kohonen map according to its developer, a Finnish Professor Teuvo Kohonen). SOM is a tool for visualization of nonlinear and multidimensional data. It is used to detect statistically significant features which are not easily seen using normal plotting methods. It is able to convert complex statistical relationships between parameters into simple geometric relationships on a low-dimensional (typically two-dimensional) display. Self-organizing maps plot the similarities of the data by grouping similar data items together. The SOM (Fig. 9) also consists of nodes or neurons. The number of neurons can vary from few dozen up to several thousand (Kohonen 1998, 2001). Each vector is represented by a d-dimensional weight vector (reference vector), where d is equal to the dimension of the input vectors. The SOM is trained iteratively, and the input vectors are usually normalized. The weights of the neurons are initially set to random values, preferably from the domain of the input samples. The procedure for placing a vector from data space onto the map is to find the neuron with the closest weight vector to the vector taken from data space and to assign the map coordinates of this neuron to our vector. In each training step, a training example is fed to the network, its Euclidean distance to all weight vectors is computed. The neuron with the weight vector most similar to

the input is called the best matching unit (BMU). The weights of the BMU and neurons close to it (neighborhood neurons) in the SOM lattice are adjusted towards the input vector. This process is repeated for each input vector, continuously decreasing the size of the neighborhood. SOM forms a semantic map where similar samples are mapped close together and dissimilar apart. The most significant difference between the above described back-propagation and self-organization models is the fact that the self-organization is trained without supervision. In self-organization, the network is not told what the correct answer is (Vesanto and Alhoniemi 2000). SOM is one of the most widely used network methods. The applications range from process monitoring to organization of document collections. Comprehensive reviews of SOM research have been conducted by Kaski *et al*. (1998) and Oja *et al*. (2002).

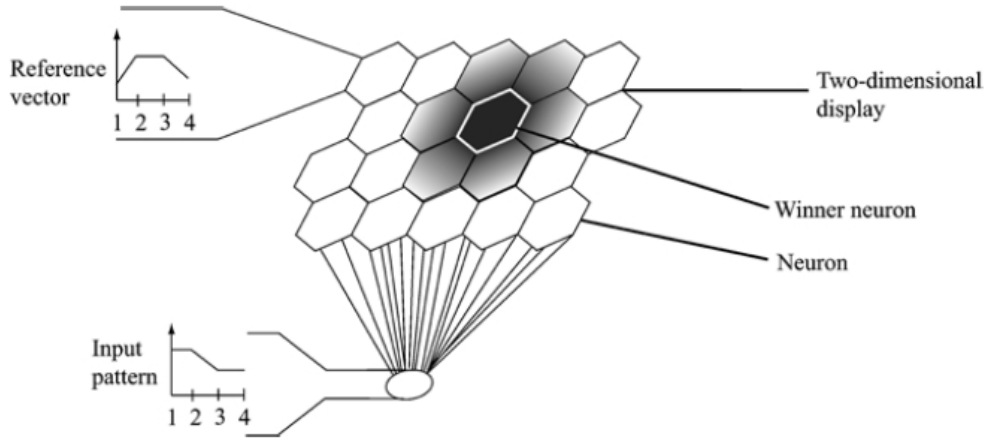


Figure 9. Idea of the SOM (Hautaniemi *et al*. 2003).

In order to effectively manage bioleaching and hydrometallurgical waste water treatment processes, models that accurately describe these systems are required. Models based on ANNs and SOMs could be successfully used in such complex applications which include large amounts of affecting parameters and nonlinear relationships between these parameters. These models could be used to design and manage bioleaching operations in which the nonlinear and complex relationships between the variables (multi-input/output) can be very difficult to otherwise identify.

5 HYPOTHESES AND OBJECTIVES OF THE PRESENT STUDY

Behavior of iron has been reported in many recent studies to be one of the most critical factors in the improvement of the efficiency of bioleaching of sulfidic minerals, especially in heaps (Dopson *et al*. 2007, Malki *et al*. 2006, Watling 2006). An additional iron oxidation stage could be utilized to alleviate the difficulties caused by the poor control over the conditions within heap bioleaching and to enhance the regeneration of the chemical oxidant, Fe^{3+}. For developing such an oxidation stage, biologically catalyzed iron oxidation processes have important advantages over the chemical alternatives. Iron oxidation is also an essential reaction in iron control, required both during heap bioleaching to precipitate the excess iron from the leach circuit and for iron removal from heap bioleaching and other hydrometallurgical effluents. Biological iron oxidation has been studied in detail, but there is very limited published information on concurrent iron biooxidation and precipitation (removal) processes, although the currently used chemical iron removal techniques have their generally acknowledged challenges.

The promising emerging technique of two-stage tank bioleaching of mineral concentrates also requires additional research efforts. High-rate biological iron oxidation at elevated metal concentrations is a prerequisite for this technique, and additional benefits could be gained by carrying out the bio-oxidation step at a low pH. However, the research on kinetics of biological iron oxidation at low pH values and in the presence of multiple inhibitory metals is limited.

This study focuses on addressing the above described research needs. It was hypothesized that efficient iron oxidation can be achieved under conditions applicable to the two-stage tank bioleaching of nickel and zinc sulfide concentrates, which are among the future targets of application (Morin 2007, Viera *et al.* 2007), and that models can be developed that can describe the kinetics of iron oxidation in the presence of single and multiple, potentially inhibitory metals.

For ferric iron regeneration and iron control during heap bioleaching and for iron and sulfate removal from bioleaching effluents an integrated laboratory-scale bioprocess was set up and studied. The process involved a fluidized bed reactor (FBR) for biological iron oxidation which was coupled with a pH adjustment unit followed by a gravity settler for precipitative iron removal. Based on the previous research at Tampere University of Technology (Kinnunen and Puhakka 2004, 2005) it was hypothesized that high-rate and high-efficiency iron oxidation can be achieved with an FBR, and a gravity settler provides the most practical way of separating the precipitated iron from the solution. It was also hypothesized that models can be developed that can be used to recognize and verify the associations between operational and performance variables in the integrated reactor system.

The overall objective of the study was to improve the effectiveness of bioleaching and iron removal from hydrometallurgical waste waters by developing bioprocesses for the oxidation, regeneration and control of iron to be used in these applications. In the development of the integrated bioprocess, the objective was to demonstrate the technical feasibility to couple biological Fe^{2+} oxidation with precipitative iron and sulfate removal, and to determine some of the key operational limits and optimal conditions for such a process on a laboratory scale. This work is seen to provide fundamental insights for the proposed process and demonstrate its feasibility in principle. However, more detailed determination of the design criteria and

scale-up principles and final optimization of the system are beyond the scope of this research as these considerations require experiments on a larger scale system.

The overall objective of the study was pursued by combined experimental work and modeling with the following specific aims:

1. To enhance the development of biological iron oxidation stage of two-stage tank bioleaching of mineral concentrates, especially zinc and nickel concentrates, and modeling of iron oxidation kinetics by
    a. determining and modeling the effect of Fe^{2+} and Fe^{3+} on iron oxidation kinetics of a *L. ferriphilum* dominated FBR culture at pH below 1 (Paper I)
    b. determining and modeling the individual and combined effects of Fe^{3+}, Ni^{2+} and Zn^{2+} on iron oxidation by a *L. ferriphilum* dominated FBR culture at pH 1 (Paper II)
2. To enhance the development of bioprocesses for iron regeneration and control in bioleach liquors by
    a. setting up, preliminary optimizing (in relation to the retention time of the FBR), and modeling the performance of an integrated reactor system consisting of an FBR for biological Fe^{3+} regeneration and a gravity settler for partial precipitative iron removal from barren heap leaching solution (Papers III–V and VII)
3. To enhance the development of bioprocesses for iron and sulfate removal from hydrometallurgical waste waters by
    a. setting up, preliminary optimizing (in relation to the pH of the system), and modeling the performance of an integrated reactor system consisting of an FBR for biological Fe^{2+} oxidation, a pH adjustment unit, and a gravity settler for precipitative iron and sulfate removal from barren heap leaching solution (Papers VI–VII)

6 MATERIALS AND METHODS

6.1 Sources of microorganisms

The acidophilic iron-oxidizing mixed culture used in all the experiments was obtained from an FBR long-term fed with 7 g Fe^{2+}/L supplemented with a mineral salts medium at pH 0.9. The inoculum was from previous FBR experiments with enrichment cultures that were originally derived from drainage water and sludge samples from the Pyhäsalmi mine, Finland (Kinnunen and Puhakka 2004, 2005).

6.2 Batch experiments - kinetics of biological iron oxidation

The kinetics of ferrous iron oxidation by *L. ferriphilum* dominated fluidized-bed culture was studied in batch experiments (Fig. 10) and the results were modeled as described in papers I and II.

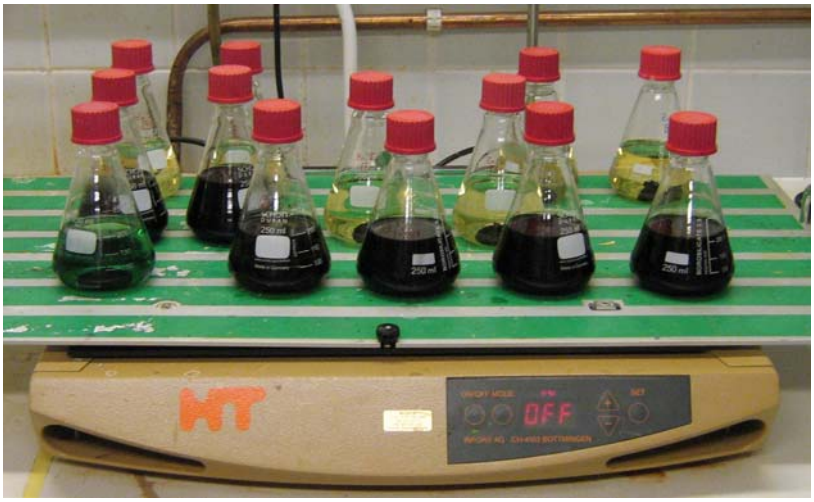


Figure 10. Batch bottles on a shaker used in ferrous iron kinetics experiments.

6.3 Reactor experiments - development of an integrated iron oxidation and precipitation bioprocess

The studies were started with batch bottle experiments in order to determine the possible toxicity effects of the constituents in the simulated heap leaching solution and the settling properties of the precipitates produced at different pHs as described in Paper III. The experiments with the integrated iron oxidation and precipitation system (Fig. 11) with an FBR connected to a gravity settler were began with simulated heap leaching solution (Paper III) and later continued with real barren heap leaching solution obtained after the recovery of valuable metals (Papers V and VI).

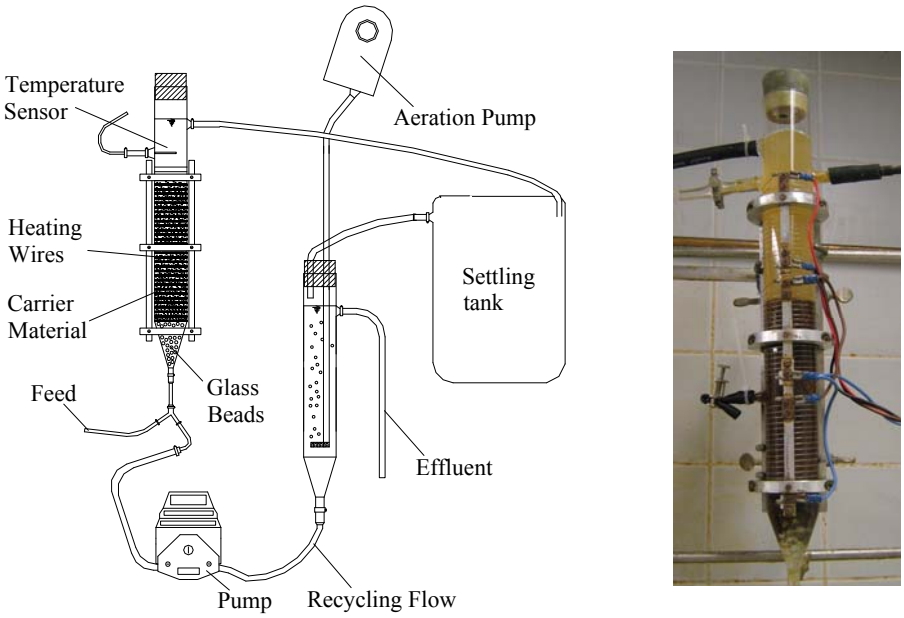


Figure 11. Schematic diagram of the integrated bioprocess with a fluidized-bed reactor (FBR) and a gravity settler and photograph of the FBR. Not drawn to the scale.

With the real barren heap leaching solution, the ferric iron regeneration and partial iron removal performance of the reactor was first preliminary optimized in relation to the loading rate (Paper V) and then the iron and sulfate removal performance was preliminary optimized in relation to the pH of the solution circulating in the reactor (Paper VI). For the pH experiments with the barren heap leaching solution the reactor configuration was modified (Fig. 12). Microbial community in the biofilm of the FBR was also monitored (Papers III, V and VI).

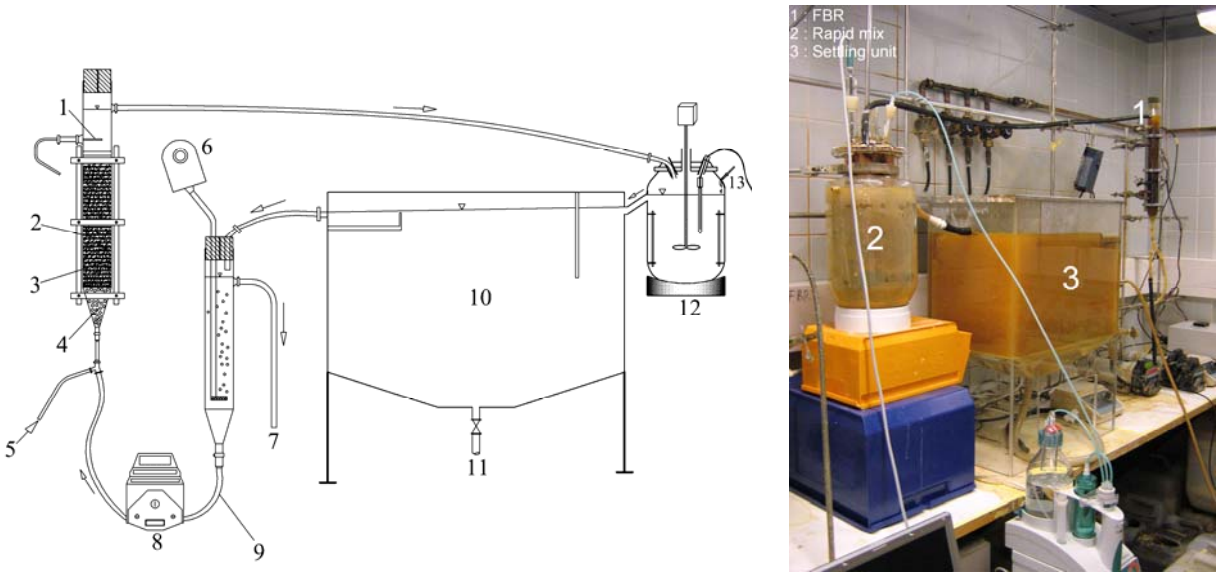


Figure 12. Schematic diagram and photograph of the modified FBR and gravity settler system. 1: Temperature sensor, 2: Heating wires, 3: Carrier material, 4: Glass beads, 5: Feed, 6: Aeration pump, 7: Final system effluent, 8: Recycle pump, 9: Recycle line, 10: Settling tank, 11: Sludge drainage, 12: Rapid mixing, 13: Base addition. Not drawn to the scale.

6.4 Modeling of the performance of the integrated iron oxidation and precipitation bioprocess

The performance of the integrated iron oxidation and precipitation system was modeled with an artificial neural network (ANN) model as described in Paper IV for the effluent Fe^{3+} concentrations with simulated heap leaching solution and with ANN, self-organizing maps and multiple regression modeling as described in Paper VII for the Fe^{3+} precipitated with the real barren heap leaching solution.

6.5 Analyses

The analyses used during the experiments are summarized in Table 11.

Table 11. Summary of the analyses used in the study.

Physiochemical and mineralogical analyses	**Method**	**Paper**
pH	Electrode	I-VII
Redox	Ag/AgCl electrode	I-VII
Dissolved oxygen	Electrode	III-VII
Temperature	Electrode	I-VII
Fe^{2+}	Spectrophotometer	I-VII
Total Fe	AAS	III-VII
Precipitates	ICP, XRD	V-VI
Volatile solids	Furnace, balance	I-II
Total suspended solids	Furnace, balance	III, V, VI
Elemental composition	ICP	V-VI
Microbiological analyses		
Cell enumeration	DAPI staining and microscopic counts	V, VI
Diversity and identification	PCR-DGGE	I-III, V, VI

7 RESULTS AND DISCUSSION

7.1 Iron oxidation kinetics

The Fe^{2+} oxidation kinetics of a *L. ferriphilum* dominated fluidized-bed culture was investigated at pH 0.85±0.15 (Papers I and II). This study was needed because of the gap in the literature on the kinetics of Fe^{2+} oxidation by *L. ferriphilum*, the effect of high Fe^{3+} concentrations on the oxidation kinetics and the fact that the previous inhibition kinetic models of Fe^{2+} oxidation apply to only single toxicants. For determining the individual and combined effects of Fe^{2+}, Fe^{3+}, Zn^{2+} and Ni^{2+} the fluidized-bed culture was subjected to different concentrations of these metals in batch cultures and the experimental results were modeled.

The threshold Fe^{2+} concentration linearly increased from 10 to 180 mg/L and from 120 to 600 mg/L in the presence of Fe^{2+} (0.1–20 g Fe^{2+}/L) and both Fe^{3+} and Fe^{2+} (0–60 g Fe^{3+}/L + 7 g Fe^{2+}/L), respectively. The threshold Fe^{2+} concentration refers to the remaining final Fe^{2+} concentration in batch cultures below which no further oxidation occurred. For comparison, in a chemostat receiving 12 g/L of Fe^{2+} at dilution rates ranging from 0.01 to 0.06 L/h, and temperatures ranging from 30 to 40°C, at a pH of 1.75, Breed *et al*. (1999) reported a threshold Fe^{2+} level of around 5 mg/L for *L. ferrooxidans*.

With Fe^{2+} only, the oxidation started without a lag phase and was completed within 1 to 60 h depending on the initial Fe^{2+} concentration. Specific Fe^{2+} oxidation rates increased up to around 4 g Fe^{2+} /L and decreased at higher concentrations implying substrate inhibition. In the presence of Fe^{3+}, Fe^{2+} oxidation started immediately without a lag phase although the rate decreased at above the initial concentration of 5 g Fe^{3+}/L. The final Fe^{2+} concentrations were obtained after 1 and 2.3 days at initial Fe^{3+} concentrations of 5 and 60 g/L, respectively. This indicated inhibition by Fe^{3+} on Fe^{2+} oxidation at high concentrations. The tolerances of mesophilic iron oxidizers to Fe^{2+} and Fe^{3+} in previous studies are shown in Table 7. The comparison shows that the present study is the first report of Fe^{2+} oxidation in the presence of 60 g/L of Fe^{3+} at pH of around 0.8. In conclusion, the use of high pulp densities in indirect (two-stage) ore concentrate leaching becomes possible as the regeneration step tolerates Fe^{3+} concentrations higher than previously reported.

The results demonstrate that the *L. ferriphilum* dominated culture also tolerated high concentrations of other metals. The culture oxidized ferrous iron even in the presence of 60 g Zn^{2+}/L or 50 g Ni^{2+}/L. The initial 4 g Fe^{2+}/L was completely oxidized within 70 days (at the rate of 0.50 g Fe^{2+}/g VS·h) in the presence of 60 g Zn^{2+}/L and within 45 days (at the rate of 0.71 g Fe^{2+}/g VS·h) in the presence of 50 g Ni^{2+}/L. Cabrera *et al*. (2005b) reported that 30 g Zn^{2+}/L and 40 g Zn^{2+}/L were the highest tolerated concentrations of Zn^{2+} for *A. ferrooxidans* and a mixed culture of *A. ferrooxidans* and *Acidiphilium* spp., respectively. The maximum tolerated concentration of Ni^{2+} by *A. ferrooxidans* has been reported to vary from 10 g/L to up to 60 g/L with some strains (Cabrera *et al*. 2005b, Dopson *et al*. 2003, Leduc *et al*. 1997).

Concerning the combined effects, Fe^{2+} oxidation proceeded by the *L. ferriphilum* dominated culture in the presence of binary combinations of 40 g Fe^{3+}/L + 10 g Ni^{2+}/L, 30 g Fe^{3+}/L + 40 g Zn^{2+}/L, 60 g Zn^{2+}/L + 10 g Ni^{2+}/L and 60 g Ni^{2+}/L + 10 g Zn^{2+}/L. Mg^{2+} and Na^{+} had a compounding effect on Zn^{2+} toxicity. These results show the potential of using high

concentrations of ferric sulfate in biohydrometallurgical applications including tank leaching systems. Very little information is available in the literature on the combined effects of several metals on the kinetics of iron oxidation by acidophilic leptospirilli or acidithiobacilli. Das *et al*. (1997) and Li and Ke (2001) tested binary and ternary combinations of potentially toxic metals on iron oxidation by *A. ferrooxidans*. While the results showed synergistic toxicity and acclimation for improved leaching, no kinetic analysis of inhibition was undertaken. Kinnunen and Puhakka (2005) studied the influences of Fe^{2+}, Fe^{3+} and Cu^{2+} ion concentrations and their different combinations on the iron oxidation by an enrichment culture dominated by *L. ferriphilum* but no detailed kinetic analysis was performed.

The experimental data was modeled by comparing the results to conventional models (Table 9) and the following combined model developed in this study:

$$\frac{dS}{dt} = \frac{q_m SX}{\left[K_s\left(1+\frac{I_c}{K_{iic}}\right)+S+S^2/K_i\right]\cdot\left(1+\frac{I_{nc1}}{K_{iinc1}}+1+\frac{I_{nc2}}{K_{iinc2}}+\ldots+1+\frac{I_{ncn}}{K_{iincn}}\right)} \quad (21)$$

where dS/dt = specific Fe^{2+} oxidation rate (g Fe^{2+}/g VS·h), S = substrate concentration (g Fe^{2+}/L), X = biomass concentration (g VS/L), q_m = maximum specific substrate oxidation rate (g Fe^{2+}/g VS·h), K_s = half saturation constant (g/L), I_c = initial competitive inhibitor concentration (g/L), I_{ncn} = initial non-competitive inhibitor concentration (g/L), K_i = self-inhibition constant (g/L), K_{iic} = competitive inhibition constant (g/L), and K_{iincn} = non-competitive inhibitor constant (g/L).

MATLAB and POLYMATH computer programs were used to find the models that most accurately describe the experimental results. The inhibition types and constants for the effects of different inhibitors on Fe^{2+} oxidation kinetics by the *L. ferriphilum* dominated fluidized-bed culture were as summarized in Table 12. A low value of K_{ii} indicates strong inhibition. In the present study, the order of increasing toxicity was Fe^{3+}>Zn^{2+}>Ni^{2+}.

For Fe^{2+} oxidation in the presence of only Fe^{2+}, the substrate inhibition model (Eq. 17) gave a reasonably good fit with R^2 value of 0.90. For self-inhibitory compounds, there is a critical substrate concentration $C_{cr} = \sqrt{K_s K_i}$ above which the substrate removal rate starts to decrease (Sahinkaya and Dilek 2007). The critical substrate concentration was calculated using observed kinetic parameters (K_s and K_i). The theoretical critical Fe^{2+} concentration was around 2 g/L with the corresponding specific oxidation rate of around 1.65 g Fe^{2+}/g VS·h. In the calculation of the Fe^{2+} oxidation rates, the initial rates were considered in order to neglect the inhibitory effect of Fe^{3+} produced during incubation. Therefore, the decrease in the Fe^{2+} oxidation rate was solely due to the inhibition by Fe^{2+} iron.

Table 12. Inhibition types for Fe^{2+} oxidation kinetics by the *L. ferriphilum* dominated fluidized-bed culture and values of the modeling constants for different inhibitors.

Inhibitor	**Type of inhibition**	**R^2 value of the fit**	**q_m (g/g VS·h)**	**K_s (g/L)**	**K_i (g/L)**	**K_{ii} (g/L) obtained in this study**	**Fe^{2+} oxidation rate at the concentration of 10 g/L (g Fe^{2+} /g VS·h)**	**Fe^{2+} oxidation rate at the concentration of 50 g/L (g Fe^{2+} /g VS·h)**
Fe^{2+}	Substrate	0.90	2.40	0.413	8.65	[a]	0.90	[b]
Fe^{3+}	Competitive	0.83	2.34	0.413	8.65	0.828	0.85	0.33
Zn^{2+}	Non-competitive	0.97	2.04	0.410	8.65	49.1	1.01	0.60
Ni^{2+}	Non-competitive	0.97	2.01	0.410	8.65	62.7	1.09	0.71

[a]Not applicable.
[b]Data not available.

For describing the combined effect of Fe^{3+} (a competitive inhibitor) and Zn^{2+} or Ni^{2+} (non-competitive inhibitors) or two non-competitive inhibitors an additional combined competitive and non-competitive model (Eq. 21) was developed based on the model by Jin and Bhattacharya (1996). The competitive and non-competitive kinetic expressions were developed to a combined model. Conceptually, the combined model can be extended to additional inhibitors. This model fairly accurately described the experimental results. Lag phases were not considered in the kinetic models and therefore the initial iron oxidation data deviated from the predicted lines in some experiments. Ferrous iron was slowly oxidized even at 40-60 g Fe^{3+}/L concentrations but these data did not fit the combined model. Thus the models cannot be used to describe situations that involve over 40 g/L Fe^{3+} concentrations.

The proposed model (Eq. 21) with observed kinetic constants can be used to predict the Fe^{2+} oxidation rate of the *L. ferriphilum* culture in the presence of Fe^{3+} and other metals. Such predictions will help to determine the solids retention time and the volume of bioreactors for Fe^{2+} oxidation in bioleaching of concentrates such as ZnS and pentlandite, in which acidophilic iron oxidizers are in contact with high Fe^{3+}, Zn and Ni concentrations. At present, the designer of a bioleaching process has to rely on limited qualitative information on inhibition.

7.2 Development of an integrated iron oxidation and precipitation bioprocess

7.2.1 Experiments with the simulated heap leaching solution

Iron re-oxidation is essential in the bioleaching of sulfide ores since Fe^{3+} is an important electron shuttle and a chemical oxidant in the process. Recirculation of Fe^{3+} and Fe^{2+} containing leach solutions back to the process is common practice in heap bioleaching but leads to the accumulation of dissolved iron. Accumulation of iron may adversely affect the process via iron precipitation on mineral surfaces, pipelines and orifices and toxicity of high iron concentrations to microorganisms. Although biological iron oxidation has been studied at depth, there is only very limited published information on simultaneous iron biooxidation and precipitation/removal processes. The existing reports are rarely reported in sufficient detail. In the present study, iron oxidation was coupled with iron precipitation in an integrated reactor system involving a low pH fluidized-bed reactor (FBR) with activated carbon matrix for continuous biological iron oxidation and a gravity settler for precipitative removal of iron and sulfate.

In the batch and continuous-flow FBR experiments (Paper III), it was found that (i) Fe^{2+} was almost completely oxidized in simulated heap leaching solution, i.e., in the presence of cations (g/L): Fe^{2+} (20); Mn^{2+} (3); Mg^{2+} (4); Al^{3+} (0.1); Na^{+} (3.6); Ca^{2+} (0.6), and (ii) iron oxidation rate by the enrichment culture dominated by *L. ferriphilum* did not significantly vary between pH of 1.5–2.5.

In a related study, Malik *et al*. (2004) studied the separate effects of various concentrations of nickel, lead, zinc, manganese, aluminum and silicon on Fe^{2+} oxidation by *A. ferrooxidans*. They demonstrated that as the concentration of aluminum was increased from 1.2 to 2.5 g/L, Fe^{2+} oxidation rate decreased from 2.8 to 0.05 g Fe^{2+}/L·d. They also determined that manganese (0.05–0.5 g/L) did not affect the Fe^{2+} oxidation rates (2.7–2.9 g Fe^{2+}/L·d).

With air aeration, the maximum Fe^{2+} oxidation rate for the simulated heap leaching solution in the FBR (Paper III) was 10 g/L·h at a retention time (τ_{FBR}) of 2 h, below which oxygen mass transfer became limiting. Ebrahimi *et al*. (2005) reported that the maximum Fe^{2+} oxidation rate corresponded to an oxygen transfer rate of 1.2 g O_2/L·h in an airlift reactor. In the present study, with the FBR the oxygen transfer rate was 1.5 g O_2/L·h. Optimum Fe^{2+} oxidation performance in FBR was achieved at a loading rate of 10.7 g Fe^{2+}/L·h with the conversion efficiency of 96% but the efficiency decreased due to precipitate formation. The results of the present study show that for < 30% Fe^{3+} precipitation, the optimum pH range is between 1.5 and 2.0. Jensen and Webb (1995) reported a decrease in iron oxidation rate with activated carbon carrier material, proposed to partly result from precipitate deposition in pore spaces at pH 2. In this study, the installation of a gravity settler in the recycle line improved the oxidation rate from 2 g Fe^{2+}/L·h to 4 g Fe^{2+}/L·h in the simulated solution and iron oxidation was almost complete (98.5%) even at pH 2.5. The Fe^{3+} precipitate had good settling characteristics (SVI values of about 30 mL/g) and the settling tank effluent had low turbidity with a suspended solid concentration of 360–480 mg/L.

7.2.2 Experiments with the barren heap leaching solution

The same integrated reactor system was fed with a real barren heap leaching solution following the recovery of valuable metals, and the effects of retention time (τ_{FBR}) (Paper V) and pH (Paper VI) on the performance of the system were determined. The iron in solution was in a completely reduced state, suggesting extensive reduction of ferric iron in the preceding stages of the leach circuit. Fourteen operational regimes were tested (Table 13).

Table 13. Operational regimes with the barren heap leaching solution.

Experiment	τ_{FBR} (h)	Flow rate of feed pump (mL/h)	Fe^{2+} concentration in the feed solution (g/L)	pH adjusted to (with)	Operation duration (days)
1	5.0	68.0	8.3±0.5	No adjustment, pH 2±0.2	12
2	2.5	136	8.0±0.1	No adjustment, pH 2±0.2	10
3	1.5	227	7.9±0.6	No adjustment, pH 2±0.2	12
4	1.0	339	8.4±0.4	No adjustment, pH 2±0.2	10
5	0.7	485	7.8±0.4	No adjustment, pH 2±0.2	7
6	1.5	227	7.7±0.1	No adjustment, pH 2±0.2	20
7	5.0	68.0	7.8±0.7	No adjustment, pH 2±0.2	36
8	2.0	170	6.7±0.2	2.5 (KOH)	11
9	2.0	170	7.0±0.2	3.0 (KOH)	18
10	2.0	170	7.3±0.2	3.5 (KOH)	24
11	2.0	170	6.4±0.9	3.2 ($CaCO_3$)	14
12	2.0	170	5.4±0.9	3.0 ($CaCO_3$)	15
13	2.0	170	6.2±0.4	2.8 ($CaCO_3$)	15
14	2.0	170	14±0.5	2.8 ($CaCO_3$)	15

Biological oxidation and control of dissolved iron in the barren heap leaching solution

Upon feeding the reactor system with the barren heap leaching solution following the recovery of valuable metals, with ambient air aeration, and at loading rates below 10 g Fe^{2+}/L·h (τ_{FBR} of 1 h), 98–99% of the Fe^{2+} in the barren heap leaching solution was oxidized in the FBR. The peak Fe^{2+} oxidation performance with 99% oxidation efficiency and average

stable oxidation rate (R_{FBR} with the fluidized-bed volume as the reference volume) of 8.2 g Fe^{2+}/L·h was achieved at τ_{FBR} of 1 h, which corresponds to the ferrous iron loading rate of 8.3 g Fe^{2+}/L·h and oxygen mass transfer of 1.2 g O_2/L·d. When taking the total volume of the reactor system as the reference volume the maximum average iron oxidation rate was 110 mg Fe^{2+}/L·h. Below τ_{FBR} of 1 h, oxygen mass transfer from gas to liquid limited iron oxidation rate. The iron oxidation rates for the FBR were higher than values previously reported for most other types of bioreactors (Table 4). Higher oxidation rates have only been reported for packed-bed bioreactors (Gómez and Cantero 2003, Grishin and Tuovinen 1988, Mazuelos *et al*. 2000, Nemati and Webb 1996). However, the rate comparison with literature data is somewhat arbitrary, as the volume bases in the calculations often remains unspecified.

The precipitation of ferric iron produced in the FBR varied from 5 to 40 % of the total dissolved iron in solution, being highest at τ_{FBR} of 5 h. The concurrent Fe^{2+} oxidation and partial precipitative iron removal were maximized at τ_{FBR} of 1.5 h, with Fe^{2+} oxidation rate (R_{FBR}) of 5.1 g Fe^{2+}/L·h (R_{tot} of 66 mg Fe^{2+}/L·h) and Fe^{3+} precipitation rate of 15 g Fe^{3+}/day in the pH adjustment unit and gravity settler, which corresponded to 37% iron removal. Gravity settler in the recycle line of the FBR was used to remove precipitates from the solution. Comparative evaluation of the iron removal performance with previous studies (Table 4) is not possible because (i) only a very limited amount of information on coupled iron biooxidation and precipitation/removal processes is available in the literature, and (ii) sufficient technical and biological details have not been disclosed in previous publications.

Compared to other process configurations, an important advantage of the integrated process consisting of an FBR and a gravity settler developed in this study is the concurrent high-rate iron oxidation and controlled partial precipitative iron removal. Some immobilized cell bioreactor types for iron oxidation have previously been reported to suffer the drawbacks of overgrowth of the cells and extensive precipitation of ferric iron compounds within the carrier matrix, causing strong diffusional resistance against the transfer of nutrients and eventually blockage (Table 4, Nemati *et al*. 1998). With the integrated process presented in this study these drawbacks are virtually non-existent. The fully mixed conditions and constant up-flow rate of the FBR result in efficient mass transfer providing sufficient oxygen supply although some Fe(III) precipitation also occurred in the FBR. Blockage of the carrier matrix did not take place. Ferric iron precipitation is beneficial to the leach circuit because partial removal of iron from regenerated leach liquors prevents iron accumulation in the overall leaching process. The integrated process described herein is robust because of high level of biomass retention. The robustness helps this bioprocess withstand transiently adverse operating conditions in heap bioleaching circuits. Compared to the most commonly used catalyzed chemical iron oxidation alternatives, the developed bioprocess has the advantages of operating under atmospheric pressure and thus keeping the bioleaching microorganisms viable, lower reagent costs and lower risks of environmental damage.

The developed integrated reactor system could also have potential to be used in two-stage tank bioleaching of mineral concentrates where a separate iron oxidation step is required. In this study the reactor was tested only with barren heap leaching solution, but as presented in section 7.1 the FBR culture tolerates high concentrations of metals, which is a prerequisite for using the reactor system in applications involving mineral concentrates.

Iron and sulfate removal from the barren heap leaching solution

Effluents from bioleaching operations and acid mine drainage cause severe problems if dispersed in the environment. Dissolved iron in bioleach liquors may also interfere with the subsequent metal recovery in bioleaching processes. Therefore, excess iron and sulfate have to be removed from these streams as stable end products. The biological or combined biological and chemical treatment of acid-mine drainage (AMD) has been studied over the years, a very limited amount of information has been published on the treatment of bioleaching solutions. In this study, the ferric iron regeneration and control process described above was further developed for iron and sulfate removal from bioleaching effluents. KOH and $CaCO_3$ were used to neutralize the pH in the range of 2.5–3.5 (Paper VI). The process was operated with 6.0±1.5 g Fe^{2+}/L and 14±0.5 g Fe^{2+}/L in the feed.

Upon increasing the pH with KOH, the oxidation rate increased from 3.3 to 3.7 g Fe^{2+}/L·h with the oxidation efficiency remaining at above 99%. The relative amount of ferric iron precipitation increased with the pH being as follows: 70% (precipitation rate of 19 g Fe^{3+}/day) at pH 2.5 and 99% (30 g Fe^{3+}/day) at pH 3.5. The extent of SO_4^{2-} precipitation in the pH adjustment unit and gravity settler was on average 14% (19 g SO_4^{2-}/day) during the KOH experiments. With $CaCO_3$ at the lower feed Fe^{2+} concentration (6.0±1.5 g Fe^{2+}/L) the Fe^{2+} oxidation rate remained close to 3 g Fe^{2+}/L·h and oxidation efficiency was >99.5%. The highest Fe^{2+} oxidation rate of 7 g Fe^{2+}/L·h was achieved with 14±0.5 g Fe^{2+}/L in the feed. Upon increasing the pH with $CaCO_3$, iron precipitation increased with the pH from 93% to 98% at pH 2.8 and 3.2, respectively. Sulfate precipitation was about 30–40 % during these experiments. With 14±0.5 g Fe^{2+}/L in the feed, 96 and 66% of the Fe^{3+} and sulfate precipitated, respectively. Iron and sulfate precipitation rates were 55 g Fe^{3+}/day and 150 g SO_4^{2-}/day, respectively. Base consumption in the KOH experiments and in the $CaCO_3$ experiments varied from 1.7 to 2.2 meq./mol Fe^{3+} removed, and from 0.77 to 2.4 meq./mol Fe^{3+} removed, respectively. Changing the base from KOH to $CaCO_3$ did not have a negative effect on the process performance; however, $CaCO_3$ is a low-cost chemical and thus preferred for neutralization. Technical challenges with the use of $CaCO_3$, which forms a suspension with water, were overcome. A shaker was used to keep the $CaCO_3$ in suspension in the slurry. Another challenge during the $CaCO_3$ experiments resulted from the precipitates adhering to the surfaces of the reactor system, causing clogging in the narrowest parts. Clogging was prevented by cleaning the reactor regularly. This challenge is likely to be alleviated in larger scale reactor configurations.

The X-ray diffraction patterns and the corresponding elemental composition of solids indicated that the precipitate formed in the reactor system without pH adjustment mainly consisted of a solid solution of Na- and H_3O-jarosites. When the pH was increased with KOH the precipitates changed to a solid solution of K- and H_3O-jarosites. In solids from the pH 3.5 experiment, the XRD patterns indicated a decline in jarosite peak intensities and presence of goethite (α-FeOOH) as a new phase. Only three goethite lines could be discerned with certainty, suggesting that it was a minor component and not well crystalline. At the same time, the background increased somewhat, indicating an increase in an amorphous phase or a loss of well crystalline material. The presence of goethite was consistent with instability of jarosites at pH values approaching 4. This shift adversely affects the extent of sulfate precipitation from the leach solution.

When the pH was increased with $CaCO_3$, the precipitates changed to a solid solution of K- and H_3O-jarosites and gypsum ($CaSO_4 \cdot 2H_2O$). At pH 3.2, the relative proportion of gypsum

increased, jarosite decreased and other amorphous ferric iron precipitates were formed. As in the KOH experiments, the peak intensities also decreased in the $CaCO_3$ experiments with increasing pH.

Comparative evaluation of the iron and sulfate precipitation performance with previous biological iron removal processes (Table 6) is difficult because of the limited amount of information on these processes available in the literature, especially for bioleaching solutions, and the lack of sufficient technical details in the publications. Processes developed for the treatment of AMD have usually been designed for solutions containing significantly lower concentrations of iron than typical in the effluents from bioleaching operations. Processes involving iron biooxidation and precipitation have been reported to be problematic, especially in a large scale. Iron oxidation rates are typically low (Table 6) and Fe(III) precipitates have been reported to cause clogging in processes designed for iron biooxidation (Nemati et al. 1998, Gómez and Cantero 2003, Jensen and Webb 1994, Omura *et al*. 1991). The integrated process developed in this study resolves these challenges. The process consists of separate biological, chemical and physical unit operations, which can be separately optimized for maximum overall performance. The developed process has bacterial biomass concentration in the range of 10^9 cells/g carrier material and 10^7-10^8 cells/mL in solution, which results in high iron oxidation rates and good tolerance to shock loadings. The previous studies on iron removal have focused on the treatment of acid mine drainage (Table 6) and not on the treatment of solutions associated with bioleaching processes.

The integrated process consisting of an FBR combined with a pH adjustment unit and a gravity settler in the recycle line can be applied to bioleaching effluents for high-rate and high-efficiency iron and sulfate removal. The bioprocess may have potential for iron and sulfate removal also from low pH AMD and other metal and sulfate containing effluent streams. Compared to the most commonly used chemical iron removal techniques, the developed bioprocess has the advantages of operating under ambient temperatures and pressures and avoiding the formation of voluminous ferric hydroxide precipitates. Compared to the bioprocess patented by Das and Das (2004) for hydrometallurgical leach liquors, the reactor system developed in this study has the advantages of operating in truly continuous mode and at higher feed flow rates, tolerating higher Fe^{3+} concentrations, having been proven for higher Fe^{2+} concentrations of the feed solution, and having higher biomass retention. Compared to the bioprocess scheme patented by Maree and Johnson (1999), the process developed in this study has the advantages of being proven for higher Fe^{2+} concentrations of the feed solution, achieving comparable iron removal performances at lower pH values, not requiring the use of flocculants, and producing mainly jarosites in stead of $Fe(OH)_3$.

The performance of the combined reactor system with barren heap leaching solution at different τ_{FBR} and pH values is summarized in Table 14.

Table 14. Performance of the combined iron oxidation and precipitative iron and sulfate removal process at different τ_{FBR} and pH values.

τ_{FBR} (h)	pH	Fe^{2+} conc. in the FBR outlet (mg/L)	Fe^{2+} oxidation rate, R_{FBR} (g/L·h)	Fe^{2+} oxidation efficiency (%)	Fe^{3+} loading rate to the settler, R_{tot} (mg/L·h)	Fe^{3+} precipit. rate (g/day)	Fe^{3+} precipit. efficiency (%)	SO_4^{2-} precipit. rate (g/day)	SO_4^{2-} precipit. (%)	Base consumption (meq./mol Fe^3 removed)
No pH adjustment										
0.7	2±0.2	60–2500	9.9	85.9	130	11	22	N.d.	N.d.	-
1	2±0.2	50–80	8.2	99.2	110	19	20	N.d.	N.d.	-
1.5	2±0.2	50–100	5.1	99.2	66	15	37	N.d.	N.d.	-
2.5	2±0.2	80–140	3.2	98.7	41	0.8	3.2	N.d.	N.d.	-
5	2±0.2	50–60	1.6	99.3	21	5.0	39	N.d.	N.d.	-
pH adjustment with KOH										
2	2.5	30–60	3.3	99.4	25	19	70	18	13	N.d.
2	3.0	15–30	3.5	99.7	26	26	92	24	17	2.2
2	3.5	15–40	3.7	99.5	27	30	99	13	11	1.7
pH adjustment with $CaCO_3$										
2	3.2	20–30	3.1	99.6	23	25	98	53	34	2.0
2	3.0	10–30	2.7	99.5	20	21	94	36	28	2.4
2	2.8	10–30	3.0	99.7	23	23	93	59	40	1.6
2	2.8	15–60	7.0	99.7	53	55	96	150	66	0.77

N.d. not determined

7.2.3 Bacterial community of the integrated iron oxidation and precipitation bioprocess

Initially, the acidophilic iron-oxidizing culture was obtained from an FBR, long-term fed with 7 g Fe^{2+}/L supplemented with a nutrient medium at pH 0.9. During the experiments, the microbial community was monitored by using denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified partial 16S rRNA genes. The PCR-DGGE followed by partial sequencing of the 16S rRNA gene showed that the bacterial community of the FBR fed with the simulated (Paper III) and real (Papers V and VI) barren heap leaching solutions and operated at different pH and τ_{FBR} values remained dominated by *L. ferriphilum* with the similarity of 100%. Occasionally, also sequences matching "*Ferrimicrobium acidiphilum*" were seen. "*Ferrimicrobium acidiphilum"* has not been formally characterized but has been found in several mine drainage environments (Bacelar-Nicolau and Johnson 1999, Bond *et al*. 2000, Brofft *et al*. 2002). The bacterial community in the FBR did not significantly change during the experiments although the system was in part of the experiments (Papers V and VI) continuously supplemented with a mixed community of bioleaching microorganisms indigenous in the barren leaching solution.

7.3 Modeling of the performance of the integrated iron oxidation and precipitation bioprocess

Management of bioleaching and hydrometallurgical effluent treatment processes requires models that accurately describe these systems. In this part of the study several visualization and regression models were evaluated in an effort to capture complex relationships between parameters of the processes described above and to predict the performance of the processes. For the data from the experiments with the simulated heap leaching solution an artificial neural network (ANN) based model was applied to model the Fe^{3+} production in the integrated reactor system (Paper IV). This study demonstrated that ANNs provide a robust tool for predicting the Fe^{3+} concentration of FBR combined with a settling tank to remove the inorganic precipitate from the solution. The model reliably predicted (R^2 value of 0.90) effluent Fe^{3+} concentration based on the selected operational parameters (influent and effluent pH, redox and dissolved oxygen concentration in the FBR, τ_{FBR}, and Fe^{2+} loading rate).

For the data from the experiments with the barren heap leaching solution, three modeling approaches were applied to examine the performance of the integrated process (Paper VII). Self-organizing maps provided a useful visualization tool which also enables the recognition of the relationships between the parameters of such complex systems. An ANN based model was also applied and it reliably predicted the quantities of ferric iron precipitated (mg/L) based on the selected operational parameters (feed pH and Fe^{2+} concentration, dissolved oxygen concentration, pH and redox in the FBR, and τ_{FBR}) in the integrated process. The best-fitting regression model also gave a good fit (R^2 value of 0.87) with the experimental data used but the ANN model was found to perform most accurately (R^2 value of 0.97).

8 CONCLUSIONS AND FUTURE RESEARCH NEEDS

Bioleaching processes involve high concentrations of dissolved metals and these metals may affect leaching efficiencies by inhibiting the action of iron and sulfur oxidizing microorganims. This is the first study to comprehensively determine the kinetics of Fe^{2+} oxidation by *L. ferriphilum*, the combined effects of several metals on iron oxidation by acidophilic leptospirilli or acidithiobacilli and to develop inhibition kinetic models of biological Fe^{2+} oxidation that are applicable to simultaneous effects of multiple toxicants. The *L. ferriphilum* dominated fluidized-bed culture tolerated high concentrations of the test metals. Fe^{2+} oxidation proceeded even at the maximum Fe^{2+} (20 g/L) or Fe^{3+} (60 g/L) concentrations tested although specific Fe^{2+} oxidation rate decreased at above 4 g Fe^{2+}/L and 5 g Fe^{3+}/L indicating substrate and product inhibition, respectively. This is the first report of Fe^{2+} oxidation in the presence of 60 g/L of Fe^{3+} at pH of around 0.9. The results demonstrate that high pulp densities thus become amenable to two-stage (indirect) ore concentrate bioleaching as the regeneration step tolerates elevated Fe^{3+} concentrations. The low pH value prevents metal precipitation. Fe^{2+} oxidation proceeded by the *L. ferriphilum* dominated culture also in the presence of binary combinations of 40 g Fe^{3+}/L + 10 g Ni^{2+}/L, 30 g Fe^{3+}/L + 40 g Zn^{2+}/L, 60 g Zn^{2+}/L + 10 g Ni^{2+}/L and 60 g Ni^{2+}/L + 10 g Zn^{2+}/L. Thus it becomes possible to use high concentrations of ferric sulfate in two-stage bioleaching applications including zinc and nickel concentrates where the concentrations of dissolved Zn^{2+} and Ni^{2+} can approach levels tested in this work.

Modeling of the data from the inhibition experiments showed that the model accounting for substrate inhibition, i.e., Haldane equation, described well the Fe^{2+} oxidation kinetics in the presence of only Fe^{2+}. Fe^{3+} competitively inhibited Fe^{2+} oxidation, and Zn^{2+} and Ni^{2+} toxicity conformed to the non-competitive inhibition model. The order of increasing toxicity was Fe^{3+}>Zn^{2+}>Ni^{2+}. For describing the combined effect of Fe^{3+} (a competitive inhibitor) and Zn^{2+} or Ni^{2+} (non-competitive inhibitors) or two non-competitive inhibitors a combined model was developed which fairly accurately described the experimental data. This is the first model to predict Fe^{2+} oxidation rates in the presence of multiple metals, and it can be used for determining the required retention times and dimensions of Fe^{2+} oxidation bioreactors. Within its range of applicability, the developed model can be used as basis for developing kinetic models for various bioleaching processes provided that the kinetic constants are determined for process-specific metals and microorganisms. The mechanisms of metal toxicity or resistance were not, however, delineated in this study. Further research efforts are needed to gain understanding on metal resistance mechanisms of acidophilic microorganisms. Genomic information and expression studies could offer the way forward in this challenge. Further research is needed also on the effects of dissolved metals on different iron- and sulfur-oxidizing microbial species and communities and on different metals relevant to the bioleaching processes in order to further develop the inhibition models of iron and sulfur oxidation kinetics.

An integrated laboratory-scale reactor system involving a low-pH fluidized-bed reactor (FBR) with activated carbon matrix for continuous biological iron oxidation coupled with a pH adjustment unit and a gravity settler for precipitative removal of iron and sulfate was set up, studied and preliminary optimized for additional ferric iron regeneration and iron control during heap bioleaching and for iron and sulfate removal from bioleaching effluents. Experiments were conducted with simulated and real barren heap leaching solution and the technical feasibility to couple Fe^{2+} oxidation with precipitative iron and sulfate removal was

demonstrated, and the effects of retention time of the FBR and pH on the performance of the process were evaluated.

Without pH adjustment, the highest Fe^{2+} oxidation rates in the FBR were 10 g/L·h (conversion efficiency of 96%) and 8.2 g/L·h (conversion efficiency of 99%) for the simulated and real heap leaching solution, respectively. Below the retention time of 1–2 h oxygen mass transfer from gas to liquid limited iron oxidation rate. For < 30% Fe^{3+} precipitation, the optimum pH range was found to be between 1.5 and 2.0. At pH 2, the concurrent Fe^{2+} oxidation and partial precipitative iron removal was optimized at the τ_{FBR} of 1.5 h, with iron oxidation rate of 5.1 g Fe^{2+}/L·h and Fe^{3+} precipitation rate of 15 g Fe^{3+}/day, which corresponded to 37% iron removal. These results demonstrate that the reactor system has potential for concurrent high-rate biological ferric iron regeneration and control, i.e., partial removal, in bioleach liquors. Scientific studies have not previously been published on combined ferric iron regeneration and control aiming at improving the efficiency of heap bioleaching. Unlike in many other bioprocess configurations for iron oxidation in the integrated process developed in this study the precipitation of ferric compounds within carrier matrix does not compromise the effectiveness of the process. Compared to the catalyzed chemical iron oxidation alternatives, the developed bioprocess has the advantages of operating under atmospheric pressure, lower reagent costs and lower risks of environmental damage.

For the treatment of effluents from bioleaching processes and possibly also acid mine drainage (AMD) and other hydrometallurgical effluents, where effective iron and sulfate removal is required, the process was further developed by using KOH and $CaCO_3$ to neutralize the pH in the range of 2.5–3.5. Upon increasing the pH, the Fe^{2+} oxidation rate remained high and ferric iron removal was significantly enhanced as the precipitation increased to optimally 96–99%. During the optimal process conditions also on average 66% of the sulfate was precipitated. Thus the integrated process has potential for high-rate and high-efficiency iron and sulfate removal from low pH, iron and sulfate containing solutions. Compared to the most commonly used chemical iron removal techniques, the developed bioprocess has the advantages of operating at ambient temperatures and pressures and avoiding the formation of voluminous ferric hydroxide precipitates. The few other iron removal methods utilizing biological iron oxidation reported in scientific journals have not been developed or tested for bioleaching solutions. Compared to the few related patented bioprocesses the bioprocess developed in this study removes also sulfate in addition iron, and achieves comparable or better iron and sulfate removal performances with simpler reactor configuration. In the present study, the integrated process was tested for iron and sulfate removal from a heap bioleaching solution. Further research would be needed on the performance of the integrated process for the treatment of other hydrometallurgical solutions.

The bacterial community of the FBR operated at different τ_{FBR} and pH values remained dominated by *L. ferriphilum* and occasionally also "*Ferrimicrobium acidiphilum*" present in the original inoculum was found although in some experiments the system was continuously supplemented with a mixed community of bioleaching microorganisms associated with the barren leaching solution.

Iron control, i.e., partial removal, is needed to prevent the excessive accumulation of dissolved iron in bioleaching circuits, and effective iron and sulfate removal is required in the treatment of hydrometallurgical effluents and AMD to prevent adverse environmental effects. It can be concluded that the process described above has the potential to be optimized either

for efficient and high-rate iron regeneration and control or for precipitative iron and sulfate removal from solutions of bioleaching operations and hydrometallurgical effluents.

Iron and sulfate removal is essential in the management of bioleaching processes and in the treatment of hydrometallurgical effluent streams. The previous studies on biological iron removal processes have focused on the treatment of AMD. This is the first published study to systematically address the treatment of solutions associated with bioleaching processes. The potential of the integrated iron oxidation and precipitative iron and sulfate removal process on industrial scale is yet to be demonstrated. Further research would be needed on the iron oxidation and iron and sulfate removal performance of the process on a larger scale to determine the detailed design criteria and scale-up principles for the individual unit operations (FBR, addition of the pH adjustment chemical and gravity settler) and to optimize the performance of the system. Especially the retention time and the mixing in the chemical addition step and the design and the retention time of the settling tank have to be carefully considered in order to prevent the accumulation of precipitates in the process. Optimization of the gravity settling process (e.g., through optimizing the retention time, temperature and design of the settler and considering the possibilities of recycling the sludge from the bottom of the settling tank to base addition) was not undertaken in this study but needs attention because the hydraulic conditions are extremely important for stability, crystallinity and crystallite growth of Fe(III) precipitates. Further research is required for determining the properties of the Fe(III) precipitates produced, the possible measures to affect these properties and the options for sludge handling and disposal. Optimization of gravity settling and subsequent sludge dewatering requires pilot-scale experimentations. Further optimization of the process is application-specific as there is considerable variation in the temperature, chemical composition and microbial community of the potential input solutions.

Effective management of complex bioleaching and hydrometallurgical effluent treatment processes requires models that accurately describe these systems. Artificial neural network (ANN) type models are problematic since they do not provide explanations of the effects of individual parameters or the mechanisms by which certain parameter affects the performance, but in certain complex processes they may still be useful. ANN based models cannot provide answers about the fundamental principles governing a process. They may, however, reliably predict the performance of complex processes based on historic data accumulated from continued measurement of the interactions of multiple interconnecting parameters, as this study demonstrated for the performance of the integrated iron oxidation and precipitation system, and thus provide a useful, although not all-inclusive, tool for the management of such processes. In this study ANN based models were used to model the performance of laboratory-scale versions of the integrated reactor system and to recognize and verify the associations between operational and performance variables when preliminary determining their optimal operational conditions. Such models can be used to determine the effects of changes in process parameters on the performance of complex process once they have been calibrated for the process in question with experimental data. ANN and multiple regression modeling successfully applied to the laboratory-scale processes suggests their applicability also in modeling and managing similar kind of larger scale processes although empirical data from larger scale experiments is needed to develop application-specific models.

The development of comprehensive models applicable to heap bioleaching processes, which operate under highly variable conditions, is one of the remaining challenges. Based on the future pilot-scale experiments with the integrated iron oxidation and precipitation bioprocess, it would be advisable to develop a mechanistic model describing the performance of the

process. This kind of model could be built by combining the kinetic models studied and developed in this study with the models describing the different unit operations and reactions occurring during the process. A mechanistic model would enable better understanding of the bioprocess and facilitate scale-up considerations. When combining the iron oxidation and precipitation bioprocess to bioleaching process circuits the model of the bioprocess could be integrated into heap bioleaching models and exploited in gaining better understanding and optimizing the overall performance of heap bioleaching.

In addition to developing mechanistic models to gain deeper understanding of the process also the possibilities of ANN type models should be evaluated. Another modeling area which could provide further insights into understanding and managing bioleaching processes is geochemical modeling based on thermodynamic equilibria. Geochemical modeling can be used to, e.g., calculate chemical speciation of metals in bioleach solutions, study the role of solubility-controlling processes and simulate bioleaching processes.

In summary, this study enables further development of bioleaching and hydrometallurgical effluent treatment processes by providing new information about the Fe^{2+} oxidation kinetics of *L. ferriphilum* at high dissolved metal concentrations at pH 1 and about the combined effects of multiple metals on Fe^{2+} oxidation kinetics, that have not been previously modeled. This is also the first published work to systematically report and model concurrent high-rate biological ferric iron regeneration and control in bioleach liquors, and iron and sulfate removal from solutions associated with bioleaching processes.

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