TAMPEREEN TEKNILLINEN YLIOPISTO
TAMPERE UNIVERSITY OF TECHNOLOGY

Mira Sulonen

Bioelectrochemical Recovery of Energy and Metals from Simulated Mining Waters

Julkaisu 1485 • Publication 1485

Tampere 2017

Tampereen teknillinen yliopisto. Julkaisu 1485
Tampere University of Technology. Publication 1485

Mira Sulonen

Bioelectrochemical Recovery of Energy and Metals from Simulated Mining Waters

Thesis for the degree of Doctor of Science in Technology to be presented with due permission for public examination and criticism in Festia Building, Auditorium Pieni Sali 1, at Tampere University of Technology, on the 15th of September 2017, at 12 noon.

Tampereen teknillinen yliopisto - Tampere University of Technology
Tampere 2017

Doctoral candidate: Mira Sulonen
Laboratory of Chemistry and Bioengineering
Faculty of Natural Sciences
Tampere University of Technology
Finland

Supervisor: Professor Jaakko Puhakka
Laboratory of Chemistry and Bioengineering
Faculty of Natural Sciences
Tampere University of Technology
Finland

Instructors: Assistant Professor Marika Kokko
Laboratory of Chemistry and Bioengineering
Faculty of Natural Sciences
Tampere University of Technology
Finland

Assistant Professor Aino-Maija Lakaniemi
Laboratory of Chemistry and Bioengineering
Faculty of Natural Sciences
Tampere University of Technology
Finland

Pre-examiners: Associate Professor Stefano Freguia
Advanced Water Management Centre
University of Queensland
Australia

Associate Professor Oskar Modin
Division of Water Environment Technology
Chalmers University of Technology
Sweden

Opponents: Professor Abraham Esteve Núñez
Department of Analytical Chemistry, Physical Chemistry
and Chemical Engineering
University of Alcalá
Spain

Associate Professor Oskar Modin
Division of Water Environment Technology
Chalmers University of Technology
Sweden

ISBN 978-952-15-3984-8 (printed)
ISBN 978-952-15-4002-8 (PDF)
ISSN 1459-2045

Abstract

Extremely acidic water with high metal concentrations is often produced during mining and processing of sulfidic ores. Sulfur-oxidizing microorganisms contribute significantly to the acidification of the water streams and oxygen depletion by oxidizing reduced inorganic sulfur compounds (RISCs) — which are released to the mining waters during the processing of sulfide minerals — to sulfuric acid. The acidic water continues leaching metals from minerals and the metal concentrations thus further increase.

Certain metals can be recovered from acidic solutions by using them as the electron acceptor at the cathode of an electrochemical system. The metal ions accept electrons from an electrode and deposit on the surface of the electrode in pure elemental form. The electrical current required for the electrodeposition of metals is conventionally drawn from the oxidation of water. However, with the assist of electroactive microorganisms, biodegradable compounds can be used as the source of the required energy. Electroactive microorganisms oxidize a substrate and donate electrons to an anode electrode. The flow of electrons from anode to cathode creates electrical current, which can be utilized in the electrodeposition of the metals. As mining waters do not usually contain organic compounds, RISCs are promising substrates for the recovery of metals from mining waters — they are present in the same stream and can be oxidized at lower potential than water. In addition, with the electrochemical treatment both metals and RISCs could be removed from the water streams simultaneously.

The aim of this work was to use tetrathionate ($S_4O_6^{2-}$) as the substrate for bioelectrochemical and electrochemical current generation. The possibility to spontaneously produce electricity from tetrathionate was first studied in microbial fuel cells (**Paper I**). After successful electricity production was obtained, a tetrathionate-fed microbial fuel cell was monitored for over 740 days to determine the long-term stability of such systems (**Paper II**). The anode potential was then externally adjusted in order to determine the minimum anode potential required for bioelectrochemical and electrochemical tetrathionate degradation (**Paper III**). Finally, the external voltage required for the simultaneous removal of tetrathionate and copper was determined (**Paper IV**).

The experiments were conducted using two-chamber flow through reactors at room temperature (22±5 °C) and highly acidic conditions (pH < 2.5). The initial lag-time for electricity production from tetrathionate was relatively long in bioelectrochemical systems (approximately 100 days), but spontaneous electricity production was proven successful with ferric iron as the cathodic electron acceptor. By optimizing the external resistance, the current density was successfully improved from 80 mA m^{-2} (1000 Ω) to 225 mA m^{-2} (100 Ω). In the long-term experiment, biofouling or accumulating reaction products were not observed to limit the electricity production even after 740 days of operation. The minimum anode potential for tetrathionate degradation was observed to be 0.3 V vs. Ag/AgCl in the bioelectrochemical systems and 0.5 V in the abiotic electrochemical systems. Higher tetrathionate degradation rates were obtained in the bioelectrochemical systems (>110 mg L^{-1} d^{-1}) than in the electrochemical systems (<35 mg L^{-1} d^{-1}). The reaction products of bioelectrochemical tetrathionate degradation were sulfate and elemental sulfur, while in electrochemical systems only sulfate was detected. For the efficient removal of tetrathionate and copper, applied voltage of above 1.0 V was required. The concentrations of tetrathionate and copper were successfully decreased below the limits set for toxicity (0.5 g $S_4O_6^{2-}$ L^{-1}) and mining effluent discharge (0.3 mg Cu^{2+} L^{-1}).

This study demonstrates for the first time that tetrathionate can be used the substrate for bioelectrochemical current generation. In bioelectrochemical systems with an efficient catholyte, tetrathionate is degraded and electricity is produced spontaneously, but abiotic electrochemical degradation requires external energy. Both bioelectrochemical and electrochemical systems provided higher current densities than a water-oxidizing control reactor when controlling the anode potential or applying external voltage. The simultaneous removal of tetrathionate and copper shows that bioelectrochemical and electrochemical systems are promising alternatives for the treatment of mining waters.

Tiivistelmä

Sulfidimineraalien louhinnan ja prosessoinnin aikana mineraalien sisältämä rikki pääsee usein kosketuksiin veden ja hapen kanssa, mikä johtaa rikkiyhdisteiden hapettumiseen. Hapettumisen tuotteena muodostuu rikkihappoa, joten sulfidimineraalialueilta peräisin olevat kaivosten prosessi- ja jätevedet ovat yleensä hyvin happamia. Rikkiyhdisteitä hapettavat mikro-organismit kiihdyttävät rikkiyhdisteiden hapettumista ja siten myös vesien happamoitumista sekä happikatoa. Metallien liukoisuus paranee usein pH:n laskiessa, joten happamat vedet liuottavat virratessaan lisää metalleja mineraaleista. Muodostuneet happamat kaivosvalumat ovat sen vuoksi ympäristölle vaarallisia sekä niiden happamuuden että korkeiden metallipitoisuuksien vuoksi.

Tiettyjä metalleja, esimerkiksi kuparia ja sinkkiä, voidaan poistaa liuoksista elektrokemiallisten kennojen avulla. Metalli-ionit toimivat elektronien vastaanottajana elektrokemiallisen kennon katodilla ja pelkistyvät elementaarisessa muodossa katodielektrodin pinnalle. Sähköenergia metallien elektrokemialliseen talteenottoon tuotetaan usein hapettamalla vettä elektrokemiallisen kennon anodielektrodilla. Veden hapetus tapahtuu korkeammassa potentiaalissa kuin useimpien metallien pelkistyminen, joten esimerkiksi kuparin talteenotto vettä hapettavassa kennossa vaatii ulkoista energiaa.

Bioelektrokemiallisten kennojen avulla metallien talteenotossa käytettävä sähköenergia voidaan kokonaan tai osittain tuottaa biohajoavista jäteyhdisteistä. Mikro-organismit hapettavat biohajoavia yhdisteitä ja luovuttavat hapettumisreaktiossa vapautuneet elektronit anodielektrodille. Elektronit siirtyvät ulkoisen virtapiirin kautta katodielektrodille, jonka pinnalla metallien pelkistyminen tapahtuu. Kaivosteollisuuden jätevedet eivät yleensä sisällä orgaanisia yhdisteitä, mutta sulfidimineraalien prosessointi vapauttaa kaivosvesiin pelkistyneitä epäorgaanisia rikkiyhdisteitä. Jos näitä epäorgaanisia rikkiyhdisteitä voitaisiin käyttää elektronien lähteenä (bio)elektrokemiallisissa kennoissa, rikkiyhdisteisiin sitoutunut kemiallinen energia voitaisiin muuntaa sähköenergiaksi ja hyödyntää esimerkiksi metallien talteenotossa. Näin myös epäorgaaniset rikkiyhdisteet ja metallit voitaisiin poistaa kaivosvesistä samanaikaisesti.

Tämän tutkimuksen tavoitteena oli hyödyntää tetrationaattia ($S_4O_6^{2-}$) sähkövirran tuottamisen lähtöaineena bioelektrokemiallisissa ja elektrokemiallisissa kennoissa. Tetrationaatin bioelektrokemiallista hapettumista tutkittiin ensin mikrobipolttokennoissa (**Julkaisu I**). Virrantiheyttä parannettiin onnistuneesti optimoimalla käytetty ulkoinen resistanssi (**Julkaisu II**). Pitkäaikaisen operoinnin vaikutusta seurattiin mikrobipolttokennossa, joka oli käynnissä yli 740 päivää (**Julkaisu II**). Anodipotentiaalin vaikutusta sähköntuotantoon ja tetrationaatin hajoamiseen tutkittiin bioelektrokemiallisissa ja elektrokemiallisissa kennoissa (**Julkaisu III**). Lopuksi tutkittiin mahdollisuutta yhdistää tetrationaatin (bio)elektrokemiallinen hajoaminen kuparin pelkistämiseen katodilla ulkoisen jännitteen avulla (**Julkaisu IV**).

Tutkimuksessa käytettiin kaksi-kammioisia läpivirtausreaktoreita, joita operoitiin huoneenlämmössä (22±5 °C) erittäin happamissa olosuhteissa ($pH < 2.5$). Tetrationaatista tuotettiin onnistuneesti sähkövirtaa käyttämällä ferrirautaa elektroniakseptorina bioelektrokemiallisessa kennossa. Kun ulkoinen resistanssi laskettiin 1000 Ω:sta 100 Ω:iin, maksimivirrantiheys nousi 80 mA m^{-2}:sta 225 mA m^{-2}:iin. Reaktiotuotteiden (H^+, SO_4^{2-}, S^0) ja biomassan muodostumisen ei havaittu rajoittavan sähköntuotantoa edes yli kahden vuoden operoinnin jälkeen. Sähkövirtaa tuotettiin bioelektrokemiallisissa kennoissa anodipotentiaalin ollessa 0.3 V vs. Ag/AgCl tai enemmän ja elektrokemiallisissa kennoissa anodipotentiaalin ollessa 0.5 V vs. Ag/AgCl tai enemmän. Tetrationaatti hajosi tehokkaammin bioelektrokemiallisessa kennoissa (>110 mg L^{-1} d^{-1}) kuin elektrokemiallisissa kennoissa (<35 mg L^{-1} d^{-1}). Bioelektrokemiallisissa kennoissa tetrationaatin hajoamistuotteina muodostui sulfaattia ja elementaarista rikkiä, kun taas elektrokemiallisessa kennoissa havaittiin reaktiotuotteena ainoastaan sulfaattia. Yhdistettäessä tetrationaattia hajottava anodi kuparia pelkistävään katodiin tetrationaatti ja kupari saatiin tehokkaasti poistettua, kun kennoon syötettiin yli 1.0 V ulkoista jännitettä. Tetrationaattipitoisuus saatiin laskettua alle toksisuusrajan (0.5 g $S_4O_6^{2-}$ L^{-1}) ja kuparipitoisuus alle yleisen kaivoseffluenteille asetetun raja-arvon (0.3 mg Cu^{2+} L^{-1}).

Tässä tutkimuksessa näytetään toteen ensimmäistä kertaa, että tetrationaattia voidaan käyttää sähköntuotannon lähtöaineena bioelektrokemiallisissa kennoissa. Sähkövirtaa voidaan tuottaa bioelektrokemiallisissa kennoissa spontaanisti käyttämällä ferrirautaa elektronien vastaanottajana katodilla, kun taas abioottinen tetrationaatin elektrokemiallinen hapetus vaatii ulkoista energiaa. Selvästi suurempi virrantiheys saavutettiin kuitenkin tetrationaattia hajottavissa bioelektrokemiallisissa ja elektrokemiallisissa kennoissa kuin vettä hapettavissa kontrollikennoissa. Tetrationaatin ja kuparin yhtäaikainen poisto osoittaa, että (bio)elektrokemialliset kennot ovat lupaava vaihtoehto kaivosvesien käsittelyyn.

Preface

The experimental work for this thesis was carried out at the Laboratory of Chemistry and Bioengineering at Tampere University of Technology (TUT), Finland. The research was conducted as a part of BioElectroMET -project, which was funded by European Union Seventh Framework Programm (Grant agreement number 282970).

I am grateful to my supervisor, Professor Jaakko Puhakka, for his excellent guidance, encouragement and support during my studies. I owe my greatest gratitude to my instructors Assistant Professor Aino-Maija Lakaniemi and Assistant Professor Marika Kokko for always giving me support, guidance, help, tips and comments. I am thankful for all the project partners for valuable collaboration and discussions. In addition, I am grateful to Professor Stefano Freguia, University of Queensland, and Professor Oskar Modin, Chalmers University of Technology, for pre-reviewing this thesis and for their valuable comments and suggestions.

I wish to thank all of my past and present co-workers and fellow students for creating amazing working atmosphere and for giving me help and support whenever needed. I am very grateful to the laboratory wizards Antti Nuottajärvi and Tarja Ylijoki-Kaiste for making everything to work smoothly in the lab. I really appreciate also all the help and assistance of the administration staff at TUT, especially from Tea Tanhuanpää, Kirsi Viitanen and Saila Kallioinen.

Finally, I want to thank my parents, siblings, grandparents and friends for their encouragement, faith and support during all these years. Special thanks to Nemät for reminding me that not all important things in life are research-related.

Tampere, August 2017

Mira Sulonen

Contents

List of Abbreviations

AC	Alternating current
ADP	Adenosine diphosphate
AMD	Acid mine drainage
AMP	Adenosine monophosphate
APS	Adenosine phosphosulfate
ATP	Adenosine triphosphate
BES	Bioelectrochemical system
CE	Coulombic efficiency
COD	Chemical oxygen demand
CV	Cyclic voltammetry
DGGE	Denaturing gradient gel electrophoresis
EC	Electrochemical cell
EIS	Electrochemical impedance spectroscopy
GSH	Glutathionate
GSSH	Sulfane sulfate
HDR	Heterodisulfide reductase
LSV	Linear sweep voltammetry
MEC	Microbial electrolysis cell
MFC	Microbial fuel cell
MSM	Mineral salts medium
NAD	Nicotinamide adenine dinucleotide
NHE	Normal hydrogen electrode
PCR	Polymerase chain reaction
PEMFC	Proton exchange membrane fuel cell
PMF	Proton motive force
RISC	Reduced inorganic sulfur compound
rRNA	Ribosomal ribonucleic acid
SAOR	Sulfite acceptor oxidoreductase
SOR	Sulfur oxygenase reductase
SQR	Sulfide quinone reductase
SRB	Sulfate reducing bacteria
TES	Trace element solution
TetH	Tetrathionate hydrolase
Tqo	Thiosulfate quinone oxidoreductase
TQR	Thiosulfate quinone reductase
Tth	Tetrathionate hydrolase

List of Publications

This thesis is based on the following original publications, which are referred to in this thesis by the roman numerals I-IV. The publications are reproduced with kind permissions from the publishers.

I	Sulonen, M.L.K., Kokko, M.E., Lakaniemi, A.-M., Puhakka, J.A. 2015. Electricity generation from tetrathionate in microbial fuel cells by acidophiles. *Journal of Hazardous Materials* 284, pp. 182-189.
II	Sulonen, M.L.K., Lakaniemi, A.-M., Kokko, M.E., Puhakka, J.A. 2016. Long-term stability of bioelectricity generation coupled with tetrathionate disproportionation. *Bioresource technology* 216, pp. 876-882.
III	Sulonen, M.L.K., Lakaniemi, A.-M., Kokko, M.E., Puhakka, J.A. 2017. The effect of anode potential on bioelectrochemical and electrochemical tetrathionate degradation. *Bioresource technology* 226, pp. 173-180.
IV	Sulonen, M.L.K., Kokko, M.E., Lakaniemi, A.-M., Puhakka, J.A. 2017. Simultaneous removal of tetrathionate and copper from simulated acidic mining water in bioelectrochemical and electrochemical systems. *Submitted for publication*.

The author’s contribution

Paper I: Mira Sulonen performed the experimental work, wrote the manuscript and is the corresponding author. Marika Kokko and Aino-Maija Lakaniemi assisted in planning of the experiments and interpretation of the results. All co-authors commented on the manuscript.

Paper II: Mira Sulonen performed the experimental work, wrote the manuscript and is the corresponding author. Aino-Maija Lakaniemi and Marika Kokko assisted in planning of the experiments and interpretation of the results. All co-authors commented on the manuscript.

Paper III: Mira Sulonen performed the experimental work, wrote the manuscript and is the corresponding author. Aino-Maija Lakaniemi and Marika Kokko assisted in planning of the experiments and interpretation of the results. All co-authors commented on the manuscript.

Paper IV: Mira Sulonen performed the experimental work, wrote the manuscript and is the corresponding author. Marika Kokko and Aino-Maija Lakaniemi assisted in planning of the experiments and interpretation of the results. All co-authors commented on the manuscript.

The experimental work was carried out under the supervision of Prof. Jaakko Puhakka (Papers I-IV).

1 Introduction

Mining has had an important effect on the development of human society, but it has also been the cause of not only intergovernmental conflicts over the territorial control and distribution of profits but also several environmental hazards (Bebbington et al. 2008). Nowadays, the mining operations and water discharges are strictly controlled by legislation in several countries (Williams 2012). However, some old mines still cause major environmental problems due to the poor practices and improper treatment of wastes at the time, when the real significance of the impacts of mining was not fully understood (Johnson 2013).

Despite the increasing control, environmental hazards related to mining have occurred also during the last decade. For example, in 2012 in Talvivaara mine in Finland, gypsum waste pond waters were leaking to the environment increasing the metal and sulfate concentrations in the nearby rivers and lakes (OTK 2014). In 2015, over 10 million liters of acidic mining water was accidentally released from Gold King Mine in Silverton, Colorado, United States (Gobla et al. 2015). The metal-rich mining water contaminated the near-by water environments (Gobla et al. 2015). The environmental accidents and increasing awareness of the environmental effects of mining have led to increasing attention and social pressure on mining industry (Johnson 2013) and to increasing search for efficient treatment methods for mining waters.

Especially, the extraction of metals from sulfide minerals possess a significant environmental risk. The processing of sulfide-containing ores often releases not only metals but also reduced sulfur compounds and sulfur oxyanions to the mining waters (Dopson & Johnson 2012, Schippers & Sand 1999). In the presence of oxygen, sulfur compounds are oxidized to sulfate via reactions that produce high amount of acidity. The formation of sulfate-rich acidic water is known as acid mine drainage (AMD) (Dold 2014, Kalin et al. 2006). AMD can form abiotically, but the presence of sulfur-oxidizing microorganisms increases the reaction rates significantly. The acidic water leaches metals from minerals, and the metal concentrations in AMD are thus also often high

(Bejan & Bunce 2015). Reduced inorganic sulfur compounds (RISCs), such as thiosulfate ($S_2O_3^{2-}$) and tetrathionate ($S_4O_6^{2-}$) can be formed as intermediates in the sulfide oxidation reactions. Aerobic oxidation of sulfide and RISCs consumes oxygen from the water environments (Dinardo & Salley 1998). To decrease the risk of oxygen depletion and uncontrolled acidification of environmental waters, RISCs should be removed from mining waters prior to their release to the environment.

Due to the population growth and industrialization, the consumption of the mineral resources will further increase. Therefore, also mining waters are becoming remunerative resources for metals. Metals can be recovered from water streams even with relatively low metal concentrations, for example, with bioelectrochemical systems (BES) (Nancharaiah et al. 2015). In BES, a microbial catalyst oxidizes a substrate compound on the anode and donates electrons released in the oxidation reaction to a solid anode electrode. The electrons flow from the anode electrode to a cathode electrode through an electric circuit. On the cathode electrode, a terminal electron acceptor accepts the electrons and becomes reduced. Microbial fuel cells (MFCs) are BESs that produce electrical energy spontaneously. In microbial electrolysis cells (MECs), external energy is applied to realize the oxidation and reduction reactions.

By using certain metal ions, such as Cu^{2+}, Zn^{2+} and Pb^{2+}, as the electron acceptor at the cathode, metals can be electrodeposited on the cathode electrode surface in pure elemental form (Modin et al. 2012, ter Heijne et al. 2010). Besides metal recovery, BESs can be used to produce electricity from waste streams. Municipal wastewater as well as several industrial wastewater streams, such as food processing and brewery wastewaters, contain significant amounts of organic compounds, which need to be removed before release to the environment (Pandey et al. 2016). Conventional methods for their removal, such as biological activated sludge treatment, are usually energy intensive (Oller et al. 2011). With BESs, the biodegradable compounds can be removed with little or no external energy (Rozendal et al. 2008). Mining waters do not contain organic compounds, but may contain RISCs due to sulfide mineral processing. By using RISCs as the anodic electron donor for metal recovery, the electron donor and the electron acceptor would be found from the same site and both RISCs and metals could be simultaneously removed from the water streams.

The aim and novelty of the study

The aim of this study was to delineate the potential of using an inorganic sulfur compound, tetrathionate, as a substrate for bioelectrochemical and electrochemical electricity generation. Current generation was first studied in MFCs and then in MECs and in abiotic electrochemical cells (ECs). This is the first study utilizing tetrathionate as the substrate for bioelectrochemical electricity production. In addition, the long-term operation of BESs in highly acidic conditions with RISCs as the substrate was studied for the first time. Furthermore, no previous studies have addressed the influence of the anode potential or cell voltage in (bio)electrochemical electrolysis cells fed with tetrathionate or the simultaneous (bio)electrochemical removal of RISCs and metals.

2 Mining waters

Earth’s crust contains several metals and minerals, which are mined and extracted for numerous industrial applications. Due to the industrialization, increasing human population, urbanization and improving global standard of living, the demand for metals and minerals is still increasing (Rogich & Matos 2008). For example, during the 21st century the yearly production of copper has increased from 12.9 million tons (2000) to 19.4 million tons (2016) (Babbit & Groat 2001, Jewell & Kimball 2017). As the resources are finite and accessible reserves are depleting, complex polymetallic ores and even streams with relatively low metal concentrations, e.g. low concentration ores, mining waste streams and urban wastes, are becoming economically viable resources. The more efficient recovery and utilization of waste streams as a source of metals and minerals are steps towards circular economy.

Two common methods used for the extraction of metals from ores are pyrometallurgy and hydrometallurgy. In pyrometallurgy, the desired metals are separated from the ores using heat (Reddy 2001), while in hydrometallurgy the metals are leached with a liquid extraction medium (Gupta 2003). Hydrometallurgical processes require significant water input. Water is used in mining operations also, for example, for milling, dust suppression and ore concentration (Lottermoser 2010). Mining waters include also the natural waters (i.e. surface waters, ground water and rainwaters) of the mining sites (Lottermoser 2010). Besides chemical leaching agents (e.g. H_2SO_4, HCl, NH_3 and Fe^{3+}), the metals can be leached utilizing biological oxidation and reduction reactions (Johnson & du Plessis 2015, Rohwerder et al. 2003).

2.1 Composition of mining waters

The characteristics of mining waters are mainly defined by the mineral depositions processed, but the location of the mine, the operational methods and chemicals used and the climate also affect the composition of mining waters (Johnson & Hallberg 2003, Lottermoser 2010). Due to seasonal changes, for example, at the production rate and temperature, the characteristics of the mining waters can also vary periodically (Edwards et al. 1999).

Even though the composition may vary at different mining sites, common typical features of mining waters at sites containing sulfide minerals are high acidity, high metal concentrations and high chloride (Cl^-) and sulfate (SO_4^{2-}) concentrations (Lottermoser 2010). The processing of sulfide minerals often releases inorganic sulfur compounds to the mining waters (Liljeqvist et al. 2011). As the processed ores or the chemicals used in the mining operations do not usually contain organic carbon, the concentrations of organic compounds in mining waters often remain negligible (Johnson & Hallberg 2003).

2.1.1 Metals

Leaching is used to extract metals in hydrometallurgical operations, but it can occur also spontaneously in sites where the minerals get in touch with water and suitable leaching agents. The lithopshere consists of a mixture of various minerals and thus the leaching operations often dissolve not only the metal of interest but also simultaneously various other metals, leading to the formation of metal-rich mine waters. In acidic mining waters, metals are usually present as metal ions or as sulfate complexes (Lottermoser 2010).

Iron is the fourth most common element on Earth's crust (Greenwood & Earnshaw 2012), and the processing of iron-bearing minerals releases iron to the mining waters. In acidic water streams, iron is usually present as ferrous iron (Fe^{2+}) or ferric iron (Fe^{3+}) ions (Lottermoser 2010). In aerobic conditions, ferrous iron can be biotically or abiotically oxidized to ferric iron (Johnson & Hallberg 2005). Ferric iron is a strong oxidizer and it is often the primary oxidizer of sulfide minerals (e.g. pyrite). The iron concentrations in mining waters have been reported to increase up to several grams per liter (Table 2.1). The typical reddish color of acidic mine waters is usually caused by the dissolved ferric iron (Johnson & Hallberg 2003). Other metals often present in mining waters at varying concentrations depending on the mineral composition of the mining sites are Cu, Zn, Al and Mg (Johnson & Hallberg 2003, Lottermoser 2010, Zou 2015).

Table 2.1: Characteristics of natural and anthropogenic acidic water streams. Concentrations are presented as mg L^{-1}.

Type	Mill effluent	Richmond mine	Mona adit	Acid mine drainage	Acid mine drainage	Yugama crater lake	Ijen lake	Cinder pool
Location	Ontario	Iron Mountain	Angsley	Zijinshan	River Tinto	Kusatsu-Shirane volcano	Crater lake	Yellowstone National Park
	Canada	California	Wales	China	Spain	Japan	Indonesia	United States
pH	3.08	1.38	2.7	1.8–2.5	2.5	1.54	0.18	4.22
T (°C)	n.r.	30	9	n.r.	n.r.	19.7	37	95
SO_4^{2-}	2500	13640	1550	5000	8260	2450	67276	96
$S_2O_3^{2-}$	n.r.	n.d.	n.r.	n.r.	n.r.	n.r.	n.r.	4.2
$S_4O_6^{2-}$	n.r.	n.d.	n.r.	n.r.	n.r.	n.r.	139	1.8
Polythionates ($S_nO_6^{2-}$)	172[a]	n.d.	n.r.	n.r.	n.r.	n.r.	412.1	n.r.
Fe(tot)	75.2	1955	490	3000	2350	320–1360	1905	0.088
Fe^{2+}	n.r.	558	475	n.r.	n.r.	n.r.	1372	0.088
Fe^{3+}	n.r.	1396	n.r.	n.r.	n.r.	25-249	n.r.	n.r.
Cu^{2+}	3.8	19	35	700	330	n.r.	0.4	n.r.
Reference	Kuyucak (2014)	Druschel et al. (2004)	Coupland & Johnson (2004)	Zou et al. (2015)	de la Torre et al. (2011)	Takano et al. (1997)	Delmelle & Bernard (1994)	Xu et al. (1998), Xu et al. (2000)

[a] includes also $S_2O_3^{2-}$, n.r. not reported, n.d. not detected

2.1.2 Sulfur compounds

Sulfur-containing sulfide minerals are widespread in the Earth's crust (Figure 2.1), and several important metals are mined from such ores. The most common sulfide mineral is pyrite, FeS_2 (Dopson & Johnson 2012). Even if the mineral of interest would not contain sulfide, the surrounding deposits often do. For example, the sulfur content in coal deposits may be up to 20% by weight (Dopson & Johnson 2012). Therefore, also the solid waste materials from mining operations can contain significant amount of sulfide (Dopson & Johnson 2012).

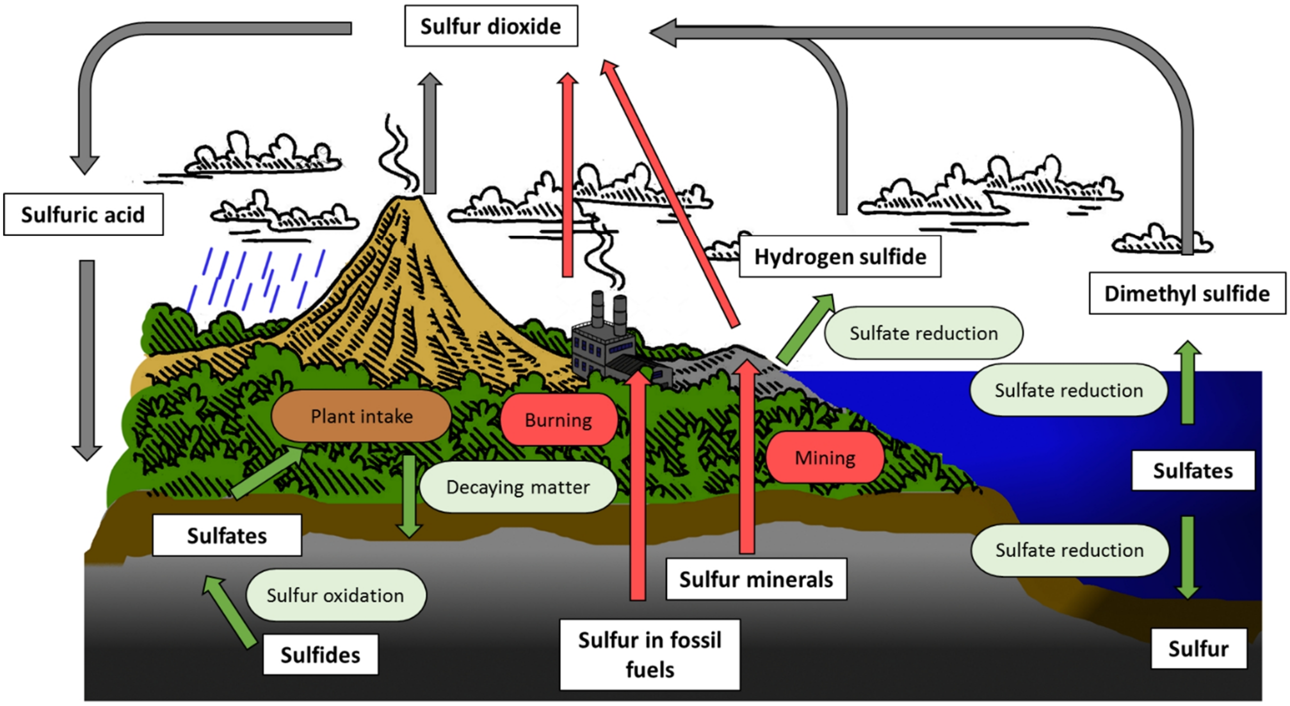

Figure 2.1: The environmental sulfur cycle. The gray arrows indicate chemical reactions, green arrows microbial reactions and red arrows anthropogenic action. Modified from Muyzer & Stams (2008).

The fragmentation of the sulfide containing ores exposes the sulfide minerals to atmospheric conditions. When exposed to oxygen and water, sulfides in the minerals will be oxidized to RISCs and then further to sulfate via acid releasing reactions. The pH of the environment decreases, if the rate of acid production is higher than the buffering capacity of the basic minerals present (Rawlings & Johnson 2002).

The reaction products of biological oxidation of metal sulfide minerals in acidic conditions depend on whether the mineral is acid soluble or acid insoluble (Dopson & Johnson 2012). Acid-insoluble sulfides are degraded via the thiosulfate mechanism (Schippers & Sand 1999). Fe^{3+} oxidizes the sulfide minerals, for example, pyrite FeS_2 to Fe^{2+}, thiosulfate ($S_2O_3^{2-}$) and protons (Equation 1, Figure 2.2). Fe^{2+} can be oxidized again to Fe^{3+} in the presence of oxygen (Equation 2). However, the abiotic oxidation of Fe^{2+} is negligible at pH below 4 (Stumm & Morgan 1995). Thiosulfate is further oxidized to tetrathionate ($S_4O_6^{2-}$), which hydrolyses to sulfate and sulfane-monosulfonic acid ($HS_3O_3^-$), which is highly reactive. The degradation of $HS_3O_3^-$ can lead formation of several different kind of sulfur compounds.

$$FeS_2 + 6\,Fe^{3+} + 3\,H_2O \rightarrow 7\,Fe^{2+} + S_2O_3^{2-} + 6\,H^+ \tag{1}$$

$$4\,Fe^{2+} + O_2 + 4\,H^+ \rightarrow 4\,Fe^{3+} + 2\,H_2O \tag{2}$$

The acid-soluble sulfide minerals (e.g. chalcosite, Cu_2S) can be solubilized by protons and Fe^{3+}. The released sulfide is oxidized first to polysulfides (S_n^{2-}) and then further to elemental sulfur. (Schippers & Sand 1999)

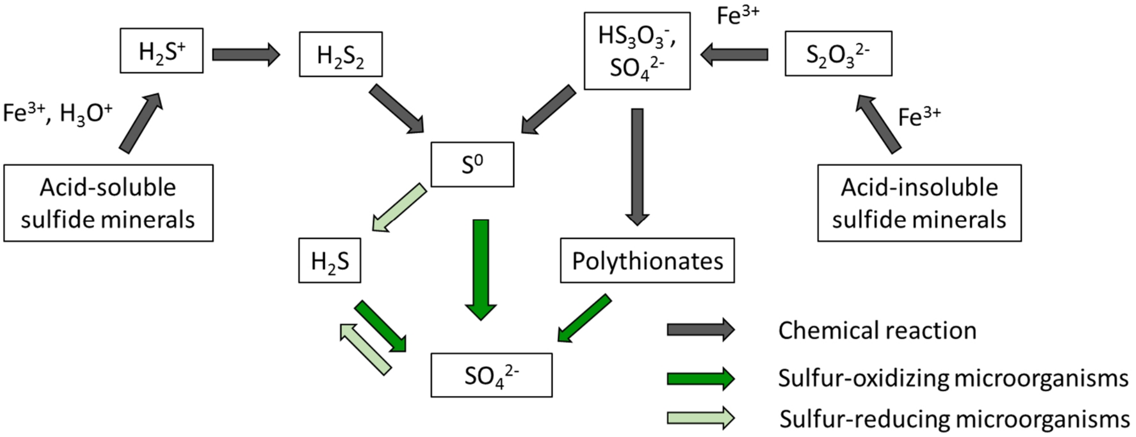

Figure 2.2: Sulfur compounds involved in the bacterial leaching of metal sulfide minerals. Modified from Dopson & Johnson (2012).

Even though sulfide and Fe^{2+} can be oxidized abiotically, the reaction rates are often significantly higher in the presence of biological catalysts (Rawlings & Johnson 2002). Iron-oxidizing microorganisms oxidize Fe^{2+} to Fe^{3+} with high efficiency even in highly acidic conditions and sulfur-oxidizing microorganisms can oxidize the formed RISCs to sulfuric acid (Equation 3) (Rawlings & Johnson 2002). The formation of AMD occurs thus much faster in the presence of iron- and sulfur-oxidizing microorganisms.

$$S_2O_3^{2-} + 2\,O_2 + H_2O \rightarrow 2\,H^+ + 2\,SO_4^{2-} \tag{3}$$

Microorganisms can also oxidize the elemental sulfur formed in the polysulfide pathway and the polythionates formed in the thiosulfate pathway to sulfate. In fact, the most common sulfur compound in mining waters is the most oxidized species, sulfate (SO_4^{2-}), and its concentration can increase up to tens or even hundreds of grams per liter (Lopez-Archilla et al. 2001, Nordstrom et al. 2000).

Thiosulfate is not stable in acidic conditions, as it decomposes to elemental sulfur and sulfite (SO_3^{2-}), which is then dehydrated to sulfur dioxide (SO_2) (Agarwala et al. 1965). Tetrathionate is abiotically oxidized in the presence of oxygen and ferric iron, but the oxidation rate was reported to remain low (below 0.002 g L^{-1} d^{-1}) at 25 °C and pH 1.5 (Druschel et al. 2003). The variety of different possible reaction products and the abiotic oxidation reactions complicate the analysis and determination of the reaction pathways of RISCs (Quatrini et al. 2009).

2.2 Microbiota

Even though the mining waters are often highly acidic ($pH < 3$), certain microorganisms thrive in such conditions. Microorganisms favoring low pH are acidophiles. Moderate acidophiles grow at pH range from 3 to 5, while extreme acidophiles prefer pH 3 or lower (Baker-Austin & Dopson 2007). Some acidophilic species have been reported to grow even at pH 0 (Dopson et al. 2004, Schleper et al. 1995).

Even though the acidophiles prefer environments with high proton concentrations, their intracellular pH remains circumneutral (Baker-Austin & Dopson 2007). The cell structure has evolved to tolerate high pH gradients across the cytoplasmic membrane. The cell membrane is highly impermeable to protons (Konings et al. 2002) and the cells have efficient systems for pumping the excess protons out from the cytoplasm (Baker-Austin & Dopson 2007). The acidophilic microorganisms can actually utilize the high pH gradient over the membrane as a source of energy. As positive charge is accumulating outside and negative charge inside the cell membrane, the pH gradient and the charge difference over the membrane cause a proton motive force (PMF). The transfer of positive ions (e.g. H^+ or Na^+) from outside the cell inside thus releases a significant amount energy, which the cell can utilize for ATP synthesis (Ferguson & Ingledew 2008).

The microbial diversity of the mining waters depends, for example, on the mineral composition, climate and available substrates and electron acceptors (e.g. oxygen, Fe^{3+}, SO_4^{2-}). Besides high acidity, high metal concentrations can limit the growth of microorganisms, even though microorganisms in mining environments usually have a high tolerance to metals (Piotrowska-Seget et al. 2005, Schmidt et al. 2009, Tuovinen et al. 1971). In moderately acidic mine waters, the microbial composition is often diverse, but in extremely acidic conditions the microbial communities have been observed to be relatively similar in different locations, common genera being *Acidithiobacillus*, *Acidiphilum* and *Leptospirillum* (Johnson & Hallberg 2003).

Due to the lack of organic compounds, the microbial communities in mining waters are usually dominated by lithotrophic microorganisms, which utilize inorganic compounds, mostly ferrous iron or inorganic sulfur compounds, as the substrate for growth. These iron-oxidizing and sulfur-oxidizing microorganisms do not only inhabit the mining waters, but they also play a significant role in the formation of acidic AMD. Therefore, one way to slow down the formation of AMD water is the adjustment of the conditions (e.g. pH, the availability of substrates and electron acceptors) in a way that they do not favor the growth of iron- and sulfur-oxidizing microorganisms (Akcil & Koldas 2006).

2.2.1 Sulfur-oxidizing microorganisms

Some microorganisms gain energy for growth by oxidizing RISCs, such as sulfide (S^{2-}), elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$) and/or polythionates ($S_nO_6^{2-}$). The group of bacteria and archaea involved in sulfur transformations in acidic conditions is phylogenetically diverse (Dopson & Johnson 2012). In bacterial metabolism, RISCs are usually oxidized to sulfite, which then further oxidizes to sulfate. In archaea, the RISC degradation occurs via disproportionation to sulfide and sulfite (Rohwerder & Sand 2007). As both of the degradation reactions produce acidity, sulfur-oxidizing microorganisms are usually acid-tolerant or acidophilic (Johnson & Hallberg 2003). Even though RISCs can be also abiotically oxidized, sulfur-oxidizing microorganisms can accelerate the formation of AMD significantly due to high reaction rates.

Many sulfur-oxidizing acidophiles are obligate aerobes and can thus utilize only oxygen as the electron acceptor (Dopson & Johnson 2012). In recipient waters, the aerobic microbial growth can lower the oxygen concentration of the water below the level supporting the growth of aquatic organisms. Besides oxygen, some species (e.g. *Acidithiobacillus ferrooxidans* (Das et al. 1992, Pronk et al. 1992) and *Acidithiobacillus ferrivorans* (Hallberg et al. 2010)) can use ferric iron as an alternative electron acceptor and grow in anoxic environments. Many chemolithotropic sulfur-oxidizing bacteria are able to use also ferrous iron and/or hydrogen as alternative electron donors (Dopson & Johnson 2012). In fact, the presence of Fe^{2+} has been observed to downregulate the expression of RISC oxidation genes in *At. ferrooxidans* (Amouric et al. 2009).

Aerobic degradation of reduced inorganic sulfur compounds

Oxygen is easily available and thus often used as the electron acceptor in the biological degradation of reduced inorganic sulfur compounds. In aerobic conditions, several acidophilic microorganisms (Hallberg et al. 1996, Isamu et al. 1993, Meulenberg et al. 1993, Pronk et al. 1990) oxidize thiosulfate first to tetrathionate (Figure 2.3). Hydrolysis of tetrathionate leads to the formation of thiosulfate, elemental sulfur and sulfate. Elemental sulfur is further oxidized to sulfite and finally to sulfate (Johnson & Hallberg 2008).

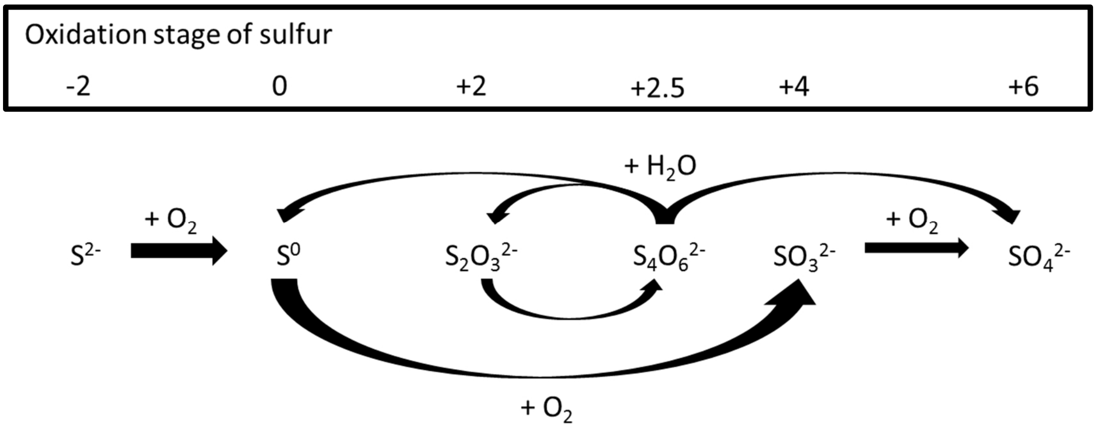

Figure 2.3: Aerobic oxidation of inorganic sulfur compounds by acidophilic microorganisms. Modified from Johnson & Hallberg (2008).

The metabolic routes for RISC oxidation have been studied in several acidophilic microorganisms (Kletzin 2008, Mangold et al. 2011, Quatrini et al. 2009, Veith et al. 2011). Quatrini et al. (2009) proposed that in aerobic oxidation of elemental sulfur, disulfide intermediates are oxidized in *At. ferrooxidans* by heterodisulfide reductase (HDR) (Figure 2.4). Tetrathionate is decomposed to thiosulfate by tetrathionate hydrolase (TetH), thiosulfate is oxidized to tetrathionate by thiosulfate quinone reductase (TQR) and sulfide is oxidized by sulfide quinone reductase (SQR). Electrons released in the oxidation reactions are donated to the quinone pool, from which they are transferred to NADH complex I for energy production or to terminal oxidases (Quatrini et al. 2009). In acidophilic archaea *Acidianus ambivalens*, the oxidation of sulfur compounds is proposed to proceed via sulfur oxygenase reductase (SOR), which catalyzes the disproportionation of sulfur to sulfite, thiosulfate and hydrogen sulfide (Figure 2.5). The formed sulfur compounds are further transformed to sulfate and elemental sulfur by TQR, TetH and SQR. (Kletzin 2008)

Anaerobic degradation of reduced inorganic sulfur compounds

Some microorganisms degrade RISCs also in anaerobic conditions. Acidic mining waters often contain ferric iron, which is an efficient electron acceptor due to its high reduction potential (0.77 V vs. normal hydrogen electrode (NHE) in standard conditions). The metabolic routes for the anaerobic oxidation of RISCs are complex, and further research is required to complete the models of metabolic routes (Kucera et al. 2016).

Similarly to aerobic metabolism, disulfide intermediates are proposed to be oxidized in *At. ferrooxidans* in anaerobic conditions by HDR (Figure 2.6). TetH and TQO catalyze the oxidation of tetrathionate and thiosulfate. From quinone pool, most of the electrons are passed to the outer membrane Fe^{3+} reductases by cytochrome complexes PetI and PetII while some of the electrons are utilized for NADH synthesis. The reduction of ferric iron on the outer membrane of the cell is proposed to occur e.g. via c4-type cytochrome Cyc2. (Kucera et al. 2016)

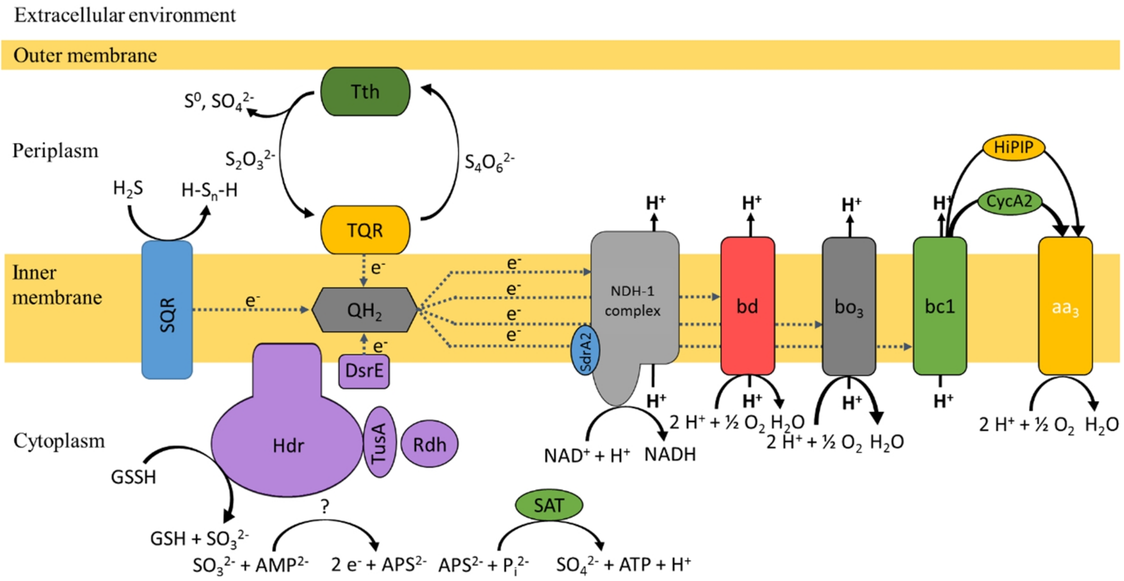

Figure 2.4: Aerobic RISC oxidation in *Acidithiobacillus ferrooxidans*. Enzymes, enzyme complexes and electron carriers predicted to be involved in the oxidation of RISCs include tetrathionate hydrolase (Tth), thiosulfate quinone reductase (TQR), sulfide quinone reductase (SQR), heterodisulfide reductase (Hdr), sulfur transferases (DsrE, TusA and Rhd), ATP sulfurylase (SAT), quinol pool (QH_2), NADH complex 1 (NDH-1), terminal oxidases (bd and bo_3), bc1 complex, high potential iron-sulfur protein (HiPIP), cytochrome c (CycA2) and aa_3 oxidase. GSSH: sulfane sulfate, GSH: glutathione, APS: adenosine 5'-phosphosulfate, ATP: adenosine triphosphate, AMP: adenosine monophosphate, NAD: Nicotinamide adenine dinucleotide. Modified from Quatrini et al. (2009).

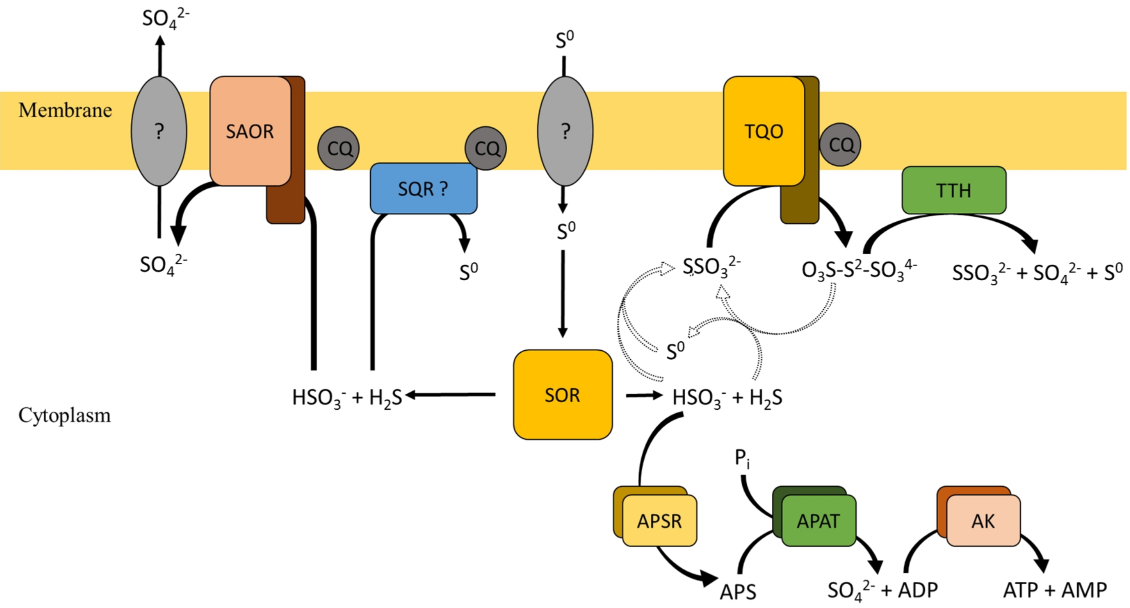

Figure 2.5: Aerobic RISC oxidation in *Acidianus ambivalens*. Enzymes, enzyme complexes and electron carriers predicted to be involved in the oxidation of RISCs include sulfite acceptor oxidoreductase (SAOR), caldariella quinones (CQ), sulfide quinone oxidoreductase (SQR), sulfur oxygenase reductase (SOR), thiosulfate quinone oxidoreductase (TQO), tetrathionate hydrolase (TTH), adenosine 5'-phosphosulfate reductase (APSR), adenosine 5'-phosphosulfate phosphate adenylyltransferase (APAT) and adenylate kinase (AK). APS: adenosine 5'-phosphosulfate, ATP: adenosine triphosphate, ADP: adenosine diphosphate, AMP: adenosine monophosphate. Modified from Kletzin (2008).

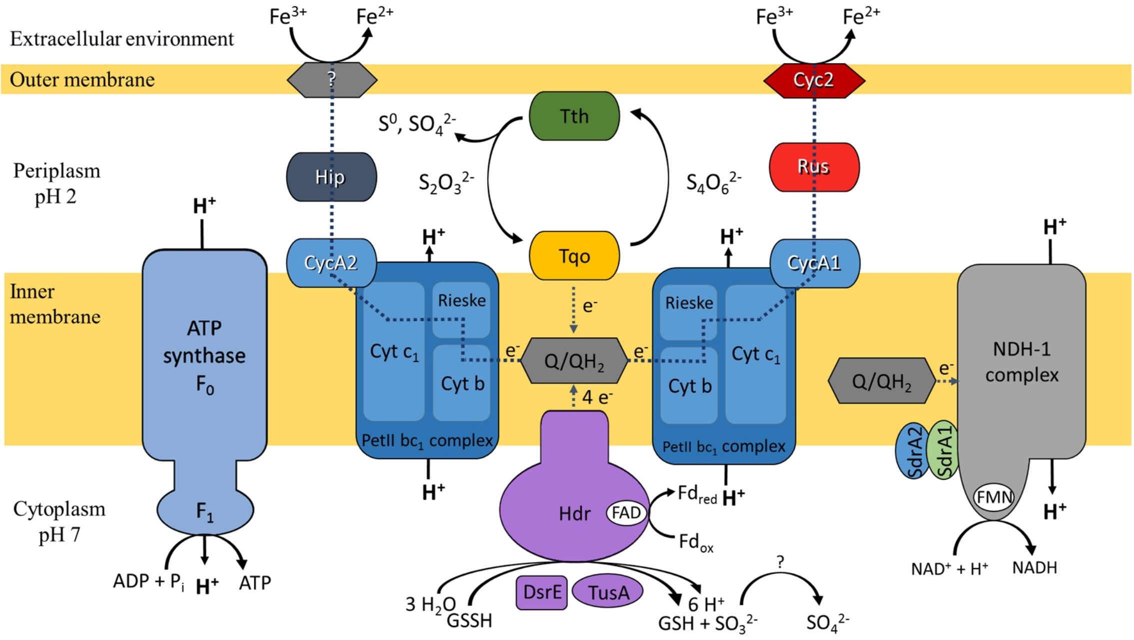

Figure 2.6: Anaerobic oxidation of inorganic sulfur compounds in *Acidithiobacillus ferrooxidans*. Enzymes, enzyme complexes and electron carriers predicted to be involved in the oxidation of RISCs include tetrathionate hydrolase (Tth), thiosulfate quinone oxidoreductase (Tqo), heterodisulfide reductase (Hdr), sulfur transferases (DsrE and TusA), quinone pool (Q/QH_2), cytochrome bc_1 complex I, cytochrome bc_1 complex II, c4-type cytochromes (CycA1 and CycA2), high potential iron-sulfur protein (Hip), rusticyanin (Rus), c-type cytochrome (Cyc2), NADH complex I (NDH-1), SdrA1 and SdrA2 proteins, ATP synthase F_0. GSSH: sulfane sulfate, GSH: glutathione, ATP: adenosine triphosphate, ADP: adenosine diphosphate, NAD: Nicotinamide adenine dinucleotide. Modified from Kucera et al. (2016).

2.2.2 Iron-oxidizing microorganisms

Due to the abundance of iron minerals, mining waters are usually rich of ferrous iron and thus also iron-oxidizing microorganisms grow well in mining waters. Moderately acidic water streams can contain various iron-oxidizing microorganisms, but in extremely acidic iron-containing streams often *Acidithiobacillus ferrooxidans* and/or *Leptospirillum* spp. are dominant (Johnson & Hallberg 2003, Schrenk et al. 1998, Walton & Johnson 1992).

Iron-oxidizing microorganisms oxidize ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). Ferric iron is a strong oxidizer, which can break the sulfur-metal bonds of sulfide minerals and oxidize RISCs thus accelerating the leaching of metals from minerals and the formation of AMD (Dopson & Johnson 2012). Due to the high reduction potential of iron ($E^0 = 0.77$ V vs. NHE), only oxygen ($E^0 = 1.23$ V vs. NHE) can serve as an electron acceptor for iron-oxidizing microorganisms. Another consequence of the high reduction potential is that the energy gains of the iron-oxidizing microorganisms, and thus the growth yields, are low (Neubauer et al. 2002).

Iron-reducing microorganisms can grow in anaerobic conditions by reducing ferric iron back to ferrous iron. Therefore, iron-reducing microorganisms can provide substrate for iron-oxidizing microorganisms while iron-oxidizing microorganisms provide electron acceptors for iron-reducing microorganisms (Weber et al. 2006).

2.3 Environmental impacts

Mining operations have significant effects on the environment; they change the landscape and can also, for example, decrease the biodiversity, cause erosion and contaminate water environments and soil (Park et al. 2015, Sengupta 1993). Due to the low pH and high metal and sulfate concentrations, AMD is a severe threat to the environment and can cause serious damage not only at the mining site but also at distant locations downstream from the point of discharge (Dopson & Johnson 2012). The impacts of AMD flowing to environmental waters are significantly affected by the buffering capacity and volume of the receiving water body (Gray 1997).The toxicity of the AMD can lower the biodiversity due to the elimination of species, which again lowers the ecological stability (Gray 1997). AMD is often a problem at mining sites still after the mining activities stop, as the oxidation reactions continue in the waste rock heaps and tailings. The formation of AMD can continue even for centuries after closing the mine (Young 1997).

2.3.1 Reduced inorganic sulfur compounds

Reduced inorganic sulfur compounds are toxic only in relatively high concentrations and, therefore, their release to the environment is not regulated (Dinardo & Salley 1998). Schwartz et al. (2006) studied the toxicity of inorganic sulfur compounds and reported that thiosulfate inhibited the growth of water flea (*Ceriodaphnia dubia*) in concentrations above 60 mg L^{-1} and tetrathionate in concentrations above 560 mg L^{-1}. For Rainbow trout (*Oncorhynchus mykiss*), Duckweed (*Lemna minor*) and Fathead minnow (*Pimephales promelas*) only concentrations above 500 mg L^{-1} of thiosulfate were observed to be inhibitory, while tetrathionate concentrations of 800–900 mg L^{-1} were not observed to inhibit the growth of these organisms. (Schwartz et al. 2006)

Therefore, RISCs do not usually cause environmental problems due to their toxicity, but due to their acid generation capacity. The biotic or abiotic oxidation of inorganic sulfur compounds to sulfuric acid decreases the pH of the water environments, causing acidification of the environment. The formed acidic water can leach metals from minerals and sediments, leading to increased metal concentrations in the water. The release of RISC-containing waters can also decrease the level of dissolved oxygen and lower the buffering capacity of the receiving water (Dinardo & Salley 1998).

2.3.2 Metals

The solubility of metals often increases with decreasing pH. AMD is thus toxic not only due to the acidity but also due to the high metal concentrations. Many metals are essential trace elements in the metabolism of living organisms, but can be acutely and chronically toxic at high concentrations (Nies 1999). To avoid environmental contamination, strict limits have been set for the discharge of metals. For example, the guideline limits for metal concentrations in effluents from ore mining operations set by the United States Environmental Protection Agency are 0.3 mg L^{-1} for copper, 0.1 mg L^{-1} for cadmium, 0.6 mg L^{-1} for lead and 1 mg L^{-1} for zinc (EPA 2011).

2.4 Treatment

The control and remediation of formed AMD is difficult and expensive. Therefore, the negative environmental impacts of AMD can be best minimized by preventing its formation (Bejan & Bunce 2015). The access of oxygen and/or water — which are both required for the formation of AMD — to the sulfidic minerals can be prevented, for example, with covers containing either organic compounds (Peppas et al. 2000), tailings (Bussière et al. 2004, Jia et al. 2013), water (Vigneault et al. 2001) or biological organisms (Kamorina et al. 2015). Moreover, by eliminating sulfide or by limiting the growth of the sulfur-oxidizing bacteria, the formation rate of AMD can be decreased (Bejan & Bunce 2015).

After reaching a water stream, contaminants can spread widely in the environment. Therefore, the contaminate-containing mining waters need to be treated before they can be released to the surrounding ground or surface waters. The toxic compounds need to be removed and the water should be neutralized. As the characteristics of AMD, the volume of the water stream, climate conditions, hydrodynamic conditions, legislation and expenses vary at different mining sites, the treatment method should be selected case-specifically for each stream (Akcil & Koldas 2006). The treatment can be done in active systems, where a reagent is constantly added, or in passive systems, in which the treatment is obtained via natural reactions (Johnson & Hallberg 2005).

2.4.1 Neutralization

The rocks of the mining site can contain minerals that have a natural capacity for neutralization (e.g. carbonates, hydroxides and silicates) (Langmuir 1997, Stumm & Morgan 1995). However, often the neutralizing capability of the minerals is not high enough to balance the protons formed in the sulfide oxidation reaction. To avoid the acidification of the environment, the formed acidic mining waters need to be neutralized before release to the natural waters.

In active treatment, the acidic streams are neutralized by continuously adding a source of alkalinity, e.g. lime (Bosman 1983, Khorasanipour et al. 2011), limestone (Maree et al. 2004, Miller et al. 2011), fly ash (Ríos et al. 2008, Xenidis et al. 2002) or caustic soda (Park et al. 2015, Wang et al. 2003). Recently, the use of also other industrial waste products as low-cost alternatives for the conventional neutralizing agents have been studied. For example, marble cutting waste (Tozsin 2016), chicken eggshells (Zhang et al. 2017) and pervious concrete (Shabalala et al. 2017) have been used for the neutralization and the removal of metals from acidic waters. Due to the increasing pH, metals will precipitate as metal hydroxides. Metals can also be selectively precipitated, as different metals precipitate at different pH ranges (Kalin et al. 2006). In addition, the increased pH inhibits the growth of the acidophilic iron- and sulfur-oxidizing microorganisms and thus slows down the rate of further acid formation (Akcil & Koldas 2006).

By adding chemical neutralizing agents, pH can be efficiently increased and the metals can be efficiently removed from the water streams. However, the operation costs of active systems are usually high (Johnson & Hallberg 2005) and the precipitates form unstable sludge that contains a mixture of various metals (Kalin et al. 2006). This metal-rich sludge also possess an environmental risk, if negligently disposed.

In passive systems, the acidic water streams are neutralized with the assist of alkalinity producing biological organisms, such as sulfate reducing microorganisms (Le Pape et al. 2017, Papirio et al. 2013), methanogenic microorganisms (Certucha-Barragán et al. 2009) or denitrifying microorganisms (Koschorreck 2007, Zou et al. 2014). Passive systems usually have low maintenance costs and produce less solid waste requiring disposal, but are expensive to install (Johnson & Hallberg 2002). In addition, such biological systems often require external organic carbon source, as the organic content of mining water is usually low (Johnson & Hallberg 2003). Moreover, precipitated metals can limit the performance of such passive systems in long-term operations due to clogging (Kalin et al. 2006).

2.4.2 Removal of inorganic sulfur compounds

Sulfur compounds can be removed from water streams with chemical or biological methods. In chemical treatment, RISCs are often oxidized to sulfate with oxidizing agents, such as chlorine (Black & Goodson 1952, Kim et al. 2008), hydrogen peroxide (Kuyucak 2014, Lu et al. 2010) or ozone (Nie et al. 2012, Sievering et al. 1992). Chemical oxidation is a fast and efficient but expensive method for RISC removal. The biological oxidation of RISCs is cost-effective, does not require high energy input and does not produce any toxic by-products. However, changes in the effluent feed or low temperatures can limit the efficiency of biological processes (Dinardo & Salley 1998).

Iglesias et al. (2016) oxidized tetrathionate in bioreactor at 31 °C and obtained oxidation rates up to 0.780 g L^{-1} h^{-1}. Liljeqvist et al. (2011) studied the removal of RISCs at low temperature (4–6 °C) in bioreactor and obtained a thiosulfate removal rate of 0.162 g L^{-1} h^{-1}, but observed residues of RISCs after treatment (Liljeqvist et al. 2011). In mesophilic and thermophilic conditions, efficient removal (~90%) has been reported (Sääf et al. 2009).

Sulfate removal

As the product of RISCs oxidation is sulfate, the sulfate concentrations in the mining waters from sulfide mineral processing are often naturally high. In environment, sulfate can enhance the biodegradation of organic soils, induce eutrophication and promote the formation of toxic and bioaccumulative methylmercury (Jacobs et al. 2014, Lamers et al. 1998). In addition, sulfate reducing bacteria (SRB) can reduce sulfate to toxic sulfide (Kaksonen & Puhakka 2007). The recommended maximum value for sulfate concentration in drinking water is 250 mg L^{-1} (WHO 2011). Efficient methods for sulfate removal are thus needed.

Methods for the removal of sulfate from solution include chemical precipitation with, for example, polyaluminum chloride (Amaral Filho et al. 2016), sodium aluminate (Tolonen et al. 2016) or barium (Kefeni et al. 2015), adsorption (Iakovleva et al. 2015, Shams et al. 2016), membrane filtration (Košutić et al. 2004, Krieg et al. 2005), electrodialysis (Sakar et al. 2015, Zhang & Angelidaki 2015) and ion exchange (Călinescu et al. 2016, Haghsheno et al. 2009). Besides chemical removal, sulfate reducing microorganisms can be used to reduce sulfate to hydrogen sulfide in active and passive systems (reviewed by Gopi Kiran et al. (2017) and Kaksonen & Puhakka (2007)). Sulfide reacts readily with metal ions, leading to the precipitation of insoluble metal sulfides (Nevatalo et al. 2010). Therefore, both metals and sulfate can be simultaneously removed from the water streams. In addition, the sulfate reducing microorganisms produce bicarbonate, which helps to neutralize the acidic waters (Kaksonen & Puhakka 2007). Most sulfate reducing microorganisms grow only in neutral conditions (Barton & Fauque 2009). However, sulfate reducing prokaryotes have been isolated also from extremely acidic mine waters (Rowe et al. 2007) and have been identified in low pH (Ňancucheo & Johnson 2012). Recently, also BESs have been used for sulfate removal (Teng et al. 2016, Zhao et al. 2008).

2.4.3 Removal of metals

Metals can be toxic to organisms even at relatively low concentrations. Therefore, the metal concentrations in the discharged waters are strictly controlled in several countries (EPA 2011, Ontario 2007). By adding neutralizing chemicals (e.g. CaO or $CaCO_3$), metals can be precipitated as metal hydroxides and metal carbonates (Chen et al. 2009, Ghosh et al. 2011). Different metal compounds precipitate at different pH values and, therefore, metals can be selectively recovered by controlling the pH (Sánchez-Andrea et al. 2016). Metals can also be precipitated as metal sulfides (Huisman et al. 2006). Sulfide can be generated from sulfate by sulfate reducing microorganisms (Machemer & Wildeman 1992, Nevatalo et al. 2010). Some microorganisms can reduce also metal ions (e.g. iron, uranium and chromium) directly to insoluble or less toxic forms (Barton et al. 2015, Lovley et al. 1993b, Wang & Shen 1995). Other metal removal methods are reviewed, for example, in Fu & Wang (2011) and include ion exchange (Abo-Farha et al. 2009, Doula 2009), adsorption (Aman et al. 2008, Jain et al. 2015, Li et al. 2010, Reyes et al. 2009), membrane filtration (Barakat & Schmidt 2010, Dialynas & Diamadopoulos 2009, Landaburu-Aguirre et al. 2010), coagulation (Chang & Wang 2007, El Samrani et al. 2008), flotation (Polat & Erdogan 2007, Yuan et al. 2008) and electrochemical treatment (Bennion & Newman 1972, ter Heijne et al. 2010, Ölmez 2009).

3 Electrochemical systems

Even though the estimations on the availability of fossil fuels vary, cleaner energy production methods are essential for reducing the greenhouse gas emissions and for responding to the increasing energy demand (Höök & Tang 2013, Shafiee & Topal 2009). Electrochemical systems enable the production of electrical energy with high efficiency from chemical compounds, such as hydrogen or methane, while the emissions of pollutants remain low. Therefore, electrochemical systems are often seen as feasible and efficient systems especially for portable energy production and transport applications (Newman & Thomas-Alyea 2004).

With electrochemical systems, chemical energy can be converted directly to electrical energy — or vice versa — via oxidation and reduction reactions. Unlike in chemical reaction, in electrochemical reactions the oxidation and reduction reactions are separate and occur at different electrodes, enabling the generation of electrical current. This chapter focuses on fuel cells, which are electricity producing electrochemical systems that are continuously fed. Electrochemical systems that require external energy to run the oxidation and reduction reactions are called electrolytic cells.

3.1 Operational principle

Fuel cells consist of two electrodes, which are separated with an electrolyte and connected via an external circuit (Newman & Thomas-Alyea 2004). A substrate is electrochemically oxidized on the surface of an anode electrode (Figure 3.1). The electrons released in the oxidation reaction are transferred to the anode electrode, from which they flow through an electric circuit to the cathode electrode. On the surface of the cathode electrode, an electron acceptor receives the electrons from the electrode and becomes reduced. To maintain the charge balance, ions (e.g. H^+, OH^-) flow from one electrode to the other through the electrolyte. The continuous feeding of the substrate and electron acceptors leads to constant flow of electrons from anode to cathode.

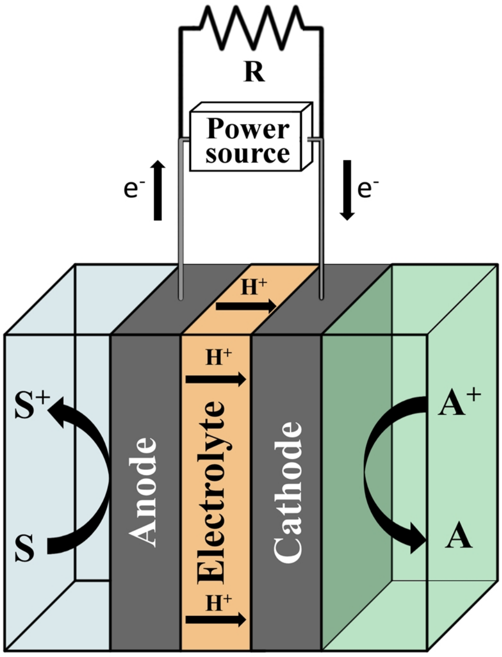

Figure 3.1: Schematic illustration of an electrochemical system. Substrate (S) is oxidized on the anode and electrons released in the oxidation reaction transfer through an electric circuit to the cathode electrode. At the cathode, an electron acceptor (A) accepts the electrons and reduces. Ions transfer through the electrolyte to maintain the charge balance. The electricity generation may be spontaneous when operated with an external load (R) or an external energy source may be required to realize the oxidation and reduction reactions.

The most commonly used anodic electron donor is hydrogen while oxygen from air is used as the cathodic electron acceptor. Alternatively, methane, methanol or hydrocarbons can be oxidized on the anode, but the oxidation reactions require high temperature (Park et al. 2000). The difference in the potentials of the anode and cathode electrodes, determined by the reduction potentials of the anodic electron donor and cathodic electron acceptor (see Chapter 3.2), define whether the system is spontaneously producing electrical energy (fuel cell) or whether external energy is required to run the oxidation and reduction reactions (electrolytic cell). In electrolytic cells, the rate of the electrochemical reactions can be controlled with an external power source, for example by applying external current or voltage. (Newman & Thomas-Alyea 2004)

Electrochemical systems enable efficient conversion between chemical and electrical energy. The systems do not produce environmentally harmful emissions and are silent and safe to use. In addition, high energy densities can be obtained. However, electrochemical systems can be expensive to manufacture and complex to operate. The system components are also vulnerable to impurities. Therefore, their durability has remained relatively low. (Winter & Brodd 2004)

The materials for the system components should be selected based on the application. The materials should not only enable the electrochemical reactions but also have high stability in the used operation conditions. Electrochemical systems are often classified based on the used electrolyte. To ensure efficient performance, the electrolyte should have high conductivity for ions and be electrically resistive, durable and chemically stable (Haile 2003). For example, polymers (Borup et al. 2007, Mehta & Cooper 2003), alkalis (McLean et al. 2002, Yu et al. 2012), acids (Stonehart 1992, Yu & Pickup 2008), molten carbonate (Antolini 2011, Kulkarni & Giddey 2012) and solid oxides (Minh 2004, Ormerod 2003) have been used as the electrolyte materials. Electrodes should be electrically and ionically conductive, electrochemically active and catalyze efficiently the electrochemical reactions (Haile 2003). Due to the high requirements, usually composite electrodes with precious metal (e.g. platinum) as a catalyst are used (Haile 2003).

3.2 Thermodynamics and kinetics

The quantity of electrical energy that can be obtained from or is required to operate an electrochemical system is defined by the anodic and cathodic reactants and the operation conditions (for review, see Zawodzinski et al. (2006) and Garrido (2004)). The maximum theoretical value for the cell voltage is determined by the thermodynamical properties, but the measured voltage remains lower than the theoretical voltage due to losses in the system.

3.2.1 Thermodynamics

The amount of energy available (Gibbs free energy, ΔG) for an electrochemical transformation can be calculated from the number of electrons (*n*) transferred (per mole of reactants) and the voltage of the cell (E) as

$$\Delta G = -nFE \tag{4}$$

where F is the Faraday constant (96 485 sA mol^{-1}). The cell voltage in standard conditions (ΔE°) can be calculated from the Gibbs free energy in standard conditions (ΔG°) (Equation 5).

$$\Delta E^\circ = -\frac{\Delta G^\circ}{nF} \tag{5}$$

The free energy for chemical reactions identified by the van’t Hoff isotherm is

$$\Delta G = \Delta G^\circ + RT \ln \left(\frac{A_P}{A_R}\right) \tag{6}$$

where R is the universal gas constant (8.31447 J mol^{-1} K^{-1}), T is temperature (K), A_P is the activity product of the products and A_R the activity product of the reactants. Combination of Equations 5 and 6 gives the cell voltage in specific conditions:

$$E = E° - \frac{RT}{nF} \ln \left(\frac{A_P}{A_R}\right) \tag{7}$$

This equation, which is known as the Nernst equation, can be used to calculate also the reduction potential of the half-cell reactions separately. The theoretical voltage of the cell can then be calculated as the difference between the potentials of the cathodic and anodic reactions.

$$E_{Cell} = E_{Cat} - E_{An} \tag{8}$$

3.2.2 Losses

In an ideal system, the measured cell voltage would equal the theoretical cell voltage, but in practice, the voltage never reaches the theoretical value, as energy is lost in the electrochemical reactions and in the electron and ion transfer. The main losses of electrochemical systems can be divided into activation losses, ohmic losses and mass transport losses. Even though the electrochemical reactions would be thermodynamically favorable, the reaction might require additional energy to activate. The activation losses can be decreased, for example, by using an efficient catalyst, by increasing the electrode surface area or by operating the system at high temperature. The resistance of the transfer of electrons in the electric circuit and the ions in the electrolyte contribute to the ohmic losses. Ways to decrease the ohmic losses include the use of highly conductive electrodes, contacts, decreasing the distance between the electrodes and increasing the electrolyte conductivity. The transfer of reactants to the electrode and the reaction products from the electrode cause mass transport losses. The main mechanisms for ion transfer from the bulk solution to the electrode are diffusion, convection and migration. The mass transport losses can be minimized by optimizing the mixing, operating conditions and the geometry of the electrodes. (Newman & Thomas-Alyea 2004)

When plotting the voltage over current, the activation losses are typically seen to decrease the voltage rapidly at low current densities, while the ohmic losses cause the voltage to decrease linearly when the current density increases (Figure 3.2). In high current densities, the mass transport losses are dominant and cause the voltage to drop rapidly. (Newman & Thomas-Alyea 2004) The contribution of different resistances to the total internal resistance of the system can be analyzed by electrochemical impedance spectroscopy (EIS). The measurement of the current while applying a small sinusoidal alternating current (AC) potential provides impedance data, based on which the contribution of different system components can be determined for a known electric circuit (for review, see e.g. Nechache et al. (2014) and Yuan et al. (2007)).

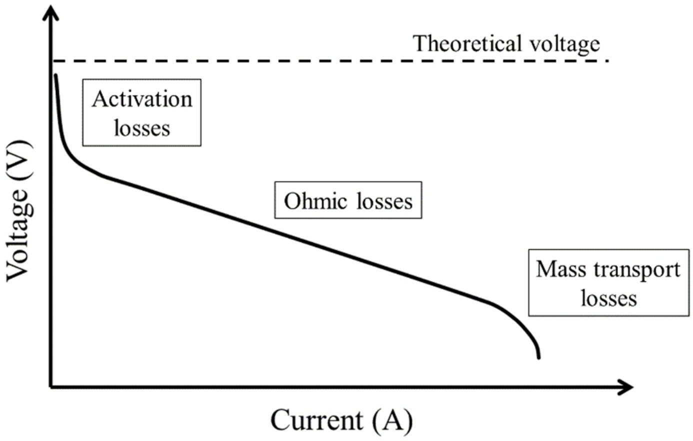

Figure 3.2: Losses in electrochemical systems. Activation losses are dominant at low currents. When the current increases, the voltage decreases linearly due to ohmic losses. With high currents, the mass transport losses start to limit the current generation. Modified from Harnisch & Schröder (2010).

3.3 Selected applications

Electrochemical systems with suitable electron donor and electron acceptor produce electrical energy spontaneously. Conversely, by applying external energy (e.g. voltage or current), thermodynamically unfavorable electrochemical reactions can be realized on the anode and cathode electrodes. Besides electrical energy production (Esquivel et al. 2017, Lim et al. 2017), electrochemical systems can be therefore used, for example, in electrosynthesis (e.g. nanomaterials, catalysts and methanol) (Albo et al. 2017, Chen et al. 2012, Yanilkin et al. 2016, Yue et al. 2017, Zaharieva et al. 2012), as sensors (Bo et al. 2017, Wang & Musameh 2003), in metal recovery (Huyen et al. 2016, Paul Chen & Lim 2005, Sarswat & Free 2016) and in corrosion protection (Fenelon & Breslin 2002, Kraljić et al. 2003). This chapter presents electrochemical applications that are directly related to the topic of this thesis.

3.3.1 Electricity production

Electrochemical systems producing electrical energy are divided into fuel cells and batteries, depending on whether the system is open (fuel cells) or closed (batteries). In fuel cells, the components involved in the redox reactions are fed to the electrochemical system from external sources. In batteries, the reacting components are stored inside the cell. (Winter & Brodd 2004) Therefore, a battery will stop producing electrical energy when the chemical reactants have been consumed, while in fuel cells the energy production will continue as long as the reactants are provided or the system malfunctions due to a mechanical failure.

Fuel cells and batteries are viable energy production systems especially for remote locations without an existing power distribution network. Another promising commercial application is the use of electrochemical systems to power automobiles. Electric vehicles work with higher efficiency and are more environmentally friendly than internal combustion vehicles (Al-Amin et al. 2016, Clark II et al. 2003, Simons & Bauer 2015). Currently, fuel cells are also used, for example, for energy production in spacecrafts (Elitzur et al. 2016, Guo et al. 2016) and as emergency power generators (Devrim et al. 2015, Fosberg 2010).

To increase the reaction rates, fuel cells are usually operated at high temperatures ranging from 100 °C (Alberti et al. 2000, Alberti et al. 2001, Belieres et al. 2006) to 1000 °C (Badwal 2001, Bonanos et al. 1995, Gönen et al. 2016). However, fuel cells with a polymer membrane as the electrolyte work efficiently also at lower temperatures (≤100 °C) (Poynton et al. 2010, Song et al. 2015, Wang et al. 2016). Lately, increasing attention has been paid on further improving the efficiency and commercializing proton exchange membrane fuel cells (PEMFC) — with a solid polymer membrane as the electrolyte — especially to replace combustion engines in transportation (Ji et al. 2017, Peng et al. 2017, Pourmahmoud et al. 2017). PEMFCs are environmentally friendly, have high efficiency and stability, can be started-up fast and operated at low temperatures.

3.3.2 Metal removal

Metal ions can be electrochemically removed from solutions by using them as the electron acceptor on the cathode of an electrochemical system. In suitable conditions, the metal ions in a solution will accept electrons from the cathode electrode and deposit on the surface of the electrode, from which metals can be recovered in pure elemental form. Alternatively, electrochemical metal removal can be obtained by reducing metal ions to an oxidation state that will precipitate in the used conditions.

The electrochemical reduction of metals is spontaneous, if the reduction potential of the cathodic half-cell reaction (metal reduction, Table 3.1) is comparable or higher than the anode potential (Equation 8). Otherwise, an external power supply is required to drive the electron flow from the anode to the cathode. Control of the electrode potentials enables also the selective recovery of metals even from mixed metal streams, as different metals are reduced at different potentials. However, the low cathodic potentials required for most metals enable hydrogen production ($E^0 = 0$ V vs. NHE) as an alternative cathodic reaction.

Table 3.1: The theoretical reduction potentials of selected metals in standard conditions.

Reaction	E^0 (V vs. NHE)	E^0 (V vs. Ag/AgCl)
$Au^{3+} + 3\ e^- \leftrightarrow Au$	+1.5	+1.3
$Ag^+ + e^- \leftrightarrow Ag$	+0.8	+0.6
$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$	+0.77	+0.57
$Cu^{2+} + 2\ e^- \leftrightarrow Cu$	+0.34	+0.14
$Pb^{2+} + 2\ e^- \leftrightarrow Pb$	-0.13	-0.33
$Sn^{2+} + 2\ e^- \leftrightarrow Sn$	-0.14	-0.34
$Ni^{2+} + 2\ e^- \leftrightarrow Ni$	-0.25	-0.45
$Co^{2+} + 2\ e^- \leftrightarrow Co$	-0.28	-0.48
$Cr^{3+} + 3\ e^- \leftrightarrow Cr$	-0.74	-0.94
$Zn^{2+} + 2\ e^- \leftrightarrow Zn$	-0.76	-0.96

Electrochemical metal recovery is a relatively simple and clean method with low operating costs (Paul Chen & Lim 2005). Electrochemical systems have been used to recover, for example, silver (Cruz et al. 2002, Paul Chen & Lim 2005, Pillai et al. 2008), lead (Allen & Chen 1993, Mecucci & Scott 2002), copper (Hunsom et al. 2005, Paul Chen & Lim 2005) and rare earth elements (Sarswat & Free 2016). Besides the type of metals present, the efficiency of electrochemical metal recovery is affected by the concentrations of the metals, pH of the solution, the presence of organic compounds (e.g. humic acid), presence of inhibiting compounds (e.g. EDTA), mixing and the ionic strength of the solution.

3.3.3 Treatment of acid mine drainage

Electrochemical systems have also been studied as a way to neutralize and remove iron from AMD. The anodic oxidation of Fe^{2+} to Fe^{3+} — which precipitates as iron hydroxides at pH above 3 — can be combined to the cathodic reduction of protons (H^+) to hydrogen gas (H_2) in the tailings pile (Bejan & Bunce 2015, Chartrand & Bunce 2003). Electrochemical treatment is often cost-efficient, as electricity is a relatively cheap energy source. However, successful treatment requires high current densities and the reactor system materials should endure the acidity of the solutions. In addition, the reactors should be designed in a way that the formed precipitates do not limit the performance even in long-term operations. (Bejan & Bunce 2015) Obtained power densities have increased up to 290 mW m^{-2} and coulombic efficiency above 97% (Cheng et al. 2007). Besides ferrous iron, also water can be used as the anodic electron donor for the hydrogen producing cathode. The oxygen producing reaction (water splitting) increases the concentration of dissolved oxygen in the water and thus increases the rate of ferrous iron oxidation to ferric iron. (Park et al. 2015)

3.3.4 Sulfur compound removal

Electrochemical systems enable the removal of some sulfur compounds (e.g. S^{2-} and $S_2O_3^{2-}$) that possess a risk to the environment (Pikaar et al. 2015, Ragauskas et al. 2003). However, electrochemical treatment is often feasible only for low-volume streams with high sulfur-compound concentrations. The anodic reactions have been reported to be complex and involve the formation of many polythionates. The electrochemical oxidation of thiosalts can be easily controlled and does not require the addition of chemical oxidants, but may require high power and the precipitates need to be regularly removed from the electrodes. (Dinardo & Salley 1998)

Dutta et al. (2008) studied the electrochemical removal of sulfide in a fuel cell with controlled anode potential of 0.2 V vs. NHE. They obtained a sulfide removal rate of 0.28 ± 0.05 kg S m^{-3} d^{-1} while generating 5 W m^{-3} power with potassium ferricyanide as the cathodic electron acceptor. The reaction product of sulfide oxidation was elemental sulfur, which was observed to deposit on the graphite anode electrode (Dutta et al. 2008). The conductivity of elemental sulfur is very low (~5 × 10^{-30} S cm^{-1} in standard conditions), and thus the deposited sulfur restricts the electron transfer on the surface of electrodes (Ateya et al. 2003, Dutta et al. 2008, Reimers et al. 2006). However, elemental sulfur can be electrochemically reduced to sulfide and polysulfides, enabling the regeneration of the electrodes (Dutta et al. 2009). In addition, sulfur can be microbiologically converted to sulfide (Dutta et al. 2009).

Besides direct oxidation, sulfide can be removed from solutions with electrochemical systems indirectly via electrochemically produced oxygen. Pikaar et al. (2011) obtained sulfide removal rate of 11.8 ± 1.7 g S m^{-2} h^{-1} with mixed metal oxide electrodes. Sulfide was mainly removed by the in-situ produced oxygen, and sulfate, thiosulfate and elemental sulfur were obtained as the final reaction products (Pikaar et al. 2011).

Thiosulfate can be electrochemically oxidized (Du et al. 2006, Feng et al. 1995, Ragauskas et al. 2003), but it also reacts spontaneously in the presence of oxygen (Glasstone & Hickling 1932). At high potentials (>1.6 V vs. NHE), the electrochemical oxidation of thiosulfate produces sulfate and elemental sulfur as the reaction products. To avoid the formation of sulfur, Feng et al. (1995) proposed that thiosulfate can be electrochemically oxidized in a two-step process. First, thiosulfate was oxidized to tetrathionate at 1.2 V vs. NHE and then tetrathionate was electrochemically oxidized to sulfate at potential above 1.6 V vs. NHE at pH 5 in acetate buffer with rotated disk electrode (Feng et al. 1995). While applying current ranging from 10 mA to 200 mA to an electrolysis cell with a carbon flow-through electrode, Ragauskas et al. (2003) reported thiosulfate and tetrathionate oxidation to occur simultaneously.

4 Bioelectrochemical systems

Bioelectrochemical systems utilize a biological catalyst, usually microorganisms, in the electrochemical oxidation and/or reduction reactions. The use of microorganisms enables the oxidation of compounds that could not be degraded in abiotic electrochemical systems or would require high potential for oxidation. In addition, in BESs electricity can be produced at ambient temperature and pressure. The bioelectrochemically degradable substrates vary from simple organic compounds, such as sugars and volatile fatty acids, to complex wastewater streams and inorganic compounds. Similarly as with abiotic electrochemical systems, electricity producing BESs are generally known as microbial fuel cells, while BESs running on external power are called microbial electrolysis cells.

4.1 Operational principle

BESs utilize the ability of microorganisms to biologically degrade organic and inorganic compounds and transfer electrons to a solid electron acceptor (Logan et al. 2006). On the anode, microorganisms, which form biofilm on the surface of the anode electrode, break down the substrate and transfer electrons from the substrate to the anode electrode to gain energy for growth (Figure 4.1). As in conventional electrochemical systems, the electrons are then transferred through the electric circuit to the cathode electrode, where an electron acceptor accepts the electrons. To maintain a charge balance, positive ions transfer from anode to cathode or negative ions from cathode to anode through the electrolyte.

Microorganisms require water for growth and thus the anolyte (anodic electrolyte) has to be a solution in BESs with bioanode. The anolyte characteristics (e.g. pH, salinity, nutrients) need to support the microbial growth. To prevent the transfer of alternative electron acceptors or growth limiting compounds from catholyte (cathodic electrolyte) to anolyte, the electrolytes can be separated with an ion exchange membrane. The reactors with only the anolyte chamber are called air-cathode BESs while reactors with separate anolyte and catholyte chambers are called two-chamber BESs (Figure 4.1).

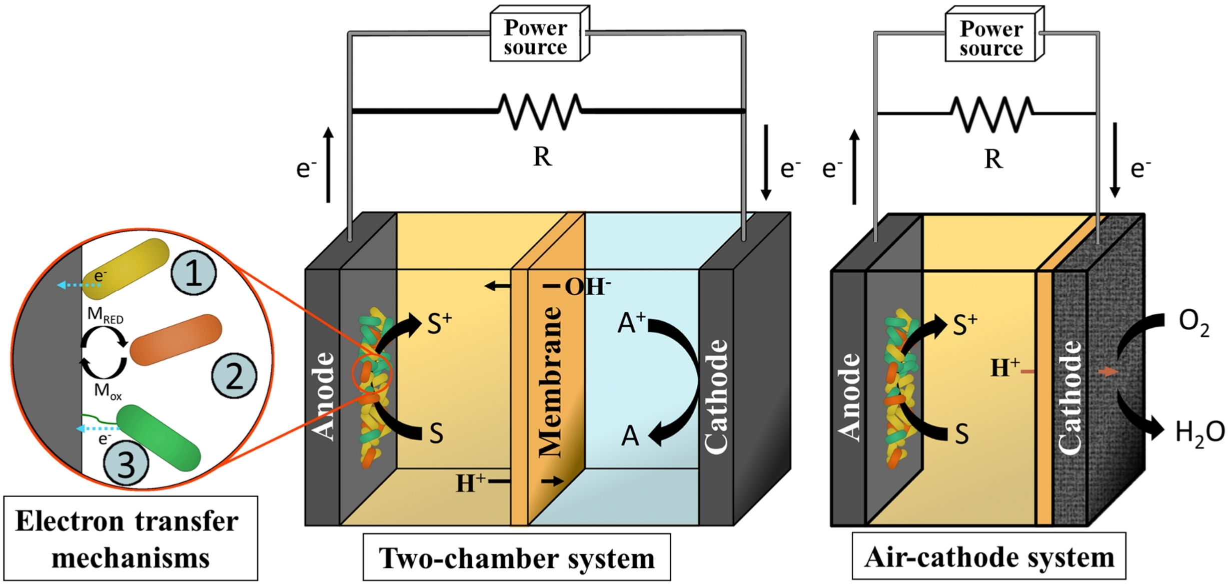

Figure 4.1: Schematic illustration of two-chamber and air-cathode bioelectrochemical systems with bioanode. Substrate (S) is oxidized on the anode with the assist of microbial catalysts. The electrons can be transferred to the electrode via three mechanisms: direct electron transfer via membrane bound cytochromes (1), via mediating electron shuttles (2) or directly via conductive nanowires (3). The electrons released in the oxidation reaction transfer through an electric circuit to the cathode electrode. At the cathode, an electron acceptor (A) accepts the electrons and reduces. Ions (e.g. H^+ or OH^-) transfer through the membrane to maintain the charge balance. In air-cathode systems, the cathodic electron acceptor is oxygen from air. The current generation may be spontaneous when operated with an external load (R) or an external energy source may be required to realize the oxidation and reduction reactions.

In addition to what is required from the electrodes of abiotic electrochemical systems (e.g. high conductivity, mechanical strength and electrochemical passivity), the electrodes used in BESs need to also be biocompatible. Carbon electrodes are popular due to their biocompatibility, low cost, stability and dimensional versatility (e.g. plate, cloth, brush) (Butti et al. 2016). Metals are often not seen as good anode materials for BESs, because trace amounts of metals may detach from the electrode during the oxidation reaction and limit the growth of the microorganisms. However, Baudler et al. (2015) obtained similar current densities with silver and copper electrodes than with graphite and concluded that the oxidative dissolution rate of metals was low at the measured potential range.

In MFCs, the cathode is often abiotic and chemical compounds, such as ferricyanide (Lay et al. 2015, Wang et al. 2013), permanganate (Kumar et al. 2017a, You et al. 2006), persulfate (Li et al. 2009, Pandit et al. 2011) and dichromate (Li et al. 2008, Pandit et al. 2011) have been used as the cathodic electron acceptors. However, the regular replacement of such solutions increases the operation cost of the system. More easily available electron acceptor is oxygen from air, but in the operational conditions that support microbial growth (e.g. temperature, electrolyte solutions) the rate of oxygen reduction remains low. Therefore, efficient, cheap and durable catalysts need to be developed to improve the energy production efficiency of air-cathode reactors. Recently, platinum alloy PtNi (Cetinkaya et al. 2015) and iron based catalysts (Santoro et al. 2016), for example, have been studied as more cost-effective alternatives to pure platinum. The cathodic reduction reaction can be utilized also in resource recovery. For example, metals (for review, see Nancharaiah et al. (2015)) and nutrients (for review, see Nancharaiah et al. (2016)) have been recovered on the cathodes of BESs.

Another important performance limiting factor in BESs is the transfer of ions in the electrolyte. The flow of negative electrons from anode to cathode needs to be counterbalanced with the flow of positive ions from anode to cathode or negative ions from cathode to anode. If the transfer of ions in the electrolyte is significantly slower than the transfer of electrons, the electrolytic ion transfer can limit the efficiency of the system. In BESs, the anode and cathode need to be often kept in different solutions to avoid the inhibition of the anodic and/or cathodic electron transfer. For example, the cathodic electron acceptors (e.g. O_2, Fe^{3+}) can work directly as the electron acceptors for the microorganisms, directing electrons away from the anode electrode. Thus, the compartments need to be separated with a membrane, which allows the transfer of the desired ions, but prevents the transfer of performance limiting ions or compounds. For example, proton exchange membranes (Oh & Logan 2006, Xu et al. 2012), cation exchange membranes (Choi et al. 2011, Liu et al. 2008), anion exchange membranes (Mo et al. 2009, Pandit et al. 2011) and bipolar membranes (ter Heijne et al. 2006, Xiang et al. 2017) have been used for this purpose. However, the membrane materials are often expensive and thus the use of membraneless systems has been proposed as an alternative design. In such systems, for example, glass-wool or glass beads can be used to separate the anode and cathode to prevent short-circuiting (Jang et al. 2004).

Besides the membrane material, the transfer of ions through the electrolyte is affected by the solution conductivity. Conductivity depends on the ion concentration (e.g. H^+, Na^+, Cl^-) in the solution. Some electroactive microorganisms tolerate high salinity (Kim & Logan 2013), but the conductivities of the treated wastewaters are often low (<2 S m^{-1}) (Huang et al. 2009, Luo et al. 2012). In addition, the chemical composition of the anolyte is often complex containing nutrients required for microbial growth and the pH is maintained near neutral (Oliot et al. 2016). At neutral pH, the transfer of protons and hydroxide ions remains negligible and the charge is balanced mainly by the other ions (e.g. Na^+, PO_4^{3-}, Cl^-) in the solution (Oliot et al. 2016).

Microorganisms can also be used to catalyze the reduction reaction at the cathode. Some microorganisms can accept electrons from the cathode electrode and donate them to an electron acceptor, such as sulfate or iron (Carbajosa et al. 2010, Luo et al. 2017, ter Heijne et al. 2007). Microorganisms can regenerate the catholyte (e.g. Fe^{2+} to Fe^{3+}), removing the need for the continuous addition of the electron acceptor (ter Heijne et al. 2007). With external energy, biocathodes can also be used for the synthetization of multicarbon compounds, such as acetic acid (Jourdin et al. 2015) and methane (Zhen et al. 2015), from CO_2.

4.2 Substrates

Even though the MFC design and operation conditions affect the efficiency of the system, the maximum power output is defined by the substrate. Both simple organic and inorganic substrates (e.g. acetate, glucose, sulfide) and complex streams with multiple substrate compounds (e.g. domestic and industrial wastewaters) have been used (for review, see Gude (2016), Pandey et al. (2016) and Pant et al. 2010)). Simple substrates are readily utilized by the microorganisms and high electricity production efficiencies have been obtained. However, waste streams are more profitable substrate sources than pure compounds based on costs, availability and possibility for simultaneous waste treatment.

4.2.1 Organic substrates

The organic compounds used as the substrates in MFCs vary from carbohydrates and alcohols to organic acids and amino acids (reviewed by Pandey et al. (2016)). However, the organic compounds are rarely available as pure compounds in nature and their use is thus relatively expensive. A more economical and environmentally friendly approach is to use waste streams that contain organic compounds as the substrate (reviewed by Gude (2016)). Waste streams rich with organic compounds include, for example, domestic wastewater (Heidrich et al. 2014, Ren et al. 2014), food processing wastewater (Fornero et al. 2010, Li et al. 2013, Oh & Logan 2005) and brewery wastewater (Cetinkaya et al. 2016, Dong et al. 2015) (Table 4.1). More complex wastewater streams used in BESs include wastewaters from dye (Kalathil et al. 2011, Nagendranatha Reddy & Venkata Mohan 2016), cellulosic (Krishna et al. 2014, Lim et al. 2012), petroleum (Mohan & Chandrasekhar 2011, Srikanth et al. 2016) and pharmaceutical (Yeruva et al. 2016) industries.

Table 4.1: Examples of wastewaters used as the substrate sources for bioelectrochemical systems in recent studies and the resulting electricity production. Oxygen was used as the cathodic electron acceptor in all of the studies.

Wastewater	COD[a] g/L	Reactor type	CD_{Max}[b] A/m^2	PD_{Max}[c] mW/m^2	R_{Ext}[d] Ω	Reference
Azo dye	1.021	MFC	0.691	122	100	Nagendranatha Reddy & Venkata Mohan (2016)
Biorefinery	2.6	MFC	5.7	4080	250	Pannell et al. (2016)
Petroleum refinery	1.04	MFC	0.544	225	460	Srikanth et al. (2016)
Pharmaceutical	~10	MFC	0.00954	0.931	100	Yeruva et al. (2016)
Tannery	1.1	MFC	0.120	7	1000	Sawasdee & Pisutpaisal (2016)

[a] chemical oxygen demand, [b] maximum current density, [c] maximum power density, [d] external resistance

The lack of substrate can limit the electricity production and even lead to death of certain important microorganisms, while high substrate concentrations can inhibit their growth. High organic loading rates have been reported to increase the organics removal rate but to lower the coulombic efficiencies (Juang et al. 2011, Rabaey et al. 2003). In such case, the organic matter is consumed for cell growth and alternative pathways producing different by-products instead of electricity production.

4.2.2 Inorganic substrates

The MFC research has mainly focused on organic substrates, but microorganisms can use also inorganic compounds, such as ferrous iron (Emerson et al. 2010), reduced inorganic sulfur compounds (Friedrich 1997), or hydrogen (Bowien & Schlegel 1981), as the energy source. MFC studies conducted with inorganic compounds are listed in Table 4.2. Inorganic compounds are promising substrates for energy production or resource recovery on sites, where no streams with organic compounds are available, e.g. mining environments. However, the electricity production efficiencies have remained lower with inorganic substrates than with organic substrates (Pandey et al. 2016). Therefore, further studies are required to optimize and improve the efficiency of the inorganic MFCs.

Table 4.2: Inorganic substrates used in microbial fuel cells.

Substrate	**Concentration**	**Anode electrode**	**Cathodic electron acceptor**	**CD_{Max}^{a} A/m²**	**PD_{Max}^{b} W/m²**	**R_{Ext}^{c}**	**Reference**
Hydrogen	30 mL/min	Graphite plate	Copper	0.48	0.25	500 Ω	Ntagia et al. (2016)
Sulfite and thiosulfate	1.27 g/L SO_3^{2-} 1.77 g/L $S_2O_3^{2-}$	Activated carbon cloth and carbon fibre veil	Oxygen	1.15	0.37	20 Ω	Zhao et al. (2009)
Sulfide	0.1 g/L S^{2-}	Granular graphite	Ferricyanide	0.042^{d}	23.7^{d}	50 Ω	Rabaey et al. (2006)
Sulfide	0.12 g/L S^{2-}	Carbon felt	Ferricyanide	0.3	0.04	1000 Ω	Lee et al. (2012)
Sulfide mineral flotation process water	0.14 g/L $S_2O_3^{2-}$	Graphite plate covered with carbon paper	Ferric iron	0.013	0	1000 Ω	Ni et al. (2016)
Tetrathionate	1.12 g/L $S_4O_6^{2-}$	Graphite plate covered with carbon paper	Ferric iron	0.034	0	1000 Ω	Ni et al. (2016)
Tetrathionate	2 g/L $S_4O_6^{2-}$	Graphite plate covered with carbon paper	Ferric iron	0.08	0.01	1000 Ω	Paper I
Tetrathionate	2 g/L $S_4O_6^{2-}$	Graphite plate covered with carbon paper	Ferric iron	0.225	0.01	100 Ω	Paper II

[a] maximum current density, [b] maximum power density, [c] external resistance, [d] calculated using an average of the presented estimated projected surface area (1770 m^2 m^{-3})

4.3 Microbial catalysts

Microorganisms gain energy for growth through oxidation and reduction reactions. After the substrate oxidation, the electrons go through several oxidation and reduction reactions within the microbial cell, before reaching the proteins that donate the electrons to a final electron acceptor. The energy gain for the microbial cell is defined by the difference of the reduction potentials of the electron donor and the electron acceptor. Compounds with low reduction potential are efficient electron donors and compounds with high reduction potential are efficient electron acceptors. Oxygen has a high reduction potential (1.23 V vs. NHE), which makes it a very good electron acceptor. Therefore, the majority of life forms on earth live through aerobic respiration. However, not all microorganisms require oxygen for growth (anaerobes) and some species do not grow at all in the presence of oxygen (obligate anaerobes). Besides oxygen, microorganisms can utilize several soluble compounds like ferric iron (Fe^{3+}), nitrate (NO_3^-) or sulfate (SO_4^{2-}) as the electron acceptor (Table 4.3). Moreover, some species utilize the organic substrates as both electron donor and electron acceptor (fermentation).

In MFCs, the anode electrode serves as the terminal electron acceptor for microorganisms. Therefore, the potential of the anode electrode defines the amount of energy that the microorganisms gain for growth: the higher the anode potential, the higher the energy gain (Schröder 2007). However, the amount of energy used for growth decreases the amount of energy available for electricity production. To maximize the electrical output, the anode potential should remain at the minimum value still supporting the growth of the microorganisms.

The anode potential might also affect the enrichment of the electroactive species, as some species can utilize the electrode as the electron acceptor only at certain potentials (Wagner et al. 2010). Microorganisms capable of direct electron transfer have been shown to enrich at low anode potentials, while higher potentials support the growth of several microorganisms (Carmona-Martínez et al. 2013, Nam et al. 2011, Wagner et al. 2010).

Table 4.3: The theoretical reduction potentials in standard conditions for common electron donors and acceptors for microbial growth.

Reaction	**E^0 (V vs. NHE)**	**E^0 (V vs. Ag/AgCl)**
$O_2 + 4\,H^+ + 4e^- \rightarrow 2\,H_2O$	1.23	1.03
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.77	0.57
$NO_3^- + 6\,H^+ + 6\,e^- \rightarrow ½\,N_2 + 3\,H_2O$	0.75	0.55
$NO_3^- + 2\,H^+ + 2\,e^- \rightarrow NO_2^- + H_2O$	0.42	0.22
$2\,H^+ + 2e^- \rightarrow H_2$	0	-0.2
$SO_4^{2-} + 10\,H^+ + 8\,e^- \rightarrow H_2S + 4\,H_2O$	-0.21	-0.41
$S^0 + 2\,H^+ + 2\,e^- \rightarrow H_2S$	-0.24	-0.44
$CO_2 + 8\,H^+ + 8\,e^- \rightarrow CH_4 + 2\,H_2O$	-0.24	-0.44
$CH_3COO^- + 2\,H_2O \rightarrow 2\,CO_2 + 7\,H^+ + 8\,e^-$	-0.29	-0.49
$C_6H_{12}O_6 + 6\,H_2O \rightarrow 6\,CO_2 + 12\,H^+ + 24\,e^-$	-0.43	-0.63

The transfer of electrons from the microorganisms to the anode electrode can occur either directly or via a mediator (Figure 4.1). In direct electron transfer, the electrons are transferred to the electrode directly from the membrane bound cytochromes (Busalmen et al. 2008, Kumar et al. 2016). Some species are able to grow conductive nanowires, which enable the formation of thicker biofilms. Alternatively, the microorganisms can donate electrons to a mediator, which becomes reduced. When the mediator reaches the electrode, it will be electrochemically oxidized. Good mediators are soluble, readily metabolized, regenerative and stable during long-term operation (Butti et al. 2016, Schröder 2007). The mediator can be synthesized by the microorganisms or externally added to the system, but the regular external addition of mediators is expensive and the mediators might be toxic to the microorganisms (Butti et al. 2016). Endogenously generated mediators include, for example, pyocyanin (Hernandez & Newman 2001) and pyoverdine (Lee et al. 2016). Potassium ferricyanide (Penteado et al. 2017), neutral red (Fathey et al. 2016), phenazines (Qiao et al. 2015) and quinones (Wong et al. 2016), for example, have been used as externally added mediators. Similarly as in bioanodes, the electron transfer in biocathodes has been proposed to occur either directly via the membrane bound cytochromes or with the assist of electron shuttles (Rimboud et al. 2016, Rosenbaum et al. 2011).

4.4 Selected applications

Even though the original concept was to utilize BESs for electricity production, several alternative applications have been discovered. Recently, increasing number of studies have been focusing on biohydrogen production (Popov et al. 2016, Rago et al. 2017, Rivera et al. 2017), hybrid processes with anaerobic digestion (Cui et al. 2017, Gajaraj et al. 2017, Liu et al. 2016, Rózsenberszki et al. 2017) and nutrient recovery (Chen et al.

2016, Iskander et al. 2016, Nagendranatha Reddy & Venkata Mohan 2016). BESs can be used also, for example, for wastewater treatment (Cui et al. 2016, Marone et al. 2016, Pannell et al. 2016, Sawasdee & Pisutpaisal 2016), for the electrosynthesis of multicarbon chemicals (Aryal et al. 2016, Bajracharya et al. 2016, Ganigué et al. 2015), for desalination (Borjas et al. 2017, Khazraee Zamanpour et al. 2017, Sophia & Bhalambaal 2015) and as biosensors (Kretzschmar et al. 2017, Ma et al. 2016, Yamashita et al. 2016). Selected applications directly related to the topic of this thesis are presented below in more detail.

4.4.1 Electricity production

If the cathodic reduction reaction occurs at higher potential than the anodic oxidation reaction, the electrochemical system will produce electricity spontaneously (Table 4.3). Even though the current and power densities obtained in BESs have remained significantly lower than in conventional fuel cells (Pandey et al. 2016, Xu & Armstrong 2013), BESs enable the electricity production from substrates that could not be utilized for electricity production in abiotic fuel cells at ambient conditions. Substrates can be found also from natural sources, e.g. soils (Kaku et al. 2008, Strik et al. 2008) and sediments (Fu et al. 2015, Schrader et al. 2016), or waste streams (Pandey et al. 2016). Electrical energy can thus be produced, for example, in remote areas that do not have existing electrical infrastructure.

The maximum power obtainable from a MFC depends on the substrate, cathodic electron acceptor and the cell configuration. In addition, multiple MFCs can be stacked to increase the total amount of the produced power (Wu et al. 2016). The electricity produced with BESs have been used to power, for example, sensors (Khaled et al. 2016, Schrader et al. 2016, Tender et al. 2008), implantable medical devices (Han et al. 2010, Roxby et al. 2016) and even robots (Ieropoulos et al. 2010, Wilkinson 2000), a mobile phone (Ieropoulos et al. 2013) and lightning in a music festival (Ieropoulos et al. 2016).

4.4.2 Wastewater treatment and environmental protection

Municipal and certain industrial wastewaters can be rich in organic compounds, which need to be removed from the wastewater before release to the environment. With BESs, the organic compounds can be removed by simultaneously converting the chemical energy stored in the compounds to electrical energy, thus combing the treatment of the water streams to electricity production.

The organic content of some wastewaters can be complex and not all organic compounds are biodegradable. The conductivity of real wastewater streams can be very low (<1 mS cm^{-1}) (Jannelli et al. 2017, Martinucci et al. 2015), which can lead to high ohmic losses caused by ion transfer in the electrolyte (Rozendal et al. 2008). The limitation of the transfer of protons from anode to cathode will also decrease the pH at the anode of the BES, and if the buffering capacity of wastewater is low, the pH can decrease rapidly to levels inhibiting the growth of the electroactive microorganisms (Rozendal et al. 2008). The microbial activity can also be limited by the presence of toxic compounds in the wastewater (Li et al. 2008, Min et al. 2005).

Treatment of real wastewater streams will require the facilities to treat high volumes of wastewater, but the scaling up of the BESs has been challenging (He et al. 2017). In large reactors, the internal resistance of the systems has been high due to the limited ion transfer in the solution. In addition, efficient low-cost electrode and separator materials should be developed to decrease the capital costs of scaled-up systems (Butti et al. 2016, Escapa et al. 2015, Logan 2010). Further studies are thus required to increase the number of bioelectrochemical technologies reaching full-scale applications.

Besides wastewaters, BESs can be used to remediate water environments by reducing the impact of environmentally harmful compounds, for example, by recovering the compounds from the water streams, by transferring them to a less toxic form or by affecting the conditions in such manner that the harmful compounds will precipitate. BESs have been used, for example, for the removal of organic (Huang et al. 2011, Liu et al. 2004), nitrogen (Virdis et al. 2008, Yu et al. 2011) and sulfur compounds (Rabaey et al. 2006, Zhao et al. 2009).

4.4.3 Bioelectrochemical anodes in acidic conditions

In abiotic electrochemical systems, higher electricity generation efficiencies have been obtained with electrolytes with either high or low pH. The high ion concentrations (H^+ or OH^-) decrease the resistance caused by the ion transfer through the electrolyte. In BESs, however, the operation conditions should be selected to support the growth of the microbial catalyst. Many heterotrophic microorganisms grow most efficiently at neutral conditions and most of the studies with BESs have been conducted at near-to-neutral pH.

The low proton concentration at the cathode can slow down the cathodic reduction reaction, if hydrogen ions are consumed in the reaction (e.g. oxygen reduction) (Erable et al. 2009, Khalfbadam et al. 2016). To increase the efficiency of the cathodic reaction, some BESs have been operated with a neutral anode and an acidic cathode. However, this creates a high pH gradient over the membrane separating the chambers, which leads to high voltage loss across the membrane. To decrease the pH gradient over the membrane, both anolyte and catholyte can be operated in acidic conditions. In this case, the microbial catalyst needs to be acidophilic. The use of acidic anolytes enables the use of acidic wastewaters, for example from mining operations, as the substrate source. Besides organic compounds (e.g. glucose, pyruvate), also inorganic sulfur compounds (e.g. sulfide, tetrathionate) have been used as the substrate for electricity production in acidic conditions. Studies conducted in highly acidic conditions are summarized in Table 4.4.

4.4.4 Metal recovery

Similarly as electrochemical systems, BESs can be used to remove and/or to recover metals from water streams by using the soluble metal ions as the electron acceptors at the cathode. Copper has a high reduction potential (0.34 V vs. NHE) and its recovery can thus be obtained with certain substrates without external energy. Studies reporting recovery of copper with different kind of BESs are compiled to Table 4.5. Besides copper, BESs have been used to remove, for example, silver (Choi & Cui 2012), cadmium (Modin et al. 2012), chromium (Li et al. 2008), lead (Modin et al. 2012), selenium (Catal et al. 2009), vanadium (Zhang et al. 2012), uranium (Gregory & Lovley 2005) and zinc (Modin et al. 2012).

Metals are often present in ionic form only in acidic conditions and precipitate at neutral and basic conditions, for example, as metal hydroxides or metal oxides (Fu & Wang 2011). Therefore, in metal reducing BESs the catholyte solution needs to be kept acidic. Most of the reported exoelectrogenic species grow only in neutral conditions (Caccavo et al. 1994, Lovley et al. 1993a, Venkateswaran et al. 1999). The anode side can be maintained neutral and the cathode side acidic, for example, by using a bipolar membrane to restrict the diffusion of ions that affect the pH from anode chamber to the cathode — and vice versa.

Metals are essential trace elementals for most organisms, but toxic in high concentrations (Nies 1999). Therefore, with metal ions as the cathodic electron acceptor, a risk of metal ions diffusion from the cathode to the anode exists. If diffusing, metals can limit the growth of the electroactive microorganisms. The influence of metal ions on the structure and function of microbial communities and the fate of metal ions in the anode chamber requires further studies.

Table 4.4: Bioelectrochemical systems operated in extremely acidic conditions (pH < 4).

Inoculum	**Substrate**	**pH**	**Reactor design**	**Electrode**	**CD_{Max}[a]** **A/m^2**	**PD_{Max}[b]** **mW/m^2**	**R_{Ext}[c]** **Ω**	**Reference**
Acidiphilium cryptum	Glucose	≤4	Two-chamber bottle MFC	Graphite felt	0.4	12.8	500	Borole et al. (2008)
Acidiphilium sp. strain 3.2 Sup 5	Glucose	2.5	MEC with controlled anode potential of 0.39 V vs. NHE	Graphite felt	3	n.r.	n.a.	Malki et al. (2008)
Sediment samples	Sediment (glucose, glycerol)	3	Sediment MFC	Graphite felt	3.5	300	10	García-Muñoz et al. (2011)
Mining water	Sulfide mineral flotation process water (thiosulfate)	<2.5	Two-chamber flow-through MFC	Graphite plate covered with carbon paper	0.013	0.37	1000	Ni et al. (2016)
Enrichment culture	Pyruvate	3.7	MFC treating AMD water (acid neutralization)	Carbon felt	0.25	8.7	100	Leiva et al. (2016)
Mining water	Tetrathionate	<2.5	Two-chamber flow-through MFC	Graphite plate covered with carbon paper	0.08	13.9	1000	Paper I
Mining water	Tetrathionate	<2.5	Two-chamber flow-through MFC	Graphite plate covered with carbon paper	0.177	26	100	Paper II

[a] maximum current density, [b] maximum power density, [c] external resistance, n.r. not reported, n.a. not applicable

Table 4.5: Selected studies demonstrating copper recovery in bioelectrochemical systems.

Reactor	Anode	Cathode	Electron donor	Metal salt	Cu^{2+} g/L	Cathodic pH	Metal removal efficiency	PD_{Max}[a] W/m^2	Reference
MFC	Graphite plate	Graphite foil on Ti plate	Acetate (20 mM)	$CuCl_2$	1	3	99.88 %	0.43 (anaerobic) 0.8 (aerobic)	ter Heijne et al. (2010)
MFC	Graphite felt	Graphite felt	Acetate (10 mM)	$CuSO_4$	0.01–0.2	2.5	>99%	0.319	Wang et al. (2010a)
MFC	Graphite felt	Graphite felt	Acetate (6.1 or 12.2 mM)	$CuSO_4$	0.6–2	2	92%, 48%	n.r.	Tao et al. (2011a)
MFC	Graphite plate	Graphite plate	Glucose (27.8 mM)	$CuSO_4$	0.05–1	4.7	>96%	0.339 W/m^3	Tao et al. (2011b)
MFC	Graphite felt	Graphite plate	Acetate (20 mM)	$CuSO_4$	2	3	n.r.	5.5	Motos et al. (2015)
MFC	Carbon felt	Titanium wire	Glucose (2.8 mM)	Leachate	2.3	<2	79 %	0.011	Fedje et al. (2015)
MEC	Graphite foil	Graphite plate	Acetate (20 mM), Hydrogen	$CuCl_2$	1	4	n.r.	0.25	Ntagia et al. (2016)
MEC	Carbon felt	Titanium wire	Acetate (20 mM)	$CuCl_2$	0.8	~0	84.30 %	n.r.	Modin et al. (2012)

[a] maximum power density, n.r. not reported

4.5 Advantages and challenges of bioelectrochemical systems

The efficiency of BESs depends on several factors, including the fuel cell configuration and design, the electrode and separator materials, pH, substrate concentration, the anolyte and catholyte solutions, mediators and the microbial culture. In addition to high fabrication and operation costs, the cost-efficiency of up-scaled applications has been limited by high internal resistance, required pH buffering, and electrode and membrane fouling (Escapa et al. 2015, Gil-Carrera et al. 2013, Gude 2016). In addition, long-term stability is required for the process to be economically feasible. Effective and low-cost materials should be developed, effective system should be designed and power densities should be improved to manufacture scalable BESs for commercial applications (Rosenbaum & Franks 2014).

Compared to electrochemical systems, the start-up time of BESs is usually longer, as in BESs biofilm formation time can vary from a few days (Wang et al. 2010b) to several weeks (Wang et al. 2009). Microorganisms also require specific operational conditions (e.g. pH, temperature) and nutrients for growth. BESs are also more vulnerable to performance limiting disturbances than abiotic electrochemical systems due to microbial catalysts (Jadhav & Ghangrekar 2009). Abiotic electrochemical systems can be operated e.g. at extreme temperatures (>900 °C) and thus higher power production and efficiencies have been obtained in electrochemical systems than in bioelectrochemical systems.

Despite the limitations, BESs provide a promising platform for the production of electrical energy from waste streams. In addition, BESs provide novel methods for the wastewater treatment, bioremediation, desalination, nutrient recovery and metal recovery, for example.

5 Hypotheses and objectives of the present work

Microorganisms, which are able to oxidize reduced inorganic sulfur compounds while simultaneously donating electrons to the solid anode electrode, were hypothised to be present in mining environments. Increasing number of studies have addressed the bioelectrochemical electricity production from different organic substrates and wastewater streams, but only few studies have been conducted with inorganic substrates. RISCs are promising substrates especially for the bioelectrochemical recovery of metals, as both RISCs and metals are often present in mining waters. Mining environments contain also solid minerals (e.g. Fe_2O_3, FeO(OH)) that microorganisms can use as terminal electron acceptors. Therefore, mining process water was used as the inoculum for RISC-fed bioelectrochemical systems in order to enrich for RISCs-oxidizing electroactive microorganisms (Paper I).

The electricity production in the BESs fed with inorganic sulfur compounds was hypothised to improve after optimization of the external resistance (Paper II). Several studies report increasing current and power densities after the external resistance has been gradually decreased (Aelterman et al. 2008, Jadhav & Ghangrekar 2009, Jang et al. 2004). Thermodynamically, the cell will produce maximum power when the external resistance equals the internal resistance. The internal resistance of the bioelectrochemical cell will decrease over time as the biofilm develops, and thus by decreasing of the external resistance, the energy production from the BES can be accordingly maximized.

The enrichment of the electroactive biofilm and the accumulating reaction products were hypothesized to affect the electricity production efficiency in long-term operation (Paper II). Long-term stability is essential for cost-efficient technical applications, but in long-term operated MFCs, accumulating reaction products may inhibit the microbial growth. In addition, the excess formation of biomass has been reported to limit the ion exchange between the chambers and the reduction of the electron acceptor at the cathode in MFCs fed with organic substrates (Choi et al. 2011, Miskan et al. 2016, Xu et al. 2012). However, in chemolithotrophic growth, biomass yields often remain lower than in heterotrophic growth (McCollom & Amend 2005). To monitor the long-term performance and accumulation reaction products, tetrathionate-fed MFC was run and monitored for 744 days (Paper II).

The anode potential of the tetrathionate-degrading MFCs remained significantly higher than the theoretical reduction potential of tetrathionate (Paper II). The microbial population was assumed to be adapted to the high anode potential and thus the anode potential did not spontaneously reach lower value. The microbial culture was hypothesized to adapt to the lower anode potential, if the anode potential was gradually decreased (Paper III).

The electrochemical degradation of tetrathionate was not spontaneous in abiotic control systems (Paper I), but electrochemical degradation was hypothesized to be efficient, if enough external energy was applied. The anode potential was gradually increased to reach the state, where current generation from the abiotic electrochemical oxidation of tetrathionate was obtained (Paper III).

Tetrathionate was hypothesized to be a suitable substrate for electrochemical recovery of copper. The recovery of copper with bioelectrochemical systems has been previously demonstrated (Modin et al. 2012, Motos et al. 2015), but no studies have addressed the use of inorganic substrates in bioelectrochemical metal recovery. Even though the anodic and cathodic reactions are separate, the formed reaction products may influence the rate and the efficiency of the oxidation and reduction reactions. The current generation was, therefore, studied in bioelectrochemical and electrochemical cells with tetrathionate degrading anode and copper reducing cathode (Paper IV).

The bioelectrochemical tetrathionate degradation occurred at lower anode potential than the abiotic electrochemical tetrathionate degradation, indicating that less external energy is required to combine tetrathionate degradation to cathodic copper reduction in bioelectrochemical system than in electrochemical system (Paper IV). Both tetrathionate-degrading systems were hypothesized to require less external energy than a water-oxidizing copper reducing electrochemical system (Paper IV).

Based on the presented hypotheses, the specific objectives of this thesis were as follows:

- To screen the microbial communities in mining process waters for electroactive chemolithotrophic microorganisms (Paper I)
- To determine whether tetrathionate can be used as the substrate for bioelectrochemical electricity production in two-chamber fuel cell with ferric iron as the cathodic electron acceptor (Paper I)
- To determine the maximum electricity production efficiency of tetrathionate-fed microbial fuel cells by optimizing the external resistance (Paper II)
- To study the long-term stability of tetrathionate-fed microbial fuel cell (Paper II)
- To determine the microbial species involved in the tetrathionate degradation and electricity production (Paper I, Paper II)
- To study the adaptation of the microbial culture to different anode potentials by controlling the anode potential with a potentiostat (Paper III)
- To determine the anode potential required for abiotic electrochemical tetrathionate degradation (Paper III, Paper IV)
- To determine the external voltage required to combine bioelectrochemical and electrochemical tetrathionate degradation to cathodic copper reduction (Paper IV)

6 Materials and methods

This chapter contains a short description of the materials and methods used in the experiments. More detailed information on the operational conditions and analyses performed is presented in the publications.

6.1 Overview of the experiments

Overview of the performed experiments is presented in Figure 6.1. The presence of tetrathionate degrading microorganisms in biohydrometallurgical process waters was first tested by cultivating the microorganisms in aerobic and anaerobic batch bottles containing tetrathionate and then in tetrathionate-fed MFCs with ferric iron as the cathodic electron acceptor (Paper I). After electricity production was successfully demonstrated, the electricity production efficiency was improved by optimizing the external resistance (Paper II). The long-term stability of the electricity production was studied by monitoring a MFC fed with tetrathionate for over two years (Paper II). The capability of the microorganisms to adapt to lower anode potentials than recorded in above-mentioned experiments was tested by gradually decreasing the anode potential with a potentiostat (Paper III). The electricity production was compared to an abiotic electrochemical tetrathionate degrading cell (Paper III). Finally, the external voltage required to combine anodic tetrathionate degradation to cathodic copper reduction was studied in bioelectrochemical and abiotic electrochemical systems (Paper IV).

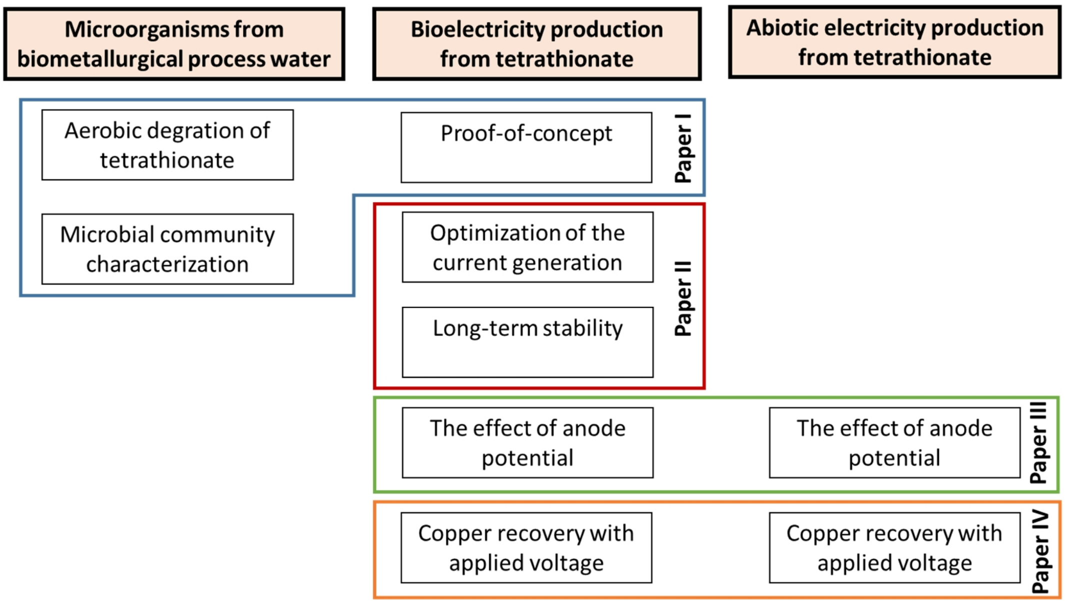

Figure 6.1: Overview of the experiments.

6.2 Reactor configuration

The two-chamber flow-through electrochemical system used has been previously described by ter Heijne et al. (2008) (Figure 6.2). An anion exchange membrane (AMI-7001, Membrane International, USA) or a monovalent cation exchange membrane (CIMS Neosepta, Astom, Japan) was used to separate the anode and cathode chambers to minimize the diffusion of alternative electron acceptors (i.e. Fe^{3+} and Cu^{2+}) from cathode to the anode. Graphite plates (MR Graphite, Germany) covered with carbon paper (Graphite foil, Coidan graphite products, USA) were used as the anode and cathode electrodes in all experiments. The volume of both anode and cathode chamber was 33 cm^3 each and the effective surface area of the electrodes was 22 cm^2. Anode and cathode potentials were measured against Ag/AgCl reference electrodes (Sentek, UK; estimated standard potential 0.205 V vs. Normal Hydrogen Electrode (NHE)) placed in 3 M KCl and connected to the anolyte or catholyte with a glass capillary (QiS, the Netherlands). All the potential values are presented against Ag/AgCl, if not otherwise stated. The reactors were operated at room temperature (22±5 °C) in a fed-batch mode. The anolyte and catholyte solutions were continuously recirculated (166–170 mL min^{-1}) over a recirculation bottle (Figure 6.3). The operational parameters of the bioelectrochemical and electrochemical systems were as summarized in Table 6.1.

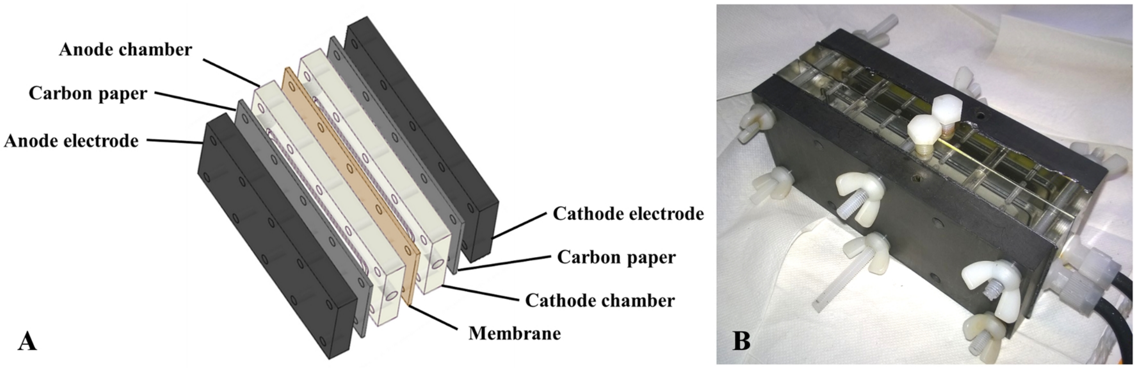

Figure 6.2: A schematic illustration (A) and a photograph (B) of the two-chamber flow-through reactor used in the experiments.

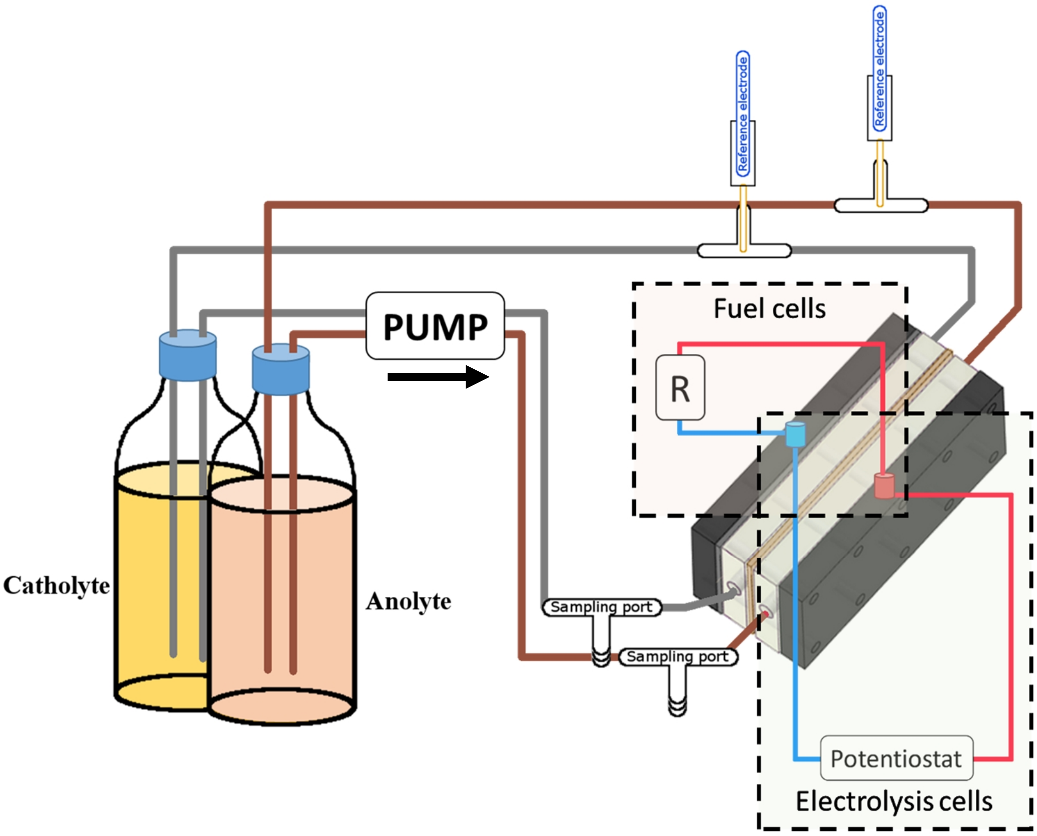

Figure 6.3: Schematic diagram of the electrochemical operation system.

Table 6.1: Operated bioelectrochemical and electrochemical systems.

Cell[a]	Type	Membrane	Catholyte	Electrochemical connection	Volume (L)	Inoculum	Used in
En1 En2 En3 En4	MFC	AEM	2 g/L Fe^{3+}	External resistance 1000 Ω	0.625	Multimetal mining process water	Paper I
MFC A (En3 2A) MFC B (En3 2B)	MFC	AEM	2 g/L Fe^{3+}	External resistance gradually decreased from 1000 Ω to 240 Ω (En3 2A) or to 100 Ω (En3 2B)	0.625	Multimetal mining process water	Paper I, Paper II
MFC LT	MFC	mCEM	2 g/L Fe^{3+}	External resistance gradually decreased from 1000 Ω to 100 Ω	0.625	Anolyte of MFC B	Paper II
BES	MEC	AEM	2 g/L Fe^{3+}	Anode potential controlled with potentiostat	0.625	Anolyte of MFC B	Paper III
ES	EC	AEM	2 g/L Fe^{3+}	Anode potential controlled with potentiostat	0.625		Paper III
MEC	MEC	AEM	1 g/L Cu^{2+} [b]	Cell voltage controlled with potentiostat	0.5	Anolyte of MFC LT	Paper IV
EC	EC	AEM	1 g/L Cu^{2+}	Cell voltage controlled with potentiostat	0.5		Paper IV

[a] cell label in the publications, [b] started up with 2 g L^{-1} Fe^{3+} as the catholyte, MFC: microbial fuel cell, MEC: microbial electrolysis cell, EC: electrochemical cell, AEM: anion exchange membrane, mCEM: monovalent cation exchange membrane.

6.3 Simulated mining water

The bioelectrochemical systems were operated with simulated acidic mining water as the anolyte. The simulated mining water contained 10% (v/v) of Mineral Salts Medium (MSM; 1 L contained 3 g $(NH_4)SO_4$, 0.1 g KCl, 0.5 g K_2HPO_4 , 0.5 g $MgSO_4 \cdot 7\ H_2O$, and 0.013 g $Ca(NO_3)_2 \cdot 4\ H_2O$) and 1% (v/v) of trace element solution (TES; 1 L contained 11 mg $FeCl_3 \cdot 6\ H_2O$, 0.5 mg $CuSO_4 \cdot 5\ H_2O$, 2 mg H_3BO_3, 2 mg $MnSO_4 \cdot H_2O$, 0.8 mg $Na_2MoO_4 \cdot 2\ H_2O$, 0.6 mg $CoCl \cdot 6\ H_2O$, 0.9 mg $ZnSO_4 \cdot 7\ H_2O$, and 0.1 mg Na_2SeO_4) in phosphate buffered (20 mM K_2HPO_4) MQ-water. The initial concentration of tetrathionate (added as $K_2S_4O_6$) was 2.5 g $S_4O_6^{2-}$ L^{-1}. Concentrated HCl (37%) was used to adjust the anolyte pH to 2.5. More tetrathionate was added to the fed-batch reactors after the tetrathionate concentration decreased below 0.5 g L^{-1}.

6.4 Inoculation and analysis of microbial communities

The bioelectrochemical reactors were inoculated with either original mining process water originating from multimetal ore heap bioleaching (Paper I) or with a culture that had been enriched in a tetrathionate-fed MFC for 8 to 18 months (Papers II-IV). To remove oxygen, anolyte and catholyte solutions were purged with nitrogen (15 minutes) prior inoculation with 10% (v/v) of inoculum.

The microbial communities were analyzed from the original multimetal mining process waters (Paper I), from the anode electrode surface (Paper I) and anolytes (Paper I, Paper II) of the tetrathionate-fed MFCs using polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) as described earlier by Mäkinen et al. (2012). Primers used in the PCR to amplify the bacterial 16S rRNA sequences were BacV3f (5’-CCT ACG GGA GGC AGC AG-3’) and 907r (5’-CCG TCA ATT CCT TTG AGT TT-3’) (Muyzer et al. 1996, Muyzer et al. 1993). The amplified samples were sequenced at Macrogen (Korea), edited with BioEdit (Version 7.1.3.0, Ibis Biosciencies, USA) and compared to GenBank database sequences using BLAST (http://blast.ncbi.nlm.nih.gov).

6.5 Analyses

The changes in the chemical composition of the anolyte and catholyte solutions and the electrochemical performance of the cells were followed periodically. Physicochemical and electrochemical analyses performed and the used analysis methods and instruments were as presented in Table 6.2.

Table 6.2: Used analysis methods and equipment.

Parameter	Method/Instrument	Reference/Manufacturer
pH	pH electrode	WTW 330 pH meter (WTW, Germany) with a Slimtrode electrode (Hamilton, Germany)
Conductivity	Conductivity meter	inoLab Multi Level 1 meter (WTW, Germany)
Electrode potentials, cell voltage	Datalogger	Agilent 34970A Data Acquisition/Switch Unit (Agilent, Canada)
Current	Potentiostat	PalmSens 3 potentiostat/galvanostat (PalmSens BV, the Netherlands)
Current	Potentiostat	µStat 8000P Multi Potentiostat (DropSens, Spain)
Cyclic voltammetry	Potentiostat	PalmSens 3 potentiostat/galvanostat (PalmSens BV, the Netherlands)
SO_4^{2-}, $S_2O_3^{2-}$	Ion chromatograph	ICS-1600 ion chromatograph (Dionex, USA) with AS-DV autosampler, Ion- Pac AS4A-SC anion exchange column and ASRS-300 suppressor (2 mm)
S^{2-}	Spectrophotometer (CuS method)	Cord-Ruwisch (1985)
$S_4O_6^{2-}$	Spectrophotometer (Cyanolysis)	Modified from Kelly et al. (1969)
Cu^{2+}	Atomic absorption spectrophotometer	AAnalyst 400 with Lumina lamp Co–Cr–Cu–Fe–Mn–Ni (PerkinElmer, USA)
Fe^{2+}	Spectrophotometer (1,10-phenantroline method)	APHA (1995)
Total Fe	Atomic absorption spectrophotometer	AAnalyst 400 with Lumina lamp Co–Cr–Cu–Fe–Mn–Ni (PerkinElmer, USA)

6.6 Calculations

In MFCs, current (I) and power (P) were calculated from the measured voltage (U) and the used external resistance (R) by following the Ohm's law:

$$I = \frac{U}{R} \tag{9}$$

$$P = UI \tag{10}$$

Current and power densities were calculated by normalizing the current or power against the effective surface area of the electrodes (22 cm^2). Performance analysis was done either by gradually decreasing the external resistance (values ranging from 50 000 Ω to 10 Ω) every 30 minutes or by linear sweep voltammetry (LSV). The internal resistance was calculated based on the polarization curve as the relation of the change in the voltage to the change in the current in the linear region (Fan et al. 2008).

The coulombic efficiency (CE) was calculated by dividing the electrical energy produced in coulombs with the electrical energy theoretically releasable from the substrate using Equation 11, where I is the generated current, n is the molar amount of substrate, b is the amount of electrons releasable from one mole of substrate and F is the Faraday constant (96 485 s A mol^{-1}).

$$CE = \frac{\int \mathrm{I}\,\mathrm{dt}}{nFb} \tag{11}$$

7 Results and discussion

This chapter highlights the main results and discusses the integrated significance of the findings. More detailed description of the results and more specific discussion is presented in the original publications.

7.1 Inoculum and microbial communities

Process water samples from multimetal mining operation were observed to contain microorganisms capable to degrade tetrathionate aerobically in batch bottles and anaerobically in bioelectrochemical systems (Paper I). The start-up time of the bioelectrochemical electricity production was relatively long (approximately 100 days) in the first experiment, but thereafter electricity was produced continuously. The long start-up time presumably resulted from low microbial density in the mining process water used as the inoculum and the acclimation of the microorganisms to the changed growth environment. When new fuel cells were inoculated with the anolyte solution of a MFC already operated on tetrathionate, the electricity production was observed to start within one to twenty days (Paper II). Microorganisms already adapted to the operation conditions were thus able to continue the electricity production with a short lag-time.

The microbial community analysis showed that the original process waters contained several iron- and sulfur-oxidizing microorganisms, which is typical for mining environments (Halinen et al. 2012, Johnson & Hallberg 2008). In MFCs, the dominant microorganisms in the anolyte and on the anode electrode surface were closely (≥99.5%) related to *Acidithiobacillus* spp. and *Ferroplasma* spp. (Paper I). *Acidithiobacillus* species are known to degrade tetrathionate aerobically and anaerobically with ferric iron as the electron acceptor (Table 7.1) (Osorio et al. 2013, Quatrini et al. 2009). *Ferroplasma* spp. oxidize ferrous iron (Dopson et al. 2004, Golyshina et al. 2000), but sulfur oxidation has not been reported. *Ferroplasma* spp. were thus presumably utilizing either iron leaking from the cathode to anode or organic excretes of other microorganisms as the substrate for growth. After 80 days of operation and onwards, only species closely related to *Acidithiobacillus* spp. were detected in the PCR-DGGE from the anolyte of long-term operated MFC (Paper II).

Table 7.1: Characteristics of the species closely related (≥99.5%) to the dominant microorganisms in the tetrathionate-fed MFCs.

Species	**Phylum**	**Carbon metabolism**[a]	**Sulfur metabolism**[a]	**Iron metabolism**[a]	**Opt. T (range)**	**Opt. pH (range)**
Acidithiobacillus ferrooxidans	Proteobacteria	Obligate autotroph	Sulfur oxidation	Iron oxidation and reduction	30–35 °C[e] (10–37 °C)[b]	2.5 [b] (1.3–4.5)[b]
Acidithiobacillus thiooxidans	Proteobacteria	Obligate autotroph	Sulfur oxidation	n.r.	28–30 °C[e] (10–37 °C)[b]	2-3 [b] (0.5–5.5)[b]
Ferroplasma acidiphilum	Euryarchaeota	Mixotroph[d]	n.r.	Iron oxidation	35 °C[c] (15–45 °C)[b]	1.7 [c] (1.3–2.2)[b]
Ferroplasma acidarmanus	Euryarchaeota	Mixotroph[d]	n.r.	Iron oxidation	42 °C [d] (23–46 °C)[b]	1.2 [d] (0–1.5)[d]

[a] Johnson & Hallberg (2008), [b] Schippers (2007), [c] Golyshina et al. (2000), [d] Dopson et al. (2004), [e] Kelly & Wood (2000), n.r. not reported

The electricity production with the type strains of pure cultures of *Acidithiobacillus ferrooxidans* and *Ferroplasma acidiphilum* was tested in sterilized two-chamber MFCs with ferric iron as the cathodic electron acceptor (unpublished data). However, the electricity production remained negligible during the whole 80-day test period. The enriched microorganisms thus had higher competence for bioelectrochemical tetrathionate degradation than the type strains.

The microbial communities were analyzed by PCR-DGGE, which enables the analysis of only the dominant species. Therefore, alternative species may have been involved in the bioelectrochemical tetrathionate degradation, which remained undetected due to their low quantity. However, as the MFCs were operated at room temperature in highly acidic conditions with only inorganic sulfur as the substrate, only extremely acidophilic chemolithotrophs are able to grow in such conditions. This leads to low microbial variation.

High K^+ and Cl^- concentrations, which resulted from the periodical addition of $K_2S_4O_6$ and replacement of the $FeCl_3$ or $CuCl_2$ catholyte, may have also limited the growth of microorganisms that do not tolerate high salinity. In the anolyte of the long-term operated MFC LT, the K^+ concentration increased close to 6 g L^{-1} and the Cl^- concentration to 4 g L^{-1} (unpublished data). *Acidithiobacillus* spp. have been reported to tolerate K^+ concentrations above 6 g L^{-1} and Cl^- concentrations up to 14 g L^{-1} (Harahuc et al. 2000, Kieft & Spence 1988, Suzuki et al. 1999). Therefore, even if the high salinity may have affected the composition of the microbial community, K^+ and Cl^- did not significantly limit the growth of the electroactive microorganisms even in the long-term operated MFC. This is supported by the stable tetrathionate degradation rates.

Chemolithoautotrophic microorganisms consume significant amount of the energy released in the substrate degradation to synthetization of the cellular components, which leads to lower biomass yield (McCollom & Amend 2005). The risk of performance limiting biofouling on the membrane or on the cathode electrode surface is thus lower in low-pH MFCs fed with inorganic substrates than in MFCs fed with organic substrates and operated at neutral pH. Even after 740 days of operation, no significant biofouling was observed in the tetrathionate-fed MFCs (Paper II).

Acidithiobacillus species have been reported to contain several type c cytochromes (Yarzábal et al. 2002), which are known to be essential for extracellular electron transfer in electroactive species (Estevez-Canales et al. 2015). No previous studies report the use of *Acidithiobacillus* spp. as the anodic microbial catalyst, but *At. ferrooxidans* has been used as the cathodic biocatalyst in BESs with ferric iron catholyte (ter Heijne et al. 2007). At the cathode, *At. ferrooxidans* oxidizes the electrochemically generated ferrous iron to ferric iron, thus regenerating the iron catholyte solution.

During the operation of the MFCs, the anodic pH decreased below one and the sulfate concentration increased to tens of grams of sulfate per liter (Paper I, Paper II). Too low pH and/or too high sulfate concentrations can affect the growth of microorganisms (Al Zuhair et al. 2008, Dopson et al. 2016). However, mining waters can be extremely acidic and the sulfate concentration in such streams can naturally increase up to several hundred grams per liter (Nordstrom et al. 2000). The low acidity and produced sulfate were not observed to inhibit the growth of the enriched microorganisms, which originated from multimetal mining process waters.

7.2 Electrochemical performance of bioelectrochemical and electrochemical systems

7.2.1 Electricity production in microbial fuel cells

The current generation started in tetrathionate-fed MFCs after 20 days of operation and in 100 days the electricity production reached the maximum level (Paper I). The current density was successfully improved by optimizing the external resistance (Paper II, Table 7.2). However, the power density decreased when operating the system with external resistance below 499 Ω, due to the decreasing voltage caused by the increasing anode potential. This indicates that low anode potentials did not support high current generation. However, continuous electricity production was obtained for over 740 days and the average current density with 100 Ω resistance was 150 mA m^{-2}. The maximum current density and power density obtained in the performance analysis improved over time as the biofilm was developing. The maximum current and power densities obtained in the performance analysis were on day 284 over two times (315 mA m^{-2} and 14.1 mW m^{-2}) and on day 711 over seven times (1120 mA m^{-2} and 44 mW m^{-2}) higher than what was obtained on day 74 (145 mA m^{-2} and 6.5 mW m^{-2}). However, the performance analysis by linear sweep voltammetry with the scan rate of 1 mV s^{-1} was observed to lead to overestimation of the current and power generation capacities in tetrathionate-fed MFCs likely due insufficient stabilization time (Paper II).

Ni et al. (2016) studied the use of tetrathionate, thiosulfate and sulfide mineral flotation process water as the substrate for MFCs at pH below 2.5. With tetrathionate as the substrate, they obtained maximum current density of 34 mA m^{-2} and power density of 2.5 mW m^{-2}. Zhao et al. (2009) reported maximum current density of 1.15 A m^{-2} with sulfite and thiosulfate as the anodic electron donors. In this system, however, electricity production resulted from the abiotic electrochemical oxidation of sulfide generated from sulfite and thiosulfate by sulfate reducing bacteria (Zhao et al. 2009).

The maximum current and power densities obtained in this study remain significantly lower than what has been obtained with MFCs degrading organic compounds (reviewed e.g. by Pandey et al. (2016)). However, the bioelectrochemical electricity production in acidic conditions and/or with inorganic sulfur compounds as the substrate has been addressed only in a few studies and the process requires further optimization. The electricity production efficiency could presumably be enhanced by optimizing the fuel cell configuration and operation conditions.

7.2.2 Internal resistance

In fuel cells, the maximum power can be obtained when the external resistance equals the internal resistance of the cell. In the performance analysis of tetrathionate-fed MFCs, the internal resistance was measured to be approximately 200 Ω after operated with the external resistance of 1000 Ω for over 100 days (Paper I). In the long-term operated MFC LT, the internal resistance decreased over time, from 420 Ω (day 74) to 190 Ω (day 284) (Paper II). In the MFC LT, the external resistance was gradually decreased, but the cell was operated otherwise in the same conditions throughout the experiment. The decrease in the internal resistance was thus presumed to result mainly from enhanced biofilm activity on the anode. Besides enriching for electroactive microorganisms (Kim et al. 2004), the cultivation of microorganisms in bioelectrochemical systems can increase the electroactivity of the microorganisms (Rabaey et al. 2004) and cause phenotypic changes in the microbial strains (Yi et al. 2009).

Microbial biomass accumulation on the membrane (Xu et al. 2012) or on the cathode electrode surface (Yang et al. 2009) can limit the ion transfer and thus increase the internal resistance. However, no performance limiting biofouling was observed even after over 740 days of operation. The accumulating potassium from added $K_2S_4O_6$, chloride ions from $FeCl_3$ and $CuCl_2$ and formed sulfate presumably increased the conductivity of the anolyte solution, and thus also led to lower ohmic resistance (Nam et al. 2010). However, the conductivity of the fresh anolyte solution was high (11.5 mS cm^{-1}) even without added K^+, Cl^-, or SO_4^{2-}. Therefore, the increasing sulfate and ion concentrations were not presumed to have a significant effect on the internal resistance.

7.2.3 Effects of anode potential and applied cell voltage

In electrochemical systems, electrons can be transferred from the substrate to the anode electrode either directly or via a mediator. The anode potential depends on the reduction potential of the final electron donor, which in electrochemical systems is usually the substrate itself. In BESs, the electrons can be transferred to the anode electrode, for example, from a cytochrome on the microbial cell surface or via an indigenous or externally added mediator.

In tetrathionate-fed MFCs, the anode potential remained significantly higher (above 0.350 V vs. Ag/AgCl) than theoretical reduction potential of tetrathionate (0.069 V vs. Ag/AgCl). The potentials of the electrodes can significantly differ from the theoretical reduction potential values due to losses caused by the MFC configuration and reaction efficiencies (Logan et al. 2006, Rabaey & Verstraete 2005). The ability of the microorganisms to adapt to lower anode potentials that what was obtained in the MFCs was tested by gradually lowering the anode potential with a potentiostat (Paper III). However, the current densities did not reach the same level that was obtained in the tetrathionate-fed MFCs (Table 7.2.), the average current density values being 70 mA m^{-2}, 25 mA m^{-2} and 3 mA m^{-2} with anode potentials of 0.4 V, 0.35 V and 0.3 V, respectively. Negative current (-6 mA m^{-2}) was obtained with anode potential of 0.275 V, which implies that with low anode potentials tetrathionate or intermediary sulfur compounds (e.g. $S_2O_3^{2-}$) were biologically or abiotically reduced at the anode.

Table 7.2: Voltage, current density (CD), power density (PD), anode potential (AP), anodic coulombic efficiency (CE_A) and tetrathionate degradation rate ($D(S_4O_6^{2-})$) in operated MFCs.

MFC	**R**	**Voltage**		**CD**		**PD**		**AP**		**CE_A**	**$D(S_4O_6^{2-})$**	**Reference**
	Ω	mV		$mA\ m^{-2}$		$mW\ m^{-2}$		mV vs. Ag/AgCl		%	$mg\ L^{-1}\ d^{-1}$	
		Max.	Avg.	Max.	Avg.	Max.	Avg.	Min.	Avg.			
En1	1000	54	29	26	15	1.5	0.5	483	512	1	64	Paper I
En2	1000	32	15	15	8	0.5	0.1	509	541	0.4	145	Paper I
En3	1000	121	43	55	22	6.7	1	394	516	1.1	119	Paper I
En4	1000	109	26	50	12	5.4	0.3	399	529	0.6	120	Paper I
En3_2A	1000	148	39	67	26	10	1.5	363	517	1.8	141	Paper I
En3_2B	1000	175	102	80	52	14	6	369	445	2.5	181	Paper I
MFC A	1000	184	58	80	26	14	2.9	355	490	2.6	89	Paper II
	499	177	115	161	105	26	12	372	400	5.1	183	Paper II
	240	100	66	177	125	20	8.5	411	426	9.2	138	Paper II
MFC B	1000	186	102	76	46	16	5.9	368	444	2.6	166	Paper II
	499	163	108	125	98	17	11	405	447	4.4	203	Paper II
	240	74	67	140	126	10	8.4	459	469	6.6	182	Paper II
	100	69	22	170	150	7	5.1	464	491	10	131	Paper II
MFC LT	1000	101	150	70	45	19	4.7	429	475	2	212	Paper II
	499	82	137	126	75	17	6.3	461	484	4.9	143	Paper II
	240	52	102	193	98	20	5.3	449	487	6.4	150	Paper II
	100	31	89	225	150	22	4.9	404	497	11	136	Paper II

The high anode potential required for electricity production indicates that the final electron donor (in the metabolic pathway or a mediator) requires significantly higher potential for oxidation than the calculated reduction potential for tetrathionate. Nevertheless, the minimum anode potential required for bioelectrochemical current generation from tetrathionate (0.3 V vs. Ag/AgCl) was lower than what was required for abiotic electrochemical tetrathionate degradation (0.5 V vs. Ag/AgCl) or for the electrochemical oxidation of water (0.88 V vs. Ag/AgCl).

No previous studies report the use of tetrathionate as the substrate for bioelectrochemical electricity production. Feng et al. (1995) studied the electrochemical oxidation of thiosulfate at pH 5 with rotated disc electrode and hypothesized that thiosulfate was oxidized to sulfate in two-step process, first thiosulfate to tetrathionate at anode potential of 0.8 V vs. Ag/AgCl and then tetrathionate to sulfate at anode potential of 1.4 V vs. Ag/AgCl. However, the effect of anode potential or the applied voltage on electrochemical tetrathionate degradation has not been previously studied.

By applying external voltage or current, unspontaneous oxidation and reduction reactions can be realized on the anode and the cathode. The theoretical reduction potential of copper (0.135 V vs. Ag/AgCl) is lower than the measured minimum anode potential for bioelectrochemical (0.3 V vs. Ag/AgCl) or electrochemical (0.5 V vs. Ag/AgCl) tetrathionate degradation. Theoretically, above 0.165 V of external voltage is thus required to combine a tetrathionate degrading anode to copper reducing cathode. The minimum reduction potential for water oxidation was measured to be 0.88 V vs. Ag/AgCl at pH 2.5 in the used electrochemical system (Paper III). The results indicate that the tetrathionate degrading (bio)electrochemical systems would require less external voltage for copper removal than the conventional water-oxidizing electrochemical systems.

The simultaneous removal of tetrathionate and copper in bioelectrochemical and electrochemical systems was studied by applying external cell voltage (Paper IV). At low applied voltages (≤0.5 V), the highest current density was obtained with MEC (15–30 mA m^{-2}). The external voltage required to increase the anode potential above 0.8 V vs. Ag/AgCl (the minimum anode potential for efficient abiotic electrochemical tetrathionate degradation) was 0.75 V in both bioelectrochemical and electrochemical systems. Therefore, current was presumably generated in MEC at applied voltages above 0.75 V via both bioelectrochemical and electrochemical tetrathionate degradation. At high applied voltages (>0.75 V), EC provided higher current density (410–3600 mA m^{-2}) than MEC. The rate of electrochemical tetrathionate degradation was thus presumably higher than the rate of bioelectrochemical tetrathionate degradation and the biofilm on the anode electrode may thus have limited the electricity production in bioelectrochemical systems by decreasing the electrode surface area available for electrochemical tetrathionate degradation. However, higher current was obtained in both bioelectrochemical and electrochemical systems than in water-oxidizing control.

7.3 Bioelectrochemical and electrochemical tetrathionate degradation and reaction products

No electricity production was observed in a control MFC operated without tetrathionate. Therefore, electricity in the MFCs was thus produced solely from tetrathionate and not from other media compounds (Paper I). No current was generated also in the abiotic control reactor fed with tetrathionate and operated with ferric iron as the cathodic electron acceptor, which demonstrates that tetrathionate does not spontaneously degrade in electrochemical systems without applied voltage or a microbial catalyst. The anodic tetrathionate concentration was decreasing in the abiotic control reactor, but the sulfate concentration remained negligible, showing that the decrease in the tetrathionate concentration mainly resulted from the diffusion of tetrathionate from the anolyte to the catholyte. After a tetrathionate-fed MFC was disconnected and operated in open circuit, the tetrathionate degradation continued, but the degradation rate decreased over time. This indicates that even if tetrathionate is degraded utilizing alternative electron acceptors leaking to the anolyte (O_2 and Fe^{3+}), current flow is required for the efficient degradation of tetrathionate (Paper III).

Tetrathionate was degraded with the degradation rate above 110 mg L^{-1} d^{-1} in BESs despite the changes in the anode potential, external resistance or applied voltage (Table 7.3). However, CE remained low (below 11%), indicating that significant amount of the electrons were directed to alternative electron acceptors, e.g. oxygen and ferric iron, were consumed for biomass growth or ended up in unmeasured intermediates. The main reaction product in the bioelectrochemical tetrathionate degradation was sulfate. Additionally, yellowish precipitation was observed to form in the reactors, which was presumed to be elemental sulfur. No sulfide or thiosulfate were detected from the anolyte. The presence of sulfate and elemental sulfur indicate that tetrathionate was degraded via disproportionation following Equation 12.

$$S_4O_6^{2-} + 6\,H_2O \rightarrow S^0 + 3\,SO_4^{2-} + 12\,H^+ + 8\,e^- \tag{12}$$

The theoretical reduction potential of this reaction is 0.069 V vs. Ag/AgCl in standard conditions (concentrations 1 M, 25 °C) and -0.17 V vs. Ag/AgCl at pH 2.5 and 20 °C. Besides the direct conversion of tetrathionate to electrical energy, the current generation may occur via intermediary reaction products, such as sulfide (S^{2-}) or thiosulfate ($S_2O_3^{2-}$), which may remain undetected due to immediate consumption or degradation. The variety of alternative pathways, the high reciprocal reactivity of sulfur compounds and the difficulty in quantification of the solid reaction products complicate the determination of the complete sulfur balance for the tetrathionate-fed reactors.

In abiotic electrochemical systems, the tetrathionate degradation rate increased with the increasing anode potential. The measured sulfate concentrations indicate that tetrathionate was directly degraded to sulfate following Equation 13.

$$S_4O_6^{2-} + 10\ H_2O \rightarrow 4\ SO_4^{2-} + 20\ H^+ + 14\ e^- \tag{13}$$

The direct electrochemical oxidation of tetrathionate to sulfate was proposed as the reaction mechanism also in previous studies, which report tetrathionate to be formed as an intermediate in electrochemical oxidation of thiosulfate (Feng et al. 1995, Ragauskas et al. 2003). Feng et al. (1995) reported tetrathionate oxidation to proceed at anode potential of 1.4 V vs. Ag/AgCl.

Table 7.3: Current density, tetrathionate degradation rate and coulombic efficiency in electrochemical systems at applied anode potential or applied voltage. The controlled parameter (anode potential or voltage) is marked with an asterisk.

Reactor	**EA**[a]	**AP**[b]	**Voltage**	**CD**[c]	**D($S_4O_6^{2-}$)**[d]	**CE$_A$**[e]	**Ref**
		V vs. Ag/AgCl	V	mA m^{-2}	mg L^{-1} d^{-1}	%	
MEC	Fe^{3+}	0.3*	n.m.	3	110	0.3	Paper III
	Fe^{3+}	0.35*	n.m.	25	120	5.7	Paper III
	Fe^{3+}	0.4*	n.m.	70	160	7.1	Paper III
	Cu^{2+}	0.57	0.2*	15	170	<1	Paper IV
	Cu^{2+}	0.76	0.5*	55	155	5	Paper IV
	Cu^{2+}	0.97	0.75*	170	150	14	Paper IV
	Cu^{2+}	1.16	1.0*	490	155	34	Paper IV
	Cu^{2+}	1.20	1.2*	900	165	54	Paper IV
	Cu^{2+}	1.15	1.5*	2280	160	140	Paper IV
EC	Fe^{3+}	0.6*	n.m.	4	20	1	Paper III
	Fe^{3+}	0.8*	n.m.	115	30	20	Paper III
	Fe^{3+}	0.9*	n.m.	315	35	48	Paper III
	Cu^{2+}	0.56	0.2*	<5	20	<2	Paper IV
	Cu^{2+}	0.72	0.5*	65	25	18	Paper IV
	Cu^{2+}	0.85	0.75*	270	35	47	Paper IV
	Cu^{2+}	1.03	1.0*	810	40	80	Paper IV
	Cu^{2+}	1.11	1.2*	1290	140	71	Paper IV
	Cu^{2+}	1.17	1.5*	2100	190	75	Paper IV

[a] cathodic electron acceptor, [b] anode potential, [c] average current density, [d] tetrathionate degradation rate, [e] anodic coulombic efficiency, n.m. not measured

When the anode and cathode chambers were separated with an anion exchange membrane, some of the tetrathionate and formed sulfate may have leaked from the anolyte to the catholyte. However, the anode electrode is positively charged and attracts the negative ions from the solution, which may have limited the diffusion of the anions from anode to cathode.

As sulfate was produced as the main reaction product in both bioelectrochemical and electrochemical systems, the sulfate concentrations increased in the anolyte solution up to several grams per liter. The recommended maximum concentration for sulfate in drinking water is 250 mg L^{-1} (WHO 2011). Therefore, also the sulfate should be removed from the treated waters before their release to the environment. Efficient methods for sulfate removal were presented in Chapter 2.4.2. Sulfate can be reduced to higher sulfur compounds, for example, with the assist of sulfate reducing bacteria, even in BESs (Teng et al. 2016, Zhao et al. 2008).

7.4 Cathodic electron acceptors

For spontaneous electricity production, the reduction potential of the cathodic reaction should be higher than the reduction potential of the anodic reaction. Cathodic electron acceptors tested in this study were oxygen (aerated phosphate buffer), ferric iron (Fe^{3+}) and copper (Cu^{2+}).

7.4.1 Oxygen and ferric iron

Oxygen is often used as the electron acceptor in bioelectrochemical and electrochemical systems, because it has a high reduction potential (E^0 = 1.03 V vs. Ag/AgCl) and because it is readily available from air. The suitability of oxygen to be used as the electron acceptor for bioelectrochemical tetrathionate degradation was tested by using aerated phosphate buffer as the catholyte solution. However, the cathode potential and thus also the cell voltage remained low (Paper I). The low solubility of oxygen to water at room temperature (Weiss 1970) may have limited the availability of oxygen on the cathode electrode surface. In addition, no catalyst, which could have increased the oxygen reduction rate, was used on the carbon-based electrode surface.

Ferric iron has been reported to be efficient cathodic electron acceptor for BESs due to its high reduction potential (0.64 V vs. Ag/AgCl at pH 2) (Kumar et al. 2017b, ter Heijne et al. 2007). In this study, the cathode potential in the electrochemical systems with ferric iron catholyte was measured to remain above 0.55 V vs. Ag/AgCl. Ferric iron was thus found to be an efficient cathodic electron acceptor and it was used as the electron acceptor in tetrathionate-fed MFCs.

In mining environments, iron-oxidizing microorganisms often oxidize ferrous iron to ferric iron and both forms of iron are thus found from mining waters. Such iron-oxidizing microorganisms can also be utilized in the regeneration of the iron catholyte. ter Heijne et al. (2007) demonstrated that *At. ferrooxidans* oxidized electrochemically generated ferrous iron back to ferric iron on the cathode of an electrochemical system by using oxygen as the electron acceptor. Similar cathodic iron regeneration, which enabled more stable electricity production, was observed in one tetrathionate-fed MFC (Paper II).

7.4.2 Copper

RISC containing mining waters often have also elevated metal concentrations. Metals can be recovered with electrochemical systems by using the metal ions as the electron acceptors at the cathode. By using metals ions as the cathodic electron acceptors for the bioelectrochemical RISC degradation, both of these environmentally harmful compounds could be removed from the water streams simultaneously. In addition, metals could be recovered in pure elemental form.

With organic substrates, copper has been spontaneously recovered in BESs while generating electrical current (Tao et al. 2011a, ter Heijne et al. 2010, Wang et al. 2010a). Copper was tested as the cathodic electron acceptor in MFCs fed with tetrathionate (Paper I), but no electricity production was observed. The theoretical reduction potential of copper is 0.135 V vs. Ag/AgCl, which is lower than the determined minimum anode potentials for bioelectrochemical and electrochemical tetrathionate degradation. External voltage is thus required to combine tetrathionate degrading anode to copper reducing cathode.

The effect of applied external voltage on current generation, tetrathionate degradation and copper removal was studied in Paper IV. External voltage of above 1.0 V was required to decrease the cathode potential below the theoretical reduction potential of copper in both bioelectrochemical and electrochemical systems, but at high applied voltages, copper was efficiently removed from the solution. At applied voltage of 1.5 V, 99.9% of the copper was removed in 7 days. The maximum copper removal rates were 440 mg L^{-1} d^{-1} in MEC and 450 mg L^{-1} d^{-1} in EC. After 9 days of operation, the cathodic copper concentration had decreased below 0.3 mg L^{-1}, which is the set limit for mining effluents (EPA 2011). The reddish brown color of the precipitate on the cathode electrode indicates that copper was electrodeposited the surface of the cathode electrode in elemental form.

7.5 Perspectives and limitations

The applicability of RISC-degrading (bio)electrochemical systems can be limited by low concentrations of RISCs in stored mining waters. Tetrathionate is relatively stable in acidic conditions even in the presence of oxidizers such as O_2 or Fe^{3+} (Druschel et al. 2003), but mining waters often contain sulfur-oxidizing microorganisms, which oxidize RISCs efficiently in both aerobic and anaerobic conditions. Therefore, the concentrations of RISCs are often low in water streams stored for extended periods. The (bio)electrochemical treatment is thus applicable only for fresh sulfide mineral process water streams, in which the dissolved oxygen has been consumed.

The (bio)electrochemical degradation of tetrathionate reduces the chemical oxygen demand of the treated water, but leads to release of acidity and sulfate. Therefore, the (bio)electrochemical treatment does not purify the water per se, but enables the recovery of part of the chemical energy stored in RISCs as electrical energy. In addition, when the RISCs are oxidized in a controlled environment, the water streams can be more easily neutralized than environmental waters. The results indicate that (bio)electrochemical treatment at its current stage can not replace the current methods used for mining water treatment. However, the integration of (B)ESs to the existing mining water treatment processes could reduce the energy requirements. The ideal position for the (B)ESs in the process flowchart should be determined for each mining or mineral processing site individually.

No previous studies have addressed the electricity production with BESs using inorganic substrates at low pH typical for mining waters. One significant drawback of acidophilic BESs as compared to neutrophilic BESs is the higher theoretical reduction potential of the anodic reaction (Equation 7), which leads to lower obtainable cell voltage. However, high acidity is correspondingly enhancing the cathodic reduction reaction. With acidophilic BESs, the pH gradient over the membrane separating an acidic cathode and the anolyte, and thus also the voltage loss caused by the pH gradient, can be decreased. Moreover, the high proton concentration increases the conductivity of the anolyte solution, thus decreasing the internal resistance of the system.

This study shows that RISCs can be used as substrates for bioelectricity generation, but the obtained current and power densities remained lower than in BES degrading organic compounds (Pandey et al. 2016). However, the electricity production efficiency of RISC-fed BESs may be enhanceable by optimization of the reactor design and the materials. The determination of the reaction pathway can also assist in identification of the factors limiting the electricity generation and thus help in construction of efficient cells for electricity production.

Copper has a relatively high reduction potential and it can thus be recovered with (bio)electrochemical systems with no or low external energy. However, most of the metals mined in high quantities (e.g. Ni, Co and Zn) have theoretical reduction potentials below 0 V vs. NHE (Table 3.1), which means that more external energy is required for their electrochemical reduction. However, the differences in the reduction potentials enable selective recovery of metals from multimetal streams with systems operated with different cathode potentials (Luo et al. 2014, Modin et al. 2012).

The treatment of industrial water streams would require reactors suitable for treating high volumes of water efficiently. However, scaling up of bioelectrochemical systems has been challenging (He et al. 2017). The increasing distance between anode and cathode electrodes combined with low solution conductivity increase the internal resistance, which decreases the obtainable power. In addition, the composition of the process water stream and the operation conditions may vary periodically. Further studies are required to develop efficient electricity production from real process waters and in upscaled reactors.

8 Conclusions

This study shows that reduced inorganic sulfur compounds from acidic water streams can serve as substrates for current generation in bioelectrochemical and electrochemical systems. In paper I, tetrathionate was demonstrated for the first time to be a suitable substrate for bioelectrochemical electricity production. With ferric iron as the cathodic electron acceptor, maximum current and power densities obtained in the performance analysis were 433 mA m^{-2} and 17.6 mW m^{-2}, respectively. The microbial community, which originated from mining waters, was dominated by species closely related (≥99.5%) to *Acidithiobacillus* spp. and *Ferroplasma* spp.

The optimization of the external resistance increased the electricity production efficiencies in tetrathionate degrading MFCs (Paper II). The average current density during operation increased from 80 mA m^{-2} to 225 mA m^{-2} when the external resistance was decreased from 1000 Ω to 100 Ω. Long-term operation (Paper II) showed that the electricity production efficiency significantly improved over time. Electricity production was not limited by reaction product accumulation (H^+ and SO_4^{2-}) or biofouling even after over 740 days of operation.

In paper III, it was demonstrated that tetrathionate is a suitable substrate also for abiotic electrochemical systems, but the efficient current generation requires an anode potential above 0.7 V vs. Ag/AgCl. When the anode potential was controlled, the tetrathionate degradation rates remained lower (below 35 mg L^{-1} d^{-1}) in the electrochemical system than in the bioelectrochemical system (above 110 mg L^{-1} d^{-1}). However, in electrochemical systems the chemical energy of tetrathionate was converted more efficiently to electrical energy.

This is the first study to show that, by applying external voltage, tetrathionate degradation can also be combined to the electrochemical reduction of copper. This enables the simultaneous removal of both of these environmentally harmful compounds, for example, from mining water streams (Paper IV). Efficient copper reduction required an applied voltage above 1.0 V in both bioelectrochemical and electrochemical systems. With applied voltage of 1.5 V, the tetrathionate and copper concentrations decreased below the set limits for toxicity or effluent discharge after 9 days of (bio)electrochemical treatment.

Mining waters that have been exposed to oxygen and/or sulfur-oxidizing microorganisms contain little or no reduced inorganic sulfur compounds. Therefore, the RISC-degrading (bio)electrochemical systems are suitable only for the treatment of fresh mining process waters, in which the sulfur compounds have not been yet oxidized to sulfate. Nevertheless, (bio)electrochemical systems can be used to convert part of the chemically bound energy of mining waters to electrical energy, while simultaneously lowering the risks of oxygen depletion and uncontrolled acidification.

Overall, both bioelectrochemical and electrochemical systems were found efficient for the electrochemical degradation of tetrathionate. In bioelectrochemical systems, tetrathionate was degraded spontaneously and electricity was produced when ferric iron was used the cathodic electron acceptor. The tetrathionate degradation rates remained high when the systems were operated as MFCs and when external potential or voltage was applied. Abiotic electrochemical systems for tetrathionate degradation seem practical for applications, where low-cost external power is available. In addition, abiotic systems can be operated without providing nutrients and operation conditions essential for the electroactive microbial community. Altogether, (bio)electrochemical systems seem promising for the removal of reduced inorganic sulfur compounds at remote mining environments. The substrate, microbial catalyst and the cathodic electron acceptor (e.g. ferric iron, metals) are all often present in mining waters and the external input required remains thus low.

9 Recommendations for further research

This study provides the proof-of-principle for (bio)electrochemical degradation of tetrathionate. The conducted studies generated basic knowledge related to the bioelectrochemical and electrochemical degradation of tetrathionate. Further studies are required to determine significance and applicability of the processes and to investigate the possibilities to improve the efficiencies of the systems.

In bioelectrochemical systems, the pathway for tetrathionate degradation should be confirmed. The reaction products indicate that tetrathionate was degraded via disproportionation, but the stable tetrathionate degradation rates at varying anode potentials indicate that the degradation reaction was not directly associated with the anode electrode. No sulfide or thiosulfate were detected from the anolyte, but the involvement of these or other indigenous mediators in the current generation is still to be clarified and all the sulfur compounds present in the anolyte and catholyte should analyzed to obtain the complete sulfur balance of the system. In addition, the microbial species responsible for the electricity generation should be verified. *Acidithiobacillus* spp. and *Ferroplasma* spp. were identified from bioelectrochemical systems, but in preliminary tests with pure cultures of the type strains of *Acidithiobacillus ferrooxidans* and *Ferroplasma acidiphilum*, no electricity production was obtained, which indicates that the electricity producing microorganisms differ from the tested strains phenotypically and/or genotypically. Further studies are required to determine the roles of the microbial catalysts.

Ferric iron is an efficient electron acceptor and iron catholytes have been successfully regenerated with the assist of iron-oxidizing microorganisms in organic bioelectrochemical systems. In this study, unintentional iron regeneration was observed to occur also in one tetrathionate-fed MFC, but its full utilization was out of the scope of this study. Therefore, further studies should be conducted to determine the requirements for the efficient iron regeneration in acidic RISC-fed bioelectrochemical systems. The tetrathionate degradation, ferric iron reduction and copper reduction were studied only with carbon paper as the electrode material. The possibility to enhance the current generation by using alternative electrode materials should be tested. However, the selected materials should be inexpensive in order to maintain the total expenses at a low level.

To disclose the suitability of the RISC-degrading (bio)electrochemical systems for the treatment of real mining waters, the RISC content of several mining process and wastewaters needs to be systematically analyzed. The toxic effects of compounds typically found from mining waters (e.g. heavy metals) on the current generation in bioelectrochemical systems should also be determined. After identification of suitable mining water stream, electricity production with real mining water as the substrate needs to be studied.

The external voltage required for the simultaneous removal of tetrathionate and copper was above 1.0 V. The possibility of using a renewable energy, for example, a solar cell, to power the tetrathionate degrading copper reducing bioelectrochemical and electrochemical systems should be studied. Moreover, in this study the electrochemical systems have been shown to work efficiently in fed-batch mode in the laboratory scale. To take a step towards industrial applications, in following experiments the process should be studied in continuous mode and in upscaled reactors.

References

Abo-Farha, S.A., Abdel-Aal, A.Y., Ashour, I.A. & Garamon, S.E. 2009. Removal of some heavy metal cations by synthetic resin purolite C100. *J Hazard Mater*, vol. 169, pp. 190-194.

Aelterman, P., Versichele, M., Marzorati, M., Boon, N. & Verstraete, W. 2008. Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes. *Bioresour Technol*, vol. 99, pp. 8895-8902.

Agarwala, U., Rees, C. & Thode, H. 1965. Sulfur isotope effects in the hydrogen ion decomposition of thiosulfate. *Can J Chem*, vol. 43, pp. 2802-2811.

Akcil, A. & Koldas, S. 2006. Acid Mine Drainage (AMD): causes, treatment and case studies. *J Clean Prod*, vol. 14, pp. 1139-1145.

Al Zuhair, S., El-Naas, M.H. & Al Hassani, H. 2008. Sulfate inhibition effect on sulfate reducing bacteria. *J Biochem Technol*, vol. 1, pp. 39-44.

Al-Amin, A.Q., Ambrose, A.F., Masud, M.M. & Azam, M.N. 2016. People purchase intention towards hydrogen fuel cell vehicles: An experiential enquiry in Malaysia. *Int J Hydrogen Energy*, vol. 41, pp. 2117-2127.

Alberti, G., Casciola, M., Massinelli, L. & Bauer, B. 2001. Polymeric proton conducting membranes for medium temperature fuel cells (110-160 °C). *J Membr Sci*, vol. 185, pp. 73-81.

Alberti, G., Casciola, M. & Palombari, R. 2000. Inorgano-organic proton conducting membranes for fuel cells and sensors at medium temperatures. *J Membr Sci*, vol. 172, pp. 233-239.

Albo, J., Beobide, G., Castaño, P. & Irabien, A. 2017. Methanol electrosynthesis from CO_2 at Cu_2O/ZnO prompted by pyridine-based aqueous solutions. *J CO2 Util*, vol. 18, pp. 164-172.

Allen, H.E. & Chen, P. 1993. Remediation of metal contaminated soil by EDTA incorporating electrochemical recovery of metal and EDTA. *Environ Prog*, vol. 12, pp. 284-293.

Aman, T., Kazi, A.A., Sabri, M.U. & Bano, Q. 2008. Potato peels as solid waste for the removal of heavy metal copper(II) from waste water/industrial effluent. *Colloids Surf B Biointerfaces*, vol. 63, pp. 116-121.

Amaral Filho, J., Azevedo, A., Etchepare, R. & Rubio, J. 2016. Removal of sulfate ions by dissolved air flotation (DAF) following precipitation and flocculation. *Int J Miner Process*, vol. 149, pp. 1-8.

American Public Health Association (APHA) 1995, *Standard methods for examination of water and wastewater*, 19th edition, American Public Health Association (APHA), American Water Works Association (AWWA) & Water Environment Federation (WEF), Washington, United States, 541 p.

Amouric, A., Appia-Ayme, C., Yarzabal, A. & Bonnefoy, V. 2009. Regulation of the iron and sulfur oxidation pathways in the acidophilic *Acidithiobacillus ferrooxidans*. *Adv Mat Res*, vol. 71-73, pp. 163-166.

Antolini, E. 2011. The stability of molten carbonate fuel cell electrodes: A review of recent improvements. *Appl Energy*, vol. 88, pp. 4274-4293.

Aryal, N., Halder, A., Tremblay, P., Chi, Q. & Zhang, T. 2016. Enhanced microbial electrosynthesis with three-dimensional graphene functionalized cathodes fabricated via solvothermal synthesis. *Electrochim Acta*, vol. 217, pp. 117-122.

Ateya, B., AlKharafi, F. & Al-Azab, A. 2003. Electrodeposition of sulfur from sulfide contaminated brines. *Electrochem Solid-State Lett*, vol. 6, pp. C137-C140.

Babbit, B. & Groat, C.G. 2001, *Mineral Commodity Summaries 2001*, U.S. Department of the Interior and U.S. Geological Survey, Washington, USA, 195 p.

Badwal, S.P.S. 2001. Stability of solid oxide fuel cell components. *Solid State Ionics*, vol. 143, pp. 39-46.

Bajracharya, S., Vanbroekhoven, K., Buisman, C.J., Pant, D. & Strik, D.P. 2016. Application of gas diffusion biocathode in microbial electrosynthesis from carbon dioxide. *Environ Sci Pollut Res*, vol. 23, pp. 22292-22308.

Baker-Austin, C. & Dopson, M. 2007. Life in acid: pH homeostasis in acidophiles. *Trends Microbiol*, vol. 15, pp. 165-171.

Barakat, M.A. & Schmidt, E. 2010. Polymer-enhanced ultrafiltration process for heavy metals removal from industrial wastewater. *Desalination*, vol. 256, pp. 90-93.

Barton, L.L. & Fauque, G.D. 2009. Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. *Adv Appl Microbiol*, vol. 68, pp. 41-98.

Barton, L.L., Tomei-Torres, F.A., Xu, H. & Zocco, T. 2015. Metabolism of metals and metalloids by the sulfate-reducing bacteria. In: Saffarini, D. Bacteria-Metal Interactions, Springer, Switzerland, pp. 57-83.

Baudler, A., Schmidt, I., Langner, M., Greiner, A. & Schröder, U. 2015. Does it have to be carbon? Metal anodes in microbial fuel cells and related bioelectrochemical systems. *Energy Environ Sci*, vol. 8, pp. 2048-2055.

Bebbington, A., Hinojosa, L., Bebbington, D.H., Burneo, M.L. & Warnaars, X. 2008. Contention and ambiguity: Mining and the possibilities of development. *Dev Change*, vol. 39, pp. 887-914.

Bejan, D. & Bunce, N.J. 2015. Acid mine drainage: electrochemical approaches to prevention and remediation of acidity and toxic metals. *J Appl Electrochem*, vol. 45, pp. 1239-1254.

Belieres, J., Gervasio, D. & Angell, C.A. 2006. Binary inorganic salt mixtures as high conductivity liquid electrolytes for >100 °C fuel cells. *Chem Commun*, vol. 46, pp. 4799-4801.

Bennion, D.N. & Newman, J. 1972. Electrochemical removal of copper ions from very dilute solutions. *J Appl Electrochem*, vol. 2, pp. 113-122.

Black, A. & Goodson, J.B. 1952. The oxidation of sulfides by chlorine in dilute aqueous solutions. *J Am Water Works Assoc*, vol. 44, pp. 309-316.

Bo, X., Zhou, M. & Guo, L. 2017. Electrochemical sensors and biosensors based on less aggregated graphene. *Biosens Bioelectron*, vol. 89, pp. 167-186.

Bonanos, N., Knight, K.S. & Ellis, B. 1995. Perovskite solid electrolytes: Structure, transport properties and fuel cell applications. *Solid State Ionics*, vol. 79, pp. 161-170.

Borjas, Z., Esteve-Núñez, A. & Ortiz, J.M. 2017. Strategies for merging microbial fuel cell technologies in water desalination processes: Start-up protocol and desalination efficiency assessment. *J Power Sources*, vol. 356, pp. 519-528.

Borole, A., O'Neill, H., Tsouris, C. & Cesar, S. 2008. A microbial fuel cell operating at low pH using the acidophile *Acidiphilium cryptum*. *Biotechnol Lett*, vol. 30, pp. 1367-1372.

Borup, R., Meyers, J., Pivovar, B., Kim, Y.S., Mukundan, R., Garland, N., Myers, D., Wilson, M., Garzon, F. & Wood, D. 2007. Scientific aspects of polymer electrolyte fuel cell durability and degradation. *Chem Rev*, vol. 107, pp. 3904-3951.

Bosman, D. 1983. Lime treatment of acid mine water and associated solids/liquid separation. *Water Sci Technol*, vol. 15, pp. 71-84.

Bowien, B. & Schlegel, H.G. 1981. Physiology and biochemistry of aerobic hydrogen-oxidizing bacteria. *Annu Rev Microbiol*, vol. 35, pp. 405-452.

Busalmen, J., Esteve-Núñez, A., Berná, A. & Feliu, J.M. 2008. C-type cytochromes wire electricity-producing bacteria to electrodes. *Angew Chem*, vol. 120, pp. 4952-4955.

Bussière, B., Benzaazoua, M., Aubertin, M. & Mbonimpa, M. 2004. A laboratory study of covers made of low-sulphide tailings to prevent acid mine drainage. *Environ Geol*, vol. 45, pp. 609-622.

Butti, S.K., Velvizhi, G., Sulonen, M.L.K., Haavisto, J.M., Koroglu, E.O., Cetinkaya, A.Y., Singh, S., Arya, D., Annie Modestra, J., Vamsi Krishna, K., Verma, A., Ozkaya, B., Lakaniemi, A., Puhakka, J.A. & Venkata Mohan, S. 2016. Microbial electrochemical technologies with the perspective of harnessing bioenergy: Maneuvering towards upscaling. *Renew Sust Energ Rev*, vol. 53, pp. 462-476.

Caccavo, F., Lonergan, D.J., Lovley, D.R., Davis, M., Stolz, J.F. & McInerney, M.J. 1994. *Geobacter sulfurreducens* sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. *Appl Environ Microbiol*, vol. 60, pp. 3752-3759.

Călinescu, O., Marin, N.M., Ioniță, D., Pascu, L.F., Tudorache, A., Surpățeanue, G., Badea, I.A. & Aboul-Enein, H.Y. 2016. Selective removal of sulfate ion from different drinking waters. *Environ Nanotechnol Monit Manage*, vol. 6, pp. 164-168.

Carbajosa, S., Malki, M., Caillard, R., Lopez, M.F., Palomares, F.J., Martín-Gago, J.A., Rodríguez, N., Amils, R., Fernández, V.M. & De Lacey, A.L. 2010. Electrochemical growth of *Acidithiobacillus ferrooxidans* on a graphite electrode for obtaining a biocathode for direct electrocatalytic reduction of oxygen. *Biosens Bioelectron*, vol. 26, pp. 877-880.

Carmona-Martínez, A.A., Harnisch, F., Kuhlicke, U., Neu, T.R. & Schröder, U. 2013. Electron transfer and biofilm formation of *Shewanella putrefaciens* as function of anode potential. *Bioelectrochemistry*, vol. 93, pp. 23-29.

Catal, T., Bermek, H. & Liu, H. 2009. Removal of selenite from wastewater using microbial fuel cells. *Biotechnol Lett*, vol. 31, pp. 1211-1216.

Cetinkaya, A.Y., Ozdemir, O.K., Demir, A. & Ozkaya, B. 2017. Electricity production and characterization of high-strength industrial wastewaters in microbial fuel cell. *Appl Biochem Biotechnol*, vol. 182, pp. 468-481.

Cetinkaya, A.Y., Ozdemir, O.K., Koroglu, E.O., Hasimoglu, A. & Ozkaya, B. 2015. The development of catalytic performance by coating Pt–Ni on CMI7000 membrane as a cathode of a microbial fuel cell. *Bioresour Technol*, vol. 195, pp. 188-193.

Chang, Q. & Wang, G. 2007. Study on the macromolecular coagulant PEX which traps heavy metals. *Chem Eng Sci*, vol. 62, pp. 4636-4643.

Chartrand, M.M.G. & Bunce, N.J. 2003. Electrochemical remediation of acid mine drainage. *J Appl Electrochem*, vol. 33, pp. 259-264.

Chen, R., Trieu, V., Zeradjanin, A.R., Natter, H., Teschner, D., Kintrup, J., Bulan, A., Schuhmann, W. & Hempelmann, R. 2012. Microstructural impact of anodic coatings on the electrochemical chlorine evolution reaction. *Phys Chem Chem Phys*, vol. 14, pp. 7392-7399.

Chen, Q., Luo, Z., Hills, C., Xue, G. & Tyrer, M. 2009. Precipitation of heavy metals from wastewater using simulated flue gas: Sequent additions of fly ash, lime and carbon dioxide. *Water Res*, vol. 43, pp. 2605-2614.

Chen, X., Liang, P., Zhang, X. & Huang, X. 2016. Bioelectrochemical systems-driven directional ion transport enables low-energy water desalination, pollutant removal, and resource recovery. *Bioresour Technol*, vol. 215, pp. 274-284.

Cheng, S., Dempsey, B.A. & Logan, B.E. 2007. Electricity generation from synthetic Acid-Mine Drainage (AMD) water using fuel cell technologies. *Environ Sci Technol*, vol. 41, pp. 8149-8153.

Choi, C. & Cui, Y. 2012. Recovery of silver from wastewater coupled with power generation using a microbial fuel cell. *Bioresour Technol*, vol. 107, pp. 522-525.

Choi, M., Chae, K., Ajayi, F.F., Kim, K., Yu, H., Kim, C. & Kim, I.S. 2011. Effects of biofouling on ion transport through cation exchange membranes and microbial fuel cell performance. *Bioresour Technol*, vol. 102, pp. 298-303.

Clark II, W.W., Paolucci, E. & Cooper, J. 2003. Commercial development of energy – environmentally sound technologies for the auto-industry: the case of fuel cells. *J Clean Prod*, vol. 11, pp. 427-437.

Cord-Ruwisch, R. 1985. A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J Microbiol Methods*, vol. 4, pp. 33-36.

Coupland, K. & Johnson, D.B. 2004. Geochemistry and microbiology of an impounded subterranean acidic water body at Mynydd Parys, Anglesey, Wales. *Geobiology*, vol. 2, pp. 77-86.

Cruz, V.R., Oropeza, M., González, I. & Ponce-De-León, C. 2002. Electrochemical recovery of silver from cyanide leaching solutions. *J Appl Electrochem*, vol. 32, pp. 473-479.

Cui, M., Cui, D., Gao, L., Cheng, H. & Wang, A. 2016. Analysis of electrode microbial communities in an up-flow bioelectrochemical system treating azo dye wastewater. *Electrochim Acta*, vol. 220, pp. 252-257.

Cui, M., Cui, D., Gao, L., Wang, A. & Cheng, H. 2017. Evaluation of anaerobic sludge volume for improving azo dye decolorization in a hybrid anaerobic reactor with built-in bioelectrochemical system. *Chemosphere*, vol. 169, pp. 18-22.

Das, A., Mishra, A.K. & Roy, P. 1992. Anaerobic growth on elemental sulfur using dissimilar iron reduction by autotrophic *Thiobacillus ferrooxidans*. *FEMS Microbiol Lett*, vol. 97, pp. 167-172.

de la Torre, M. L., Grande, J.A., Graiño, J., Gómez, T. & Cerón, J.C. 2011. Characterization of AMD pollution in the river Tinto (SW Spain). Geochemical comparison between generating source and receiving environment. *Water Air Soil Pollut*, vol. 216, pp. 3-19.

Delmelle, P. & Bernard, A. 1994. Geochemistry, mineralogy, and chemical modeling of the acid crater lake of Kawah Ijen Volcano, Indonesia. *Geochim Cosmochim Acta*, vol. 58, pp. 2445-2460.

Devrim, Y., Devrim, H. & Eroglu, I. 2015. Development of 500 W PEM fuel cell stack for portable power generators. *Int J Hydrogen Energy*, vol. 40, pp. 7707-7719.

Dialynas, E. & Diamadopoulos, E. 2009. Integration of a membrane bioreactor coupled with reverse osmosis for advanced treatment of municipal wastewater. *Desalination*, vol. 238, pp. 302-311.

Dinardo, O. & Salley, J. 1998, *Treatment of thiosalts in milling effluents: a review of treatment processes*, Mining and Mineral Sciences Laboratories, Canada, 118 p.

Dold, B. 2014. Evolution of Acid Mine Drainage formation in sulphidic mine tailings. *Minerals*, vol. 4, pp. 621-641.

Dong, Y., Qu, Y., He, W., Du, Y., Liu, J., Han, X. & Feng, Y. 2015. A 90-liter stackable baffled microbial fuel cell for brewery wastewater treatment based on energy self-sufficient mode. *Bioresour Technol*, vol. 195, pp. 66-72.

Dopson, M., Baker-Austin, C., Hind, A., Bowman, J.P. & Bond, P.L. 2004. Characterization of *Ferroplasma* isolates and *Ferroplasma acidarmanus* sp. nov., extreme acidophiles from Acid Mine Drainage and industrial bioleaching environments. *Appl Environ Microbiol*, vol. 70, pp. 2079-2088.

Dopson, M. & Johnson, D.B. 2012. Biodiversity, metabolism and applications of acidophilic sulfur-metabolizing microorganisms. *Environ Microbiol*, vol. 14, pp. 2620-2631.

Dopson, M., Ni, G. & Sleutels, T.H.J.A. 2016. Possibilities for extremophilic microorganisms in microbial electrochemical systems. *FEMS Microbiol Rev*, vol. 40, pp. 164-181.

Doula, M.K. 2009. Simultaneous removal of Cu, Mn and Zn from drinking water with the use of clinoptilolite and its Fe-modified form. *Water Res*, vol. 43, pp. 3659-3672.

Druschel, G.K., Baker, B.J., Gihring, T.M. & Banfield, J.F. 2004. Acid mine drainage biogeochemistry at Iron Mountain, California. *Geochem Trans*, vol. 5, pp. 13-32.

Druschel, G.K., Hamers, R.J. & Banfield, J.F. 2003. Kinetics and mechanism of polythionate oxidation to sulfate at low pH by O_2 and Fe^{3+}. *Geochim Cosmochim Acta*, vol. 67, pp. 4457-4469.

Du, Z., Gao, Q., Feng, J., Lu, Y. & Wang, J. 2006. Dynamic instabilities and mechanism of the electrochemical oxidation of thiosulfate. *J Phys Chem B*, vol. 110, pp. 26098-26104.

Dutta, P.K., Keller, J., Yuan, Z., Rozendal, R.A. & Rabaey, K. 2009. Role of sulfur during acetate oxidation in biological anodes. *Environ Sci Technol*, vol. 43, pp. 3839-3845.

Dutta, P.K., Rabaey, K., Yuan, Z. & Keller, J. 2008. Spontaneous electrochemical removal of aqueous sulfide. *Water Res*, vol. 42, pp. 4965-4975.

Dutta, P.K., Rozendal, R.A., Yuan, Z., Rabaey, K. & Keller, J. 2009. Electrochemical regeneration of sulfur loaded electrodes. *Electrochem Commun*, vol. 11, pp. 1437-1440.

Edwards, K.J., Gihring, T.M. & Banfield, J.F. 1999. Seasonal variations in microbial populations and environmental conditions in an extreme acid mine drainage environment. *Appl Environ Microbiol*, vol. 65, pp. 3627-3632.

El Samrani, A.G., Lartiges, B.S. & Villiéras, F. 2008. Chemical coagulation of combined sewer overflow: Heavy metal removal and treatment optimization. *Water Res*, vol. 42, pp. 951-960.

Elitzur, S., Rosenband, V. & Gany, A. 2016. Combined energy production and waste management in manned spacecraft utilizing on-demand hydrogen production and fuel cells. *Acta Astronaut*, vol. 128, pp. 580-583.

Emerson, D., Fleming, E.J. & McBeth, J.M. 2010. Iron-oxidizing bacteria: an environmental and genomic perspective. *Annu Rev Microbiol*, vol. 64, pp. 561-583.

EPA 2011, *Ore Mining and Dressing Preliminary Study Report*, United States Environmental Publication Agency, 54 p.

Erable, B., Etcheverry, L. & Bergel, A. 2009. Increased power from a two-chamber microbial fuel cell with a low-pH air-cathode compartment. *Electrochem Commun*, vol. 11, pp. 619-622.

Escapa, A., San-Martín, M.I., Mateos, R. & Morán, A. 2015. Scaling-up of membraneless microbial electrolysis cells (MECs) for domestic wastewater treatment: Bottlenecks and limitations. *Bioresour Technol*, vol. 180, pp. 72-78.

Esquivel, J., Buser, J., Lim, C., Domínguez, C., Rojas, S., Yager, P. & Sabaté, N. 2017. Single-use paper-based hydrogen fuel cells for point-of-care diagnostic applications. *J Power Sources*, vol. 342, pp. 442-451.

Estevez-Canales, M., Kuzume, A., Borjas, Z., Füeg, M., Lovley, D., Wandlowski, T. & Esteve-Núñez, A. 2015. A severe reduction in the cytochrome C content of *Geobacter sulfurreducens* eliminates its capacity for extracellular electron transfer. *Environ Microbiol Rep*, vol. 7, pp. 219-226.

Fan, Y., Sharbrough, E. & Liu, H. 2008. Quantification of the internal resistance distribution of microbial fuel cells. *Environ Sci Technol*, vol. 42, pp. 8101-8107.

Fathey, R., Gomaa, O.M., Ali, A.E., El Kareem, H.A. & Zaid, M.A. 2016. Neutral red as a mediator for the enhancement of electricity production using a domestic wastewater double chamber microbial fuel cell. *Ann Microbiol*, vol. 66, pp. 695-702.

Fedje, K.K., Modin, O. & Strömvall, A. 2015. Copper recovery from polluted soils using acidic washing and bioelectrochemical systems. *Metals*, vol. 5, pp. 1328-1348.

Fenelon, A.M. & Breslin, C.B. 2002. The electrochemical synthesis of polypyrrole at a copper electrode: corrosion protection properties. *Electrochim Acta*, vol. 47, pp. 4467-4476.

Feng, J., Johnson, D.C. & Lowery, S.N. 1995. Oxidation of thiosulfate to sulfate at glassy carbon electrodes. *J Electrochem Soc*, vol. 142, pp. 2618-2620.

Ferguson, S.J. & Ingledew, W.J. 2008. Energetic problems faced by micro-organisms growing or surviving on parsimonious energy sources and at acidic pH: I. *Acidithiobacillus ferrooxidans* as a paradigm. *Biochim Biophys Acta Bioenerg*, vol. 1777, pp. 1471-1479.

Fornero, J.J., Rosenbaum, M. & Angenent, L.T. 2010. Electric power generation from municipal, food, and animal wastewaters using microbial fuel cells. *Electroanalysis*, vol. 22, pp. 832-843.

Fosberg, K. 2010. Fuel cell systems provide backup power in telecom applications. *Fuel Cells Bulletin*, vol. 2010, pp. 12-14.

Friedrich, C.G. 1997. Physiology and genetics of sulfur-oxidizing bacteria. *Adv Microb Physiol*, vol. 39, pp. 235-289.

Fu, F. & Wang, Q. 2011. Removal of heavy metal ions from wastewaters: A review. *J Environ Manage*, vol. 92, pp. 407-418.

Fu, Y., Lu, Z., Zai, X. & Wang, J. 2015. Carbon foam anode modified by urea and its higher electrochemical performance in marine benthic microbial fuel cell. *J Ocean Univ China*, vol. 14, pp. 663-668.

Gajaraj, S., Huang, Y., Zheng, P. & Hu, Z. 2017. Methane production improvement and associated methanogenic assemblages in bioelectrochemically assisted anaerobic digestion. *Biochem Eng J*, vol. 117, Part B, pp. 105-112.

Ganigué, R., Puig, S., Batlle-Vilanova, P., Balaguer, M.D. & Colprim, J. 2015. Microbial electrosynthesis of butyrate from carbon dioxide. *Chem Commun*, vol. 51, pp. 3235-3238.

García-Muñoz, J., Fernández, V.M., De Lacey, A.L., Malki, M. & Amils, R. 2011. Electricity generation by microorganisms in the sediment-water interface of an extreme acidic microcosm. *Int Microbiol*, vol. 14, pp. 73-81.

Garrido, J. 2004. Thermodynamics of electrochemical systems. *J Phys Chem B*, vol. 108, pp. 18336-18340.

Ghosh, P., Samanta, A.N. & Ray, S. 2011. Reduction of COD and removal of Zn^{2+} from rayon industry wastewater by combined electro-Fenton treatment and chemical precipitation. *Desalination*, vol. 266, pp. 213-217.

Gil-Carrera, L., Escapa, A., Mehta, P., Santoyo, G., Guiot, S.R., Morán, A. & Tartakovsky, B. 2013. Microbial electrolysis cell scale-up for combined wastewater treatment and hydrogen production. *Bioresour Technol*, vol. 130, pp. 584-591.

Glasstone, S. & Hickling, A. 1932. 336. Studies in electrolytic oxidation. Part I. The electrolytic oxidation of sodium thiosulphate at a platinum anode. *J Chem Soc*, vol. 1, pp. 2345-2356.

Gobla, M.J., Gemperline, C.M. & Stone, L.W. 2015, *Technical Evaluation of the Gold King Mine Incident*, U.S. Department of the Interior - Bureau of Reclamation, 132 p.

Golyshina, O.V., Pivovarova, T.A., Karavaiko, G.I., Kondratéva, T.F., Moore, E.R., Abraham, W.R., Lünsdorf, H., Timmis, K.N., Yakimov, M.M. & Golyshin, P.N. 2000. *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the Archaea. *Int J Syst Evol Microbiol*, vol. 50, pp. 997-1006.

Gönen, Y.E., Ermiş, I. & Ari, M. 2016. Electrical properties of triple-doped bismuth oxide electrolyte for solid oxide fuel cells. *Phase Transitions*, vol. 89, pp. 1129-1136.

Gopi Kiran, M., Pakshirajan, K. & Das, G. 2017. An overview of sulfidogenic biological reactors for the simultaneous treatment of sulfate and heavy metal rich wastewater. *Chem Eng Sci*, vol. 158, pp. 606-620.

Gray, N. 1997. Environmental impact and remediation of acid mine drainage: a management problem. *Environ Geol*, vol. 30, pp. 62-71.

Greenwood, N.N. & Earnshaw, A. 2012, *Chemistry of the Elements*, 2nd edition, Elsevier, India, 1600 p.

Gregory, K.B. & Lovley, D.R. 2005. Remediation and recovery of uranium from contaminated subsurface environments with electrodes. *Environ Sci Technol*, vol. 39, pp. 8943-8947.

Gude, V.G. 2016. Wastewater treatment in microbial fuel cells – an overview. *J Clean Prod*, vol. 122, pp. 287-307.

Guo, Q., Ye, F., Guo, H. & Ma, C.F. 2016. Gas/water and heat management of PEM-based fuel cell and electrolyzer systems for space applications. *Microgravity Sci Technol*, vol. 29, pp. 63-15.

Gupta, C.K. 2003, *Chemical metallurgy: principles and practice*, John Wiley & Sons, Weinheim, Germany, 811 p.

Haghsheno, R., Mohebbi, A., Hashemipour, H. & Sarrafi, A. 2009. Study of kinetic and fixed bed operation of removal of sulfate anions from an industrial wastewater by an anion exchange resin. *J Hazard Mater*, vol. 166, pp. 961-966.

Haile, S.M. 2003. Fuel cell materials and components. *Acta Materialia*, vol. 51, pp. 5981-6000.

Halinen, A., Beecroft, N.J., Määttä, K., Nurmi, P., Laukkanen, K., Kaksonen, A.H., Riekkola-Vanhanen, M. & Puhakka, J.A. 2012. Microbial community dynamics during a demonstration-scale bioheap leaching operation. *Hydrometallurgy*, vol. 125-126, pp. 34-41.

Hallberg, K., Dopson, M. & Lindström, E. 1996. Reduced sulfur compound oxidation by *Thiobacillus caldus*. *J Bacteriol*, vol. 178, pp. 6-11.

Hallberg, K.,B., González-Toril, E. & Johnson, D.B. 2010. *Acidithiobacillus ferrivorans*, sp. nov.; facultatively anaerobic, psychrotolerant iron-, and sulfur-oxidizing acidophiles isolated from metal mine-impacted environments. *Extremophiles*, vol. 14, pp. 9-19.

Han, Y., Yu, C. & Liu, H. 2010. A microbial fuel cell as power supply for implantable medical devices. *Biosens Bioelectron*, vol. 25, pp. 2156-2160.

Harahuc, L., Lizama, H.M. & Suzuki, I. 2000. Selective inhibition of the oxidation of ferrous iron or sulfur in *Thiobacillus ferrooxidans*. *Applied and Environmental Microbiology*, vol. 66, pp. 1031-1037.

Harnisch, F. & Schröder, U. 2010. From MFC to MXC: chemical and biological cathodes and their potential for microbial bioelectrochemical systems. *Chem Soc Rev*, vol. 39, pp. 4433-4448.

He, L., Du, P., Chen, Y., Lu, H., Cheng, X., Chang, B. & Wang, Z. 2017. Advances in microbial fuel cells for wastewater treatment. *Renew Sust Energ Rev*, vol. 71, pp. 388-403.

Heidrich, E.S., Edwards, S.R., Dolfing, J., Cotterill, S.E. & Curtis, T.P. 2014. Performance of a pilot scale microbial electrolysis cell fed on domestic wastewater at ambient temperatures for a 12 month period. *Bioresour Technol*, vol. 173, pp. 87-95.

Hernandez, M.E. & Newman, D.K. 2001. Extracellular electron transfer. *Cell Mol Life Sci*, vol. 58, pp. 1562-1571.

Höök, M. & Tang, X. 2013. Depletion of fossil fuels and anthropogenic climate change—A review. *Energy Policy*, vol. 52, pp. 797-809.

Huang, D., Zhou, S., Chen, Q., Zhao, B., Yuan, Y. & Zhuang, L. 2011. Enhanced anaerobic degradation of organic pollutants in a soil microbial fuel cell. *Chem Eng J*, vol. 172, pp. 647-653.

Huang, L., Cheng, S., Rezaei, F. & Logan, B.E. 2009. Reducing organic loads in wastewater effluents from paper recycling plants using microbial fuel cells. *Environ Technol*, vol. 30, pp. 499-504.

Huisman, J.L., Schouten, G. & Schultz, C. 2006. Biologically produced sulphide for purification of process streams, effluent treatment and recovery of metals in the metal and mining industry. *Hydrometallurgy*, vol. 83, pp. 106-113.

Hunsom, M., Pruksathorn, K., Damronglerd, S., Vergnes, H. & Duverneuil, P. 2005. Electrochemical treatment of heavy metals (Cu^{2+}, Cr^{6+}, Ni^{2+}) from industrial effluent and modeling of copper reduction. *Water Res*, vol. 39, pp. 610-616.

Huyen, P.T., Dang, T.D., Tung, M.T., Huyen, N.T.T., Green, T.A. & Roy, S. 2016. Electrochemical copper recovery from galvanic sludge. *Hydrometallurgy*, vol. 164, pp. 295-303.

Iakovleva, E., Mäkilä, E., Salonen, J., Sitarz, M. & Sillanpää, M. 2015. Industrial products and wastes as adsorbents for sulphate and chloride removal from synthetic alkaline solution and mine process water. *Chem Eng J*, vol. 259, pp. 364-371.

Ieropoulos, I.A., Ledezma, P., Stinchcombe, A., Papaharalabos, G., Melhuish, C. & Greenman, J. 2013. Waste to real energy: the first MFC powered mobile phone. *Phys Chem Chem Phys*, vol. 15, pp. 15312-15316.

Ieropoulos, I.A., Stinchcombe, A., Gajda, I., Forbes, S., Merino-Jimenez, I., Pasternak, G., Sanchez-Herranz, D. & Greenman, J. 2016. Pee power urinal–microbial fuel cell technology field trials in the context of sanitation. *Environ Sci Water Res Technol*, vol. 2, pp. 336-343.

Ieropoulos, I., Greenman, J., Melhuish, C. & Horsfield, I. 2010. EcoBot-III: A Robot with Guts. *Proceedings of the Alife Conference*, pp. 733-740.

Iglesias, N., Romero, R., Mazuelos, A., Montes-Rosua, C. & Carranza, F. 2016. Treatment of tetrathionate effluents by continuous oxidation in a flooded packed-bed bioreactor. *Int J Miner Process*, vol. 155, pp. 91-98.

Isamu, S., Chan, C.W. & Takeuchi, T.L. 1993. Oxidation of Inorganic Sulfur Compounds by *Thiobacilli*. In: Alpers, C.N. & Blowes, D.W. Environmental Geochemistry of Sulfide Oxidation, American Chemical Society, Washington, DC, pp. 60-67.

Iskander, S.M., Brazil, B., Novak, J.T. & He, Z. 2016. Resource recovery from landfill leachate using bioelectrochemical systems: Opportunities, challenges, and perspectives. *Bioresour Technol*, vol. 201, pp. 347-354.

Jacobs, J.A., Lehr, J.H. & Testa, S.M. 2014. Acid drainage and aquatic resources. In: Neuman, D.R., Jennings, S.R. & Jacobs, J.A. Acid mine drainage, rock drainage, and acid sulfate soils: Causes, assessment, prediction, prevention, and remediation, Wiley Online Library, Hoboken, New Jersey, pp. 131-137.

Jadhav, G.S. & Ghangrekar, M.M. 2009. Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. *Bioresour Technol*, vol. 100, pp. 717-723.

Jain, R., Jordan, N., Schild, D., van Hullebusch, E.D., Weiss, S., Franzen, C., Farges, F., Hübner, R. & Lens, P.N.L. 2015. Adsorption of zinc by biogenic elemental selenium nanoparticles. *Chem Eng J*, vol. 260, pp. 855-863.

Jang, J.K., Pham, T.H., Chang, I.S., Kang, K.H., Moon, H., Cho, K.S. & Kim, B.H. 2004. Construction and operation of a novel mediator- and membrane-less microbial fuel cell. *Process Biochemistry*, vol. 39, pp. 1007-1012.

Jannelli, N., Anna Nastro, R., Cigolotti, V., Minutillo, M. & Falcucci, G. 2017. Low pH, high salinity: Too much for microbial fuel cells? *Appl Energy*, vol. 192, pp. 543-550.

Jewell, S. & Kimball, S.M. 2017, *Mineral Commodity Summaries 2017*, U.S. Department of the Interior and U.S. Geological Survey, Reston, Virginia, USA, 206 p.

Ji, Y., Cho, Y.i., Jeon, Y., Lee, C., Park, D. & Shul, Y. 2017. Design of active Pt on TiO_2 based nanofibrous cathode for superior PEMFC performance and durability at high temperature. *Appl Catal B*, vol. 204, pp. 421-429.

Jia, Y., Stenman, D., Mäkitalo, M., Maurice, C. & Öhlander, B. 2013. Use of amended tailings as mine waste cover. *Waste Biomass Valorization*, vol. 4, pp. 709-718.

Johnson, D.B. 2013. Development and application of biotechnologies in the metal mining industry. *Environ Sci Pollut Res*, vol. 20, pp. 7768-7776.

Johnson, D.B. & Hallberg, K.B. 2002. Pitfalls of passive mine water treatment. *Rev Environ Sci Biotechnol*, vol. 1, pp. 335-343.

Johnson, D.B. & Hallberg, K.B. 2008. Carbon, iron and sulfur metabolism in acidophilic micro-organisms. *Adv Microb Physiol*, vol. 54, pp. 201-255.

Johnson, D.B. & du Plessis, C.A. 2015. Biomining in reverse gear: Using bacteria to extract metals from oxidised ores. *Minerals Eng*, vol. 75, pp. 2-5.

Johnson, D.B. & Hallberg, K.B. 2005. Acid mine drainage remediation options: a review. *Sci Total Environ*, vol. 338, pp. 3-14.

Johnson, D.B. & Hallberg, K.B. 2003. The microbiology of acidic mine waters. *Res Microbiol*, vol. 154, pp. 466-473.

Jourdin, L., Grieger, T., Monetti, J., Flexer, V., Freguia, S., Lu, Y., Chen, J., Romano, M., Wallace, G.G. & Keller, J. 2015. High acetic acid production rate obtained by microbial electrosynthesis from carbon dioxide. *Environ Sci Technol*, vol. 49, pp. 13566-13574.

Juang, D., Yang, P., Chou, H. & Chiu, L. 2011. Effects of microbial species, organic loading and substrate degradation rate on the power generation capability of microbial fuel cells. *Biotechnol Lett*, vol. 33, pp. 2147.

Kaksonen, A.H. & Puhakka, J.A. 2007. Sulfate reduction based bioprocesses for the treatment of Acid Mine Drainage and the recovery of metals. *Eng Life Sci*, vol. 7, pp. 541-564.

Kaku, N., Yonezawa, N., Kodama, Y. & Watanabe, K. 2008. Plant/microbe cooperation for electricity generation in a rice paddy field. *Appl Microbiol Biotechnol*, vol. 79, pp. 43-49.

Kalathil, S., Lee, J. & Cho, M.H. 2011. Granular activated carbon based microbial fuel cell for simultaneous decolorization of real dye wastewater and electricity generation. *New Biotechnol*, vol. 29, pp. 32-37.

Kalin, M., Fyson, A. & Wheeler, W.N. 2006. The chemistry of conventional and alternative treatment systems for the neutralization of acid mine drainage. *Sci Total Environ*, vol. 366, pp. 395-408.

Kamorina, G., Tremblay, F., Bussière, B., Smirnova, E. & Thiffault, N. 2015. Bluejoint is an effective bio-barrier species on mine covers. *J Environ Qual*, vol. 44, pp. 1791-1799.

Kefeni, K.K., Msagati, T.M., Maree, J.P. & Mamba, B.B. 2015. Metals and sulphate removal from acid mine drainage in two steps via ferrite sludge and barium sulphate formation. *Minerals Eng*, vol. 81, pp. 79-87.

Kelly, D.P., Chambers, L.A. & Trudinger, P.A. 1969. Cyanolysis and spectrophotometric estimation of trithionate in mixture with thiosulfate and tetrathionate. *Anal Chem*, vol. 41, pp. 898-901.

Kelly, D. & Wood, A. 2000. Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov. *Int J Syst Evol Microbiol*, vol. 50, pp. 511-516.

Khaled, F., Ondel, O. & Allard, B. 2016. Microbial fuel cells as power supply of a low-power temperature sensor. *J Power Sources*, vol. 306, pp. 354-360.

Khalfbadam, H.M., Ginige, M.P., Sarukkalige, R., Kayaalp, A.S. & Cheng, K.Y. 2016. Bioelectrochemical system as an oxidising filter for soluble and particulate organic matter removal from municipal wastewater. *Chem Eng J*, vol. 296, pp. 225-233.

Khazraee Zamanpour, M., Kariminia, H. & Vosoughi, M. 2017. Electricity generation, desalination and microalgae cultivation in a biocathode-microbial desalination cell. *J Environ Chem Eng*, vol. 5, pp. 843-848.

Khorasanipour, M., Moore, F. & Naseh, R. 2011. Lime treatment of mine drainage at the Sarcheshmeh porphyry copper mine, Iran. *Mine Water Environ*, vol. 30, pp. 216-230.

Kieft, T.L. & Spence, S.D. 1988. Osmoregulation in *Thiobacillus ferrooxidans*: Stimulation of iron oxidation by proline and betaine under salt stress. *Curr Microbiol*, vol. 17, pp. 255-258.

Kim, B., Lee, H., Park, J., Sohn, J. & Lee, J. 2008. Oxidative sulfur removal from complex copper concentrate. *Mater Trans*, vol. 49, pp. 1889-1892.

Kim, B.H., Park, H.S., Kim, H.J., Kim, G.T., Chang, I.S., Lee, J. & Phung, N.T. 2004. Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. *Appl Microbiol Biotechnol*, vol. 63, pp. 672-681.

Kim, Y. & Logan, B.E. 2013. Simultaneous removal of organic matter and salt ions from saline wastewater in bioelectrochemical systems. *Desalination*, vol. 308, pp. 115-121.

Kletzin, A. 2008. Oxidation of sulfur and inorganic sulfur compounds in *Acidianus ambivalens*. In: Dahl, C. & Friedrich, C.G. Microbial sulfur metabolism, Springer, Berlin, Heidelberg, pp. 184-201.

Konings, W., Albers, S., Koning, S. & Driessen, A.M. 2002. The cell membrane plays a crucial role in survival of bacteria and archaea in extreme environments. *Antonie Van Leeuwenhoek*, vol. 81, pp. 61-72.

Koschorreck, M. 2007. Natural alkalinity generation in neutral lakes affected by acid mine drainage. *J Environ Qual*, vol. 36, pp. 1163-1171.

Košutić, K., Novak, I., Sipos, L. & Kunst, B. 2004. Removal of sulfates and other inorganics from potable water by nanofiltration membranes of characterized porosity. *Sep Purif Technol*, vol. 37, pp. 177-185.

Kraljić, M., Mandić, Z. & Duić, L. 2003. Inhibition of steel corrosion by polyaniline coatings. *Corros Sci*, vol. 45, pp. 181-198.

Kretzschmar, J., Koch, C., Liebetrau, J., Mertig, M. & Harnisch, F. 2017. Electroactive biofilms as sensor for volatile fatty acids: Cross sensitivity, response dynamics, latency and stability. *Sensors Actuators B: Chem*, vol. 241, pp. 466-472.

Krieg, H.M., Modise, S.J., Keizer, K. & Neomagus, H.W.J.P. 2005. Salt rejection in nanofiltration for single and binary salt mixtures in view of sulphate removal. *Desalination*, vol. 171, pp. 205-215.

Krishna, K.V., Sarkar, O. & Venkata Mohan, S. 2014. Bioelectrochemical treatment of paper and pulp wastewater in comparison with anaerobic process: Integrating chemical coagulation with simultaneous power production. *Bioresour Technol*, vol. 174, pp. 142-151.

Kucera, J., Pakostova, E., Lochman, J., Janiczek, O. & Mandl, M. 2016. Are there multiple mechanisms of anaerobic sulfur oxidation with ferric iron in *Acidithiobacillus ferrooxidans*? *Res Microbiol*, vol. 167, pp. 357-366.

Kulkarni, A. & Giddey, S. 2012. Materials issues and recent developments in molten carbonate fuel cells. *J Solid State Electrochem*, vol. 16, pp. 3123-3146.

Kumar, R., Singh, L. & Zularisam, A.W. 2016. Exoelectrogens: Recent advances in molecular drivers involved in extracellular electron transfer and strategies used to improve it for microbial fuel cell applications. *Renew Sust Energ Rev*, vol. 56, pp. 1322-1336.

Kumar, S.S., Basu, S. & Bishnoi, N.R. 2017a. Effect of cathode environment on bioelectricity generation using a novel consortium in anode side of a microbial fuel cell. *Biochem Eng J*, vol. 121, pp. 17-24.

Kumar, S.S., Malyan, S.K. & Bishnoi, N.R. 2017b. Performance of buffered ferric chloride as terminal electron acceptor in dual chamber microbial fuel cell. *J Environ Chem Eng*, vol. 5, pp. 1238-1243.

Kuyucak, N. 2014. Management of thiosalts in mill effluents by chemical oxidation or buffering in the lime neutralization process. *Minerals Eng*, vol. 60, pp. 41-50.

Lamers, L.P., Tomassen, H.B. & Roelofs, J.G. 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. *Environ Sci Technol*, vol. 32, pp. 199-205.

Landaburu-Aguirre, J., Pongrácz, E., Perämäki, P. & Keiski, R.L. 2010. Micellar-enhanced ultrafiltration for the removal of cadmium and zinc: Use of response surface methodology to improve understanding of process performance and optimisation. *J Hazard Mater*, vol. 180, pp. 524-534.

Langmuir, D. 1997, *Aqueous environmental geochemistry*, Prentice Hall, Upper Saddle River, New Jersey, USA, 600 p.

Lay, C., Kokko, M.E. & Puhakka, J.A. 2015. Power generation in fed-batch and continuous up-flow microbial fuel cell from synthetic wastewater. *Energy*, vol. 91, pp. 235-241.

Le Pape, P., Battaglia-Brunet, F., Parmentier, M., Joulian, C., Gassaud, C., Fernandez-Rojo, L., Guigner, J., Ikogou, M., Stetten, L., Olivi, L., Casiot, C. & Morin, G. 2017. Complete removal of arsenic and zinc from a heavily contaminated acid mine drainage via an indigenous SRB consortium. *J Hazard Mater*, vol. 321, pp. 764-772.

Lee, Y., Kim, T.G. & Cho, K. 2016. Enhancement of electricity production in a mediatorless air–cathode microbial fuel cell using *Klebsiella* sp. IR21. *Bioprocess Biosyst Eng*, vol. 39, pp. 1005-1014.

Lee, C., Ho, K., Lee, D., Su, A. & Chang, J. 2012. Electricity harvest from nitrate/sulfide-containing wastewaters using microbial fuel cell with autotrophic denitrifier, *Pseudomonas* sp. C27. *Int J Hydrogen Energy*, vol. 37, pp. 15827-15832.

Leiva, E., Leiva-Aravena, E. & Vargas, I. 2016. Acid water neutralization using Microbial Fuel Cells: An alternative for Acid Mine Drainage treatment. *Water*, vol. 8, pp. 536.

Li, J., Fu, Q., Liao, Q., Zhu, X., Ye, D. & Tian, X. 2009. Persulfate: A self-activated cathodic electron acceptor for microbial fuel cells. *J Power Sources*, vol. 194, pp. 269-274.

Li, X.M., Cheng, K.Y. & Wong, J.W.C. 2013. Bioelectricity production from food waste leachate using microbial fuel cells: Effect of NaCl and pH. *Bioresour Technol*, vol. 149, pp. 452-458.

Li, Y., Liu, F., Xia, B., Du, Q., Zhang, P., Wang, D., Wang, Z. & Xia, Y. 2010. Removal of copper from aqueous solution by carbon nanotube/calcium alginate composites. *J Hazard Mater*, vol. 177, pp. 876-880.

Li, Z., Zhang, X. & Lei, L. 2008. Electricity production during the treatment of real electroplating wastewater containing Cr^{6+} using microbial fuel cell. *Process Biochem*, vol. 43, pp. 1352-1358.

Liljeqvist, M., Sundkvist, J., Saleh, A. & Dopson, M. 2011. Low temperature removal of inorganic sulfur compounds from mining process waters. *Biotechnol Bioeng*, vol. 108, pp. 1251-1259.

Lim, H., Yun, Y.S., Cho, S.Y., Park, K., Song, M.Y., Jin, H. & Kang, K. 2017. All-carbon-based cathode for a true high-energy-density Li-O_2 battery. *Carbon*, vol. 114, pp. 311-316.

Lim, S.J., Park, W., Kim, T. & Shin, I.H. 2012. Swine wastewater treatment using a unique sequence of ion exchange membranes and bioelectrochemical system. *Bioresour Technol*, vol. 118, pp. 163-169.

Liu, D., Zhang, L., Chen, S., Buisman, C. & ter Heijne, A. 2016. Bioelectrochemical enhancement of methane production in low temperature anaerobic digestion at 10 °C. *Water Res*, vol. 99, pp. 281-287.

Liu, H., Ramnarayanan, R. & Logan, B.E. 2004. Production of electricity during wastewater treatment using a single chamber Microbial Fuel Cell. *Environ Sci Technol*, vol. 38, pp. 2281-2285.

Liu, Z., Liu, J., Zhang, S. & Su, Z. 2008. A novel configuration of microbial fuel cell stack bridged internally through an extra cation exchange membrane. *Biotechnol Lett*, vol. 30, pp. 1017-1023.

Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W. & Rabaey, K. 2006. Microbial Fuel Cells: Methodology and technology. *Environ Sci Technol*, vol. 40, pp. 5181-5192.

Logan, B. 2010. Scaling up microbial fuel cells and other bioelectrochemical systems. *Appl Microbiol Biotechnol*, vol. 85, pp. 1665-1671.

Lopez-Archilla, A.I., Marin, I. & Amils, R. 2001. Microbial community composition and ecology of an acidic aquatic environment: The Tinto river, Spain. *Microb Ecol*, vol. 41, pp. 20-35.

Lottermoser, B.G. 2010, *Mine wastes: characterization, treatment and environmental impacts*, 2nd edition, Springer-Verlag, Berlin, 400 p.

Lovley, D.R., Giovannoni, S.J., White, D.C., Champine, J.E., Phillips, E.J.P., Gorby, Y.A. & Goodwin, S. 1993a. *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. *Arch Microbiol*, vol. 159, pp. 336-344.

Lovley, D.R., Roden, E.E., Phillips, E.J.P. & Woodward, J.C. 1993b. Enzymatic iron and uranium reduction by sulfate-reducing bacteria. *Mar Geol*, vol. 113, pp. 41-53.

Lu, Y., Gao, Q., Xu, L., Zhao, Y. & Epstein, I.R. 2010. Oxygen-sulfur species distribution and kinetic analysis in the hydrogen peroxide-thiosulfate system. *Inorg Chem*, vol. 49, pp. 6026-6034.

Luo, H., Qin, B., Liu, G., Zhang, R., Tang, Y. & Hou, Y. 2014. Selective recovery of Cu^{2+} and Ni^{2+} from wastewater using bioelectrochemical system. *Front Environ Sci Eng*, vol. 9, pp. 522-527.

Luo, H., Xu, P., Roane, T.M., Jenkins, P.E. & Ren, Z. 2012. Microbial desalination cells for improved performance in wastewater treatment, electricity production, and desalination. *Bioresour Technol*, vol. 105, pp. 60-66.

Luo, H., Teng, W., Liu, G., Zhang, R. & Lu, Y. 2017. Sulfate reduction and microbial community of autotrophic biocathode in response to acidity. *Process Biochem*, vol. 54, pp. 120-127.

Ma, W., Luo, C., Lin, J., Chou, S., Chen, P., Syu, M., Kuo, S. & Lai, S. 2016. A Portable low-power acquisition system with a urease bioelectrochemical sensor for potentiometric detection of urea concentrations. *Sensors*, vol. 16, pp. 474-488.

Machemer, S.D. & Wildeman, T.R. 1992. Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland. *J Contam Hydrol*, vol. 9, pp. 115-131.

Mäkinen, A.E., Nissilä, M.E. & Puhakka, J.A. 2012. Dark fermentative hydrogen production from xylose by a hot spring enrichment culture. *Int J Hydrogen Energ*, vol. 37, pp. 12234-12240.

Malki, M., De Lacey, A.L., Rodríguez, N., Amils, R. & Fernandez, V.M. 2008. Preferential use of an anode as an electron acceptor by an acidophilic bacterium in the presence of oxygen. *Appl Environ Microbiol*, vol. 74, pp. 4472-4476.

Mangold, S., Valdes, J., Holmes, D.S. & Dopson, M. 2011. Sulfur metabolism in the extreme acidophile *Acidithiobacillus caldus*. *Front Microbiol*, vol. 2, pp. 7-24.

Maree, J., De Beer, M., Strydom, W., Christie, A. & Waanders, F. 2004. Neutralizing coal mine effluent with limestone to decrease metals and sulphate concentrations. *Mine Water Environ*, vol. 23, pp. 81-86.

Marone, A., Carmona-Martínez, A.A., Sire, Y., Meudec, E., Steyer, J.P., Bernet, N. & Trably, E. 2016. Bioelectrochemical treatment of table olive brine processing wastewater for biogas production and phenolic compounds removal. *Water Res*, vol. 100, pp. 316-325.

Martinucci, E., Pizza, F., Perrino, D., Colombo, A., Trasatti, S., Barnabei, A.L., Liberale, A. & Cristiani, P. 2015. Energy balance and microbial fuel cells experimentation at wastewater treatment plant Milano-Nosedo. *Int J Hydrogen Energy*, vol. 40, pp. 14683-14689.

McCollom, T. & Amend, J. 2005. A thermodynamic assessment of energy requirements for biomass synthesis by chemolithoautotrophic micro-organisms in oxic and anoxic environments. *Geobiology*, vol. 3, pp. 135-144.

McLean, G.F., Niet, T., Prince-Richard, S. & Djilali, N. 2002. An assessment of alkaline fuel cell technology. *Int J Hydrogen Energy*, vol. 27, pp. 507-526.

Mecucci, A. & Scott, K. 2002. Leaching and electrochemical recovery of copper, lead and tin from scrap printed circuit boards. *J Chem Technol Biotechnol*, vol. 77, pp. 449-457.

Mehta, V. & Cooper, J.S. 2003. Review and analysis of PEM fuel cell design and manufacturing. *J Power Sources*, vol. 114, pp. 32-53.

Meulenberg, R., Scheer, E.J., Pronk, J.T., Hazeu, W., Bos, P. & Gijs Kuenen, J. 1993. Metabolism of tetrathionate in *Thiobacillus acidophilus*. *FEMS Microbiol Lett*, vol. 112, pp. 167-172.

Miller, A., Figueroa, L. & Wildeman, T. 2011. Zinc and nickel removal in simulated limestone treatment of mining influenced water. *Appl Geochem*, vol. 26, pp. 125-132.

Min, B., Kim, J., Oh, S., Regan, J.M. & Logan, B.E. 2005. Electricity generation from swine wastewater using microbial fuel cells. *Water Res*, vol. 39, pp. 4961-4968.

Minh, N.Q. 2004. Solid oxide fuel cell technology—features and applications. *Solid State Ionics*, vol. 174, pp. 271-277.

Miskan, M., Ismail, M., Ghasemi, M., Md Jahim, J., Nordin, D. & Abu Bakar, M.H. 2016. Characterization of membrane biofouling and its effect on the performance of microbial fuel cell. *Int J Hydrogen Energy*, vol. 41, pp. 543-552.

Mo, Y., Liang, P., Huang, X., Wang, H. & Cao, X. 2009. Enhancing the stability of power generation of single-chamber microbial fuel cells using an anion exchange membrane. *J Chem Technol Biotechnol*, vol. 84, pp. 1767-1772.

Modin, O., Wang, X., Wu, X., Rauch, S. & Fedje, K.K. 2012. Bioelectrochemical recovery of Cu, Pb, Cd, and Zn from dilute solutions. *J Hazard Mater*, vol. 235-236, pp. 291-297.

Mohan, S.V. & Chandrasekhar, K. 2011. Self-induced bio-potential and graphite electron accepting conditions enhances petroleum sludge degradation in bio-electrochemical system with simultaneous power generation. *Bioresour Technol*, vol. 102, pp. 9532-9541.

Motos, P.R., ter Heijne, A., van der Weijden, R., Saakes, M., Buisman, C.J. & Sleutels, T.H. 2015. High rate copper and energy recovery in microbial fuel cells. *Front Microbiol*, vol. 6, pp. 1-8.

Muyzer, G., Hottenträger, S., Teske, A. & Waver, C. 1996. Denaturing gradien gel electrophoresis of PCR-amplified 16S rDNA - a new molecular approach to analyze the genetic diversity of mixed microbial communities. In: Akkermans, D.L., van Elsas, J.D. & De Brujin, F. Molecular microbial ecology manual, Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 1-23.

Muyzer, G. & Stams, A.J. 2008. The ecology and biotechnology of sulphate-reducing bacteria. *Nat Rev Microbiol*, vol. 6, pp. 441-454.

Muyzer, G., de Waal, E.C. & Uitterlinden, A.G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol*, vol. 59, pp. 695-700.

Nagendranatha Reddy, C. & Venkata Mohan, S. 2016. Integrated bio-electrogenic process for bioelectricity production and cathodic nutrient recovery from azo dye wastewater. *Renewable Energy*, vol. 98, pp. 188-196.

Nam, J., Kim, H., Lim, K., Shin, H. & Logan, B.E. 2010. Variation of power generation at different buffer types and conductivities in single chamber microbial fuel cells. *Biosens Bioelectron*, vol. 25, pp. 1155-1159.

Nam, J., Tokash, J.C. & Logan, B.E. 2011. Comparison of microbial electrolysis cells operated with added voltage or by setting the anode potential. *Int J Hydrogen Energy*, vol. 36, pp. 10550-10556.

Nancharaiah, Y.V., Venkata Mohan, S. & Lens, P.N.L. 2016. Recent advances in nutrient removal and recovery in biological and bioelectrochemical systems. *Bioresour Technol*, vol. 215, pp. 173-185.

Nancharaiah, Y.V., Venkata Mohan, S. & Lens, P.N.L. 2015. Metals removal and recovery in bioelectrochemical systems: A review. *Bioresour Technol*, vol. 195, pp. 102-114.

Ňancucheo, I. & Johnson, D.B. 2012. Selective removal of transition metals from acidic mine waters by novel consortia of acidophilic sulfidogenic bacteria. *Microb Biotechnol*, vol. 5, pp. 34-44.

Nechache, A., Cassir, M. & Ringuedé, A. 2014. Solid oxide electrolysis cell analysis by means of electrochemical impedance spectroscopy: A review. *J Power Sources*, vol. 258, pp. 164-181.

Neubauer, S.C., Emerson, D. & Megonigal, J.P. 2002. Life at the energetic edge: kinetics of circumneutral iron oxidation by lithotrophic iron-oxidizing bacteria isolated from the wetland-plant rhizosphere. *Appl Environ Microbiol*, vol. 68, pp. 3988-3995.

Nevatalo, L.M., Bijmans, M.F.M., Lens, P.N.L., Kaksonen, A.H. & Puhakka, J.A. 2010. The effect of sub-optimal temperature on specific sulfidogenic activity of mesophilic SRB in an H_2-fed membrane bioreactor. *Process Biochem*, vol. 45, pp. 363-368.

Newman, J. & Thomas-Alyea, K.E. 2004, *Electrochemical systems*, 3rd edition, John Wiley & Sons, Hoboken, New Jersey, 672 p.

Ni, G., Christel, S., Roman, P., Wong, Z.L., Bijmans, M.F.M. & Dopson, M. 2016. Electricity generation from an inorganic sulfur compound containing mining wastewater by acidophilic microorganisms. *Res Microbiol*, vol. 167, pp. 568-575.

Nie, M., Gu, C., Zhong, K. & Fang, Y. 2012. Catalytic oxidation removal of sulfur dioxide by ozone in the presence of metallic ions. *Energy Fuels*, vol. 26, pp. 5590-5595.

Nies, D.H. 1999. Microbial heavy-metal resistance. *Appl Microbiol Biotechnol*, vol. 51, pp. 730-750.

Nordstrom, D.K., Alpers, C.N., Ptacek, C.J. & Blowes, D.W. 2000. Negative pH and extremely acidic mine waters from Iron Mountain, California. *Environ Sci Technol*, vol. 34, pp. 254-258.

Ntagia, E., Rodenas, P., ter Heijne, A., Buisman, C.J.N. & Sleutels, T.H.J.A. 2016. Hydrogen as electron donor for copper removal in bioelectrochemical systems. *Int J Hydrogen Energy*, vol. 41, pp. 5758-5764.

Oh, S.E. & Logan, B.E. 2005. Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. *Water Res*, vol. 39, pp. 4673-4682.

Oh, S. & Logan, B. 2006. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. *Appl Microbiol Biotechnol*, vol. 70, pp. 162-169.

Oliot, M., Galier, S., Roux de Balmann, H. & Bergel, A. 2016. Ion transport in microbial fuel cells: Key roles, theory and critical review. *Appl Energy*, vol. 183, pp. 1682-1704.

Oller, I., Malato, S. & Sánchez-Pérez, J.A. 2011. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination—A review. *Sci Total Environ*, vol. 409, pp. 4141-4166.

Ölmez, T. 2009. The optimization of Cr(VI) reduction and removal by electrocoagulation using response surface methodology. *J Hazard Mater*, vol. 162, pp. 1371-1378.

Ontario 2007, *Effluent monitoring and effluent limits - metal mining sector under*, Environmental Protection Act, R.S.O., Ontario, Canada, 16 p.

Ormerod, R.M. 2003. Solid oxide fuel cells. *Chem Soc Rev*, vol. 32, pp. 17-28.

Osorio, H., Mangold, S., Denis, Y., Ñancucheo, I., Esparza, M., Johnson, D.B., Bonnefoy, V., Dopson, M. & Holmes, D.S. 2013. Anaerobic sulfur metabolism coupled to dissimilatory iron reduction in the extremophile *Acidithiobacillus ferrooxidans*. *Appl Environ Microbiol*, vol. 79, pp. 2172-2181.

OTK 2014, *Ympäristöonnettomuus Talvivaaran kaivoksella marraskuussa 2012*, Safety Investigation Authority, Finland, 93 p.

Pandey, P., Shinde, V.N., Deopurkar, R.L., Kale, S.P., Patil, S.A. & Pant, D. 2016. Recent advances in the use of different substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery. *Appl Energy*, vol. 168, pp. 706-723.

Pandit, S., Sengupta, A., Kale, S. & Das, D. 2011. Performance of electron acceptors in catholyte of a two-chambered microbial fuel cell using anion exchange membrane. *Bioresour Technol*, vol. 102, pp. 2736-2744.

Pannell, T.C., Goud, R.K., Schell, D.J. & Borole, A.P. 2016. Effect of fed-batch vs. continuous mode of operation on microbial fuel cell performance treating biorefinery wastewater. *Biochem Eng J*, vol. 116, pp. 85-94.

Pant, D., Van Bogaert, G., Diels, L. & Vanbroekhoven, K. 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresour Technol*, vol. 101, pp. 1533-1543.

Papirio, S., Villa-Gomez, D., Esposito, G., Pirozzi, F. & Lens, P. 2013. Acid mine drainage treatment in fluidized-bed bioreactors by sulfate-reducing bacteria: a critical review. *Crit Rev Environ Sci Technol*, vol. 43, pp. 2545-2580.

Park, S., Yoo, J., Ji, S., Yang, J. & Baek, K. 2015. Selective recovery of dissolved Fe, Al, Cu, and Zn in acid mine drainage based on modeling to predict precipitation pH. *Environ Sci Pollut Res*, vol. 22, pp. 3013-3022.

Park, S., Vohs, J.M. & Gorte, R.J. 2000. Direct oxidation of hydrocarbons in a solid-oxide fuel cell. *Nature*, vol. 404, pp. 265-267.

Park, S., Shin, S., Yang, J., Ji, S. & Baek, K. 2015. Selective recovery of dissolved metals from mine drainage using electrochemical reactions. *Electrochim Acta*, vol. 181, pp. 248-254.

Paul Chen, J. & Lim, L.L. 2005. Recovery of precious metals by an electrochemical deposition method. *Chemosphere*, vol. 60, pp. 1384-1392.

Peng, X., Zhao, S., Omasta, T.J., Roller, J.M. & Mustain, W.E. 2017. Activity and durability of Pt-Ni nanocage electocatalysts in proton exchange membrane fuel cells. *Appl Catal B*, vol. 203, pp. 927-935.

Penteado, E.D., Fernandez-Marchante, C.M., Zaiat, M., Gonzalez, E.R. & Rodrigo, M.A. 2017. On the effects of ferricyanide as cathodic mediator on the performance of Microbial Fuel Cells. *Electrocatalysis*, vol. 8, pp. 59-66.

Peppas, A., Komnitsas, K. & Halikia, I. 2000. Use of organic covers for acid mine drainage control. *Minerals Eng*, vol. 13, pp. 563-574.

Pikaar, I., Likosova, E.M., Freguia, S., Keller, J., Rabaey, K. & Yuan, Z. 2015. Electrochemical abatement of hydrogen sulfide from waste streams. *Crit Rev Environ Sci Technol*, vol. 45, pp. 1555-1578.

Pikaar, I., Rozendal, R.A., Yuan, Z., Keller, J. & Rabaey, K. 2011. Electrochemical sulfide oxidation from domestic wastewater using mixed metal-coated titanium electrodes. *Water Res*, vol. 45, pp. 5381-5388.

Pillai, K.C., Chung, S.J. & Moon, I. 2008. Studies on electrochemical recovery of silver from simulated waste water from Ag(II)/Ag(I) based mediated electrochemical oxidation process. *Chemosphere*, vol. 73, pp. 1505-1511.

Piotrowska-Seget, Z., Cycoń, M. & Kozdrój, J. 2005. Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. *Appl Soil Ecol*, vol. 28, pp. 237-246.

Polat, H. & Erdogan, D. 2007. Heavy metal removal from waste waters by ion flotation. *J Hazard Mater*, vol. 148, pp. 267-273.

Popov, A.L., Michie, I.S., Kim, J.R., Dinsdale, R.M., Guwy, A.J., Esteves, S.R. & Premier, G.C. 2016. Enrichment strategy for enhanced bioelectrochemical hydrogen production and the prevention of methanogenesis. *Int J Hydrogen Energy*, vol. 41, pp. 4120-4131.

Pourmahmoud, N., Sadeghifar, H. & Torkavannejad, A. 2017. A novel, state-of-the-art tubular architecture for polymer electrolyte membrane fuel cells: Performance enhancement, size and cost reduction. *Int J Heat Mass Transfer*, vol. 108, Part A, pp. 577-584.

Poynton, S.D., Kizewski, J.P., Slade, R.C.T. & Varcoe, J.R. 2010. Novel electrolyte membranes and non-Pt catalysts for low temperature fuel cells. *Solid State Ionics*, vol. 181, pp. 219-222.

Pronk, J.T., de Bruyn, J.C., Bos, P. & Kuenen, J.G. 1992. Anaerobic growth of *Thiobacillus ferrooxidans*. *Appl Environ Microbiol*, vol. 58, pp. 2227-2230.

Pronk, J.T., Meulenberg, R., Hazeu, W., Bos, P. & Kuenen, J.G. 1990. Oxidation of reduced inorganic sulphur compounds by acidophilic thiobacilli. *FEMS Microbiol Lett*, vol. 75, pp. 293-306.

Qiao, Y., Qiao, Y., Zou, L., Ma, C. & Liu, J. 2015. Real-time monitoring of phenazines excretion in *Pseudomonas aeruginosa* microbial fuel cell anode using cavity microelectrodes. *Bioresour Technol*, vol. 198, pp. 1-6.

Quatrini, R., Appia-Ayme, C., Denis, Y., Jedlicki, E., Holmes, D. & Bonnefoy, V. 2009. Extending the models for iron and sulfur oxidation in the extreme acidophile *Acidithiobacillus ferrooxidans*. *BMC Genomics*, vol. 10, pp. 394-413.

Rabaey, K., Boon, N., Siciliano, S.D., Verhaege, M. & Verstraete, W. 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. *Appl Environ Microbiol*, vol. 70, pp. 5373-5382.

Rabaey, K., Lissens, G., Siciliano, S.D. & Verstraete, W. 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol Lett*, vol. 25, pp. 1531-1535.

Rabaey, K., Van de Sompel, K., Maignien, L., Boon, N., Aelterman, P., Clauwaert, P., De Schamphelaire, L., Pham, H.T., Vermeulen, J., Verhaege, M., Lens, P. & Verstraete, W. 2006. Microbial Fuel Cells for sulfide removal. *Environ Sci Technol*, vol. 40, pp. 5218-5224.

Rabaey, K. & Verstraete, W. 2005. Microbial fuel cells: novel biotechnology for energy generation. *Trends Biotechnol*, vol. 23, pp. 291-298.

Ragauskas, R., Dikčius, A., Vengris, T. & Padarauskas, A. 2003. Degradation of color photographic waste fix solution by anodic oxidation. *J Chem Technol Biotechnol*, vol. 78, pp. 81-86.

Rago, L., Baeza, J.A. & Guisasola, A. 2017. Bioelectrochemical hydrogen production with cheese whey as sole substrate. *J Chem Technol Biotechnol*, vol. 92, pp. 173-179.

Rawlings, D.E. & Johnson, D.B. 2002. Ecology and biodiversity of extremely acidophilic microorganisms. In: Gerday, C. & Glansdorff, N. Extremophiles, Eolls Publishers Co. Ltd., Oxford, United Kingdom, pp. 89-118.

Reddy, L.K. 2001, *Principles of engineering metallurgy*, New Age International, New Delhi, India, 249 p.

Reimers, C., Girguis, P., Stecher, H., Tender, L., Ryckelynck, N. & Whaling, P. 2006. Microbial fuel cell energy from an ocean cold seep. *Geobiology*, vol. 4, pp. 123-136.

Ren, L., Ahn, Y. & Logan, B.E. 2014. A two-stage Microbial Fuel Cell and Anaerobic Fluidized Bed Membrane Bioreactor (MFC-AFMBR) system for effective domestic wastewater treatment. *Environ Sci Technol*, vol. 48, pp. 4199-4206.

Reyes, I., Villarroel, M., Diez, M.C. & Navia, R. 2009. Using lignimerin (a recovered organic material from Kraft cellulose mill wastewater) as sorbent for Cu and Zn retention from aqueous solutions. *Bioresour Technol*, vol. 100, pp. 4676-4682.

Rimboud, M., Bergel, A. & Erable, B. 2016. Multiple electron transfer systems in oxygen reducing biocathodes revealed by different conditions of aeration/agitation. *Bioelectrochemistry*, vol. 110, pp. 46-51.

Ríos, C.A., Williams, C.D. & Roberts, C.L. 2008. Removal of heavy metals from acid mine drainage (AMD) using coal fly ash, natural clinker and synthetic zeolites. *J Hazard Mater*, vol. 156, pp. 23-35.

Rivera, I., Bakonyi, P. & Buitrón, G. 2017. H_2 production in membraneless bioelectrochemical cells with optimized architecture: The effect of cathode surface area and electrode distance. *Chemosphere*, vol. 171, pp. 379-385.

Rogich, D.G. & Matos, G.R. 2008, *The global flows of metals and minerals*, US Geological Survey, Reston, Virginia, 11 p.

Rohwerder, T., Gehrke, T., Kinzler, K. & Sand, W. 2003. Bioleaching review part A: Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. *Appl Microbiol Biotechnol*, vol. 63, pp. 239-248.

Rohwerder, T. & Sand, W. 2007. Oxidation of inorganic sulfur compounds in acidophilic prokaryotes. *Eng Life Sci*, vol. 7, pp. 301-309.

Rosenbaum, M.A. & Franks, A.E. 2014. Microbial catalysis in bioelectrochemical technologies: status quo, challenges and perspectives. *Appl Microbiol Biotechnol*, vol. 98, pp. 509-518.

Rosenbaum, M., Aulenta, F., Villano, M. & Angenent, L.T. 2011. Cathodes as electron donors for microbial metabolism: Which extracellular electron transfer mechanisms are involved? *Bioresour Technol*, vol. 102, pp. 324-333.

Rowe, O.F., Sánchez-España, J., Hallberg, K.B. & Johnson, D.B. 2007. Microbial communities and geochemical dynamics in an extremely acidic, metal-rich stream at an abandoned sulfide mine (Huelva, Spain) underpinned by two functional primary production systems. *Environ Microbiol*, vol. 9, pp. 1761-1771.

Roxby, D.N., Tran, N., Yu, P. & Nguyen, H.T. 2016. Effect of growth solution, membrane size and array connection on microbial fuel cell power supply for medical devices. *IEEE 38th Annual International Conference of the Engineering in Medicine and Biology Society (EMBC)*, pp. 1946-1949.

Rozendal, R.A., Hamelers, H.V.M., Rabaey, K., Keller, J. & Buisman, C.J.N. 2008. Towards practical implementation of bioelectrochemical wastewater treatment. *Trends Biotechnol*, vol. 26, pp. 450-459.

Rózsenberszki, T., Koók, L., Bakonyi, P., Nemestóthy, N., Logroño, W., Pérez, M., Urquizo, G., Recalde, C., Kurdi, R. & Sarkady, A. 2017. Municipal waste liquor treatment via bioelectrochemical and fermentation ($H_2 + CH_4$) processes: Assessment of various technological sequences. *Chemosphere*, vol. 171, pp. 692-701.

Sääf, S., Sundkvist, J. & Dopson, M. 2009. Psychrotrophic, mesophilic and moderate thermophilic removal of reduced inorganic sulfur compounds from process waters. *8th International Conference on Acid Rock Drainage (ICARD) and Securing the Future*, pp. 1-10.

Sakar, H., Balcik Canbolat, C., Karagunduz, A. & Keskinler, B. 2015. Sulfate removal from nanofiltration concentrate of alkaloid wastewater by electrodialysis. *Desalination Water Treat*, vol. 57, pp. 21003-21014.

Sánchez-Andrea, I., Stams, A.J., Weijma, J., Contreras, P.G., Dijkman, H., Rozendal, R.A. & Johnson, D.B. 2016. A case in support of implementing innovative bio-processes in the metal mining industry. *FEMS Microbiol Lett*, vol. 363, pp. 1-4.

Santoro, C., Serov, A., Stariha, L., Kodali, M., Gordon, J., Babanova, S., Bretschger, O., Artyushkova, K. & Atanassov, P. 2016. Iron based catalysts from novel low-cost organic precursors for enhanced oxygen reduction reaction in neutral media microbial fuel cells. *Energy Environ Sci*, vol. 9, pp. 2346-2353.

Sarswat, P.K. & Free, M.L. 2016. Frequency and atomic mass based selective electrochemical recovery of rare earth metals and isotopes. *Electrochim Acta*, vol. 219, pp. 435-446.

Sawasdee, V. & Pisutpaisal, N. 2016. Simultaneous pollution treatment and electricity generation of tannery wastewater in air-cathode single chamber MFC. *Int J Hydrogen Energy*, vol. 41, pp. 15632-15637.

Schippers, A. & Sand, W. 1999. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. *Appl Environ Microbiol*, vol. 65, pp. 319-321.

Schippers, A. 2007. Microorganisms involved in bioleaching and nucleic acid-based molecular methods for their identification and quantification. In: Donati, E. & Sand, W. Microbial Processing of Metal Sulfides, Springer, Netherlands, pp. 3-33.

Schleper, C., Puehler, G., Holz, I., Gambacorta, A., Janekovic, D., Santarius, U., Klenk, H.P. & Zillig, W. 1995. *Picrophilus* gen. nov., fam. nov.: a novel aerobic, heterotrophic, thermoacidophilic genus and family comprising archaea capable of growth around pH 0. *J Bacteriol*, vol. 177, pp. 7050-7059.

Schmidt, A., Haferburg, G., Schmidt, A., Lischke, U., Merten, D., Ghergel, F., Büchel, G. & Kothe, E. 2009. Heavy metal resistance to the extreme: *Streptomyces* strains from a former uranium mining area. *Chem Erde Geochem*, vol. 69, pp. 35-44.

Schrader, P.S., Reimers, C.E., Girguis, P., Delaney, J., Doolan, C., Wolf, M. & Green, D. 2016. Independent benthic Microbial Fuel Cells powering sensors and acoustic communications with the MARS underwater observatory. *J Atmos Ocean Technol*, vol. 33, pp. 607-617.

Schrenk, M.O., Edwards, K.J., Goodman, R.M., Hamers, R.J. & Banfield, J.F. 1998. Distribution of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*: implications for generation of acid mine drainage. *Science*, vol. 279, pp. 1519-1522.

Schröder, U. 2007. Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. *Phys Chem Chem Phys*, vol. 9, pp. 2619-2629.

Schwartz, M., Vigneault, B. & McGeer, J. 2006, *Evaluating the potential for thiosalts to contribute to toxicity in mine effluents*, Natural Resources, Canada, 101 p.

Sengupta, M. 1993, *Environmental impacts of mining: Monitoring, restoration, and control*, Lewis Publishers, Unites States, 512 p.

Shabalala, A.N., Ekolu, S.O., Diop, S. & Solomon, F. 2017. Pervious concrete reactive barrier for removal of heavy metals from acid mine drainage − column study. *J Hazard Mater*, vol. 323, Part B, pp. 641-653.

Shafiee, S. & Topal, E. 2009. When will fossil fuel reserves be diminished? *Energy Policy*, vol. 37, pp. 181-189.

Shams, M., Qasemi, M., Afsharnia, M. & Hossein Mahvi, A. 2016. Sulphate removal from aqueous solutions by granular ferric hydroxide. *Desalination Water Treat*, vol. 57, pp. 23800-23807.

Sievering, H., Boatmani, J., Gorman, E., Kimf, Y., Anderson, L., Ennis, G., Luriaj, M. & Pandis, S. 1992. Removal of sulphur from the marine boundary layer by ozone. *Nature*, vol. 360, pp. 571-573.

Simons, A. & Bauer, C. 2015. A life-cycle perspective on automotive fuel cells. *Appl Energy*, vol. 157, pp. 884-896.

Song, F., Fu, Y., Gao, Y., Li, J., Qiao, J., Zhou, X. & Liu, Y. 2015. Novel alkaline anion-exchange membranes based on chitosan/ethenylmethylimidazoliumchloride polymer with ethenylpyrrolidone composites for low temperature polymer electrolyte fuel cells. *Electrochim Acta*, vol. 177, pp. 137-144.

Sophia, A.C. & Bhalambaal, V.M. 2015. Utilization of coconut shell carbon in the anode compartment of microbial desalination cell (MDC) for enhanced desalination and bio-electricity production. *J Environ Chem Eng*, vol. 3, pp. 2768-2776.

Srikanth, S., Kumar, M., Singh, D., Singh, M.P. & Das, B.P. 2016. Electro-biocatalytic treatment of petroleum refinery wastewater using microbial fuel cell (MFC) in continuous mode operation. *Bioresour Technol*, vol. 221, pp. 70-77.

Stonehart, P. 1992. Development of alloy electrocatalysts for phosphoric acid fuel cells (PAFC). *J Appl Electrochem*, vol. 22, pp. 995-1001.

Strik, D.P., Snel, J.F. & Buisman, C.J. 2008. Green electricity production with living plants and bacteria in a fuel cell. *Int J Energy Res*, vol. 32, pp. 870-876.

Stumm, W. & Morgan, J.J. 1995, *Aquatic chemistry: chemical equilibria and rates in natural waters*, 3rd edition, John Wiley & Sons, USA, 1040 p.

Suzuki, I., Lee, D., Mackay, B., Harahuc, L. & Oh, J.K. 1999. Effect of various ions, pH, and osmotic pressure on oxidation of elemental sulfur by *Thiobacillus thiooxidans*. *Appl Environ Microbiol*, vol. 65, pp. 5163-5168.

Takano, B., Koshida, M., Fujiwara, Y., Sugimori, K. & Takayanagi, S. 1997. Influence of sulfur-oxidizing bacteria on the budget of sulfate in Yugama crater lake, Kusatsu-Shirane volcano, Japan. *Biogeochemistry*, vol. 38, pp. 227-253.

Tao, H., Liang, M., Li, W., Zhang, L., Ni, J. & Wu, W. 2011a. Removal of copper from aqueous solution by electrodeposition in cathode chamber of microbial fuel cell. *J Hazard Mater*, vol. 189, pp. 186-192.

Tao, H., Zhang, L., Gao, Z. & Wu, W. 2011b. Copper reduction in a pilot-scale membrane-free bioelectrochemical reactor. *Bioresour Technol*, vol. 102, pp. 10334-10339.

Tender, L.M., Gray, S.A., Groveman, E., Lowy, D.A., Kauffman, P., Melhado, J., Tyce, R.C., Flynn, D., Petrecca, R. & Dobarro, J. 2008. The first demonstration of a microbial fuel cell as a viable power supply: Powering a meteorological buoy. *J Power Sources*, vol. 179, pp. 571-575.

Teng, W., Liu, G., Luo, H., Zhang, R. & Xiang, Y. 2016. Simultaneous sulfate and zinc removal from acid wastewater using an acidophilic and autotrophic biocathode. *J Hazard Mater*, vol. 304, pp. 159-165.

ter Heijne, A., Hamelers, H.V.M. & Buisman, C.J.N. 2007. Microbial fuel cell operation with continuous biological ferrous iron oxidation of the catholyte. *Environ Sci Technol*, vol. 41, pp. 4130-4134.

ter Heijne, A., Hamelers, H.V.M., de Wilde, V., Rozendal, R.A. & Buisman, C.J.N. 2006. A bipolar membrane combined with ferric iron reduction as an efficient cathode system in Microbial Fuel Cells. *Environ Sci Technol*, vol. 40, pp. 5200-5205.

ter Heijne, A., Hamelers, H.V.M., Saakes, M. & Buisman, C.J.N. 2008. Performance of non-porous graphite and titanium-based anodes in microbial fuel cells. *Electrochim Acta*, vol. 53, pp. 5697-5703.

ter Heijne, A., Liu, F., van der Weijden, R., Weijma, J., Buisman, C.J.N. & Hamelers, H.V.M. 2010. Copper recovery combined with electricity production in a Microbial Fuel Cell. *Environ Sci Technol*, vol. 44, pp. 4376-4381.

Certucha-Barragán, M.T., Acedo-Félix, E., Monge-Amaya, O., Leobardo Valenzuela-Garcíab, J., Javier Almendariz-Tapia, F. & Lilia Leal-Cruz, A. 2009. Copper bioaccumulation in an upflow anaerobic sludge blanket reactor. *Chem Speciation Bioavailability*, vol. 21, pp. 161-164.

Tolonen, E., Hu, T., Rämö, J. & Lassi, U. 2016. The removal of sulphate from mine water by precipitation as ettringite and the utilisation of the precipitate as a sorbent for arsenate removal. *J Environ Manage*, vol. 181, pp. 856-862.

Tozsin, G. 2016. Inhibition of acid mine drainage and immobilization of heavy metals from copper flotation tailings using a marble cutting waste. *Int J Miner Metall Mater*, vol. 23, pp. 1-6.

Tuovinen, O.H., Niemelä, S. & Gyllenberg, H. 1971. Tolerance of *Thiobacillus ferrooxidans* to some metals. *Antonie Van Leeuwenhoek*, vol. 37, pp. 489-496.

Veith, A., Urich, T., Seyfarth, K., Protze, J., Frazão, C. & Kletzin, A. 2011. Substrate pathways and mechanisms of inhibition in the sulfur oxygenase reductase of *Acidianus ambivalens*. *Front Microbiol*, vol. 2, pp. 25-36.

Venkateswaran, K., Moser, D.P., Dollhopf, M.E., Lies, D.P., Saffarini, D.A., MacGregor, B.J., Ringelberg, D.B., White, D.C., Nishijima, M., Sano, H., Burghardt, J., Stackebrandt, E. & Nealson, K.H. 1999. Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. *Int J Syst Bacteriol*, vol. 49, pp. 705-724.

Vigneault, B., Campbell, P.G.C., Tessier, A. & De Vitre, R. 2001. Geochemical changes in sulfidic mine tailings stored under a shallow water cover. *Water Res*, vol. 35, pp. 1066-1076.

Virdis, B., Rabaey, K., Yuan, Z. & Keller, J. 2008. Microbial fuel cells for simultaneous carbon and nitrogen removal. *Water Res*, vol. 42, pp. 3013-3024.

Wagner, R.C., Call, D.F. & Logan, B.E. 2010. Optimal set anode potentials vary in bioelectrochemical systems. *Environ Sci Technol*, vol. 44, pp. 6036-6041.

Walton, K.C. & Johnson, D.B. 1992. Microbiological and chemical characteristics of an acidic stream draining a disused copper mine. *Environ Pollut*, vol. 76, pp. 169-175.

Wang, H., Wang, G., Ling, Y., Qian, F., Song, Y., Lu, X., Chen, S., Tong, Y. & Li, Y. 2013. High power density microbial fuel cell with flexible 3D graphene–nickel foam as anode. *Nanoscale*, vol. 5, pp. 10283-10290.

Wang, J.W., Bejan, D. & Bunce, N.J. 2003. Removal of arsenic from synthetic acid mine drainage by electrochemical pH adjustment and coprecipitation with iron hydroxide. *Environ Sci Technol*, vol. 37, pp. 4500-4506.

Wang, J. & Musameh, M. 2003. Carbon nanotube/teflon composite electrochemical sensors and biosensors. *Anal Chem*, vol. 75, pp. 2075-2079.

Wang, Y. & Shen, H. 1995. Bacterial reduction of hexavalent chromium. *J Ind Microbiol*, vol. 14, pp. 159-163.

Wang, Z., Lim, B., Lu, H., Fan, J. & Choi, C. 2010a. Cathodic reduction of Cu^{2+} and electric power generation using a microbial fuel cell. *Bull Korean Chem Soc*, vol. 31, pp. 2025-2030.

Wang, A., Sun, D., Ren, N., Liu, C., Liu, W., Logan, B.E. & Wu, W. 2010b. A rapid selection strategy for an anodophilic consortium for microbial fuel cells. *Bioresour Technol*, vol. 101, pp. 5733-5735.

Wang, B., Zhu, Y., Zhou, T. & Xie, K. 2016. Synthesis and properties of chitosan membranes modified by reactive cationic dyes as a novel alkaline exchange membrane for low temperature fuel cells. *Int J Hydrogen Energy*, vol. 41, pp. 18166-18177.

Wang, X., Feng, Y., Ren, N., Wang, H., Lee, H., Li, N. & Zhao, Q. 2009. Accelerated start-up of two-chambered microbial fuel cells: Effect of anodic positive poised potential. *Electrochim Acta*, vol. 54, pp. 1109-1114.

Weber, K.A., Achenbach, L.A. & Coates, J.D. 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nat Rev Microbiol*, vol. 4, pp. 752-764.

Weiss, R.F. 1970. The solubility of nitrogen, oxygen and argon in water and seawater. *Deep Sea Res Oceanogr Abstr*, vol. 17, pp. 721-735.

WHO 2011, *Guidelines for drinking-water quality*, World Health Organization, Malta, 564 p.

Wilkinson, S. 2000. “Gastrobots”—Benefits and challenges of Microbial Fuel Cells in food powered robot applications. *Autonomous Robots*, vol. 9, pp. 99-111.

Williams, J.P. 2012. Global trends and tribulations in mining regulation. *J Energy Nat Resour Law*, vol. 30, pp. 391-422.

Winter, M. & Brodd, R.J. 2004. What are batteries, fuel cells, and supercapacitors? *Chem Rev*, vol. 104, pp. 4245-4270.

Wong, M.T., Cheng, D., Wang, R. & Hsing, I. 2016. Modifying the endogenous electron fluxes of *Rhodobacter sphaeroides* 2.4.1 for improved electricity generation. *Enzyme Microb Technol*, vol. 86, pp. 45-51.

Wu, S., Li, H., Zhou, X., Liang, P., Zhang, X., Jiang, Y. & Huang, X. 2016. A novel pilot-scale stacked microbial fuel cell for efficient electricity generation and wastewater treatment. *Water Res*, vol. 98, pp. 396-403.

Xenidis, A., Mylona, E. & Paspaliaris, I. 2002. Potential use of lignite fly ash for the control of acid generation from sulphidic wastes. *Waste Manage*, vol. 22, pp. 631-641.

Xiang, Y., Liu, G., Zhang, R., Lu, Y. & Luo, H. 2017. High-efficient acetate production from carbon dioxide using a bioanode microbial electrosynthesis system with bipolar membrane. *Bioresour Technol*, vol. 233, pp. 227-235.

Xu, L. & Armstrong, F.A. 2013. Optimizing the power of enzyme-based membrane-less hydrogen fuel cells for hydrogen-rich H_2–air mixtures. *Energy Environ Sci*, vol. 6, pp. 2166-2171.

Xu, J., Sheng, G., Luo, H., Li, W., Wang, L. & Yu, H. 2012. Fouling of proton exchange membrane (PEM) deteriorates the performance of microbial fuel cell. *Water Res*, vol. 46, pp. 1817-1824.

Xu, Y., Schoonen, M.A.A., Nordstrom, D.K., Cunningham, K.M. & Ball, J.W. 2000. Sulfur geochemistry of hydrothermal waters in Yellowstone National Park, Wyoming, USA. II. Formation and decomposition of thiosulfate and polythionate in Cinder Pool. *J Volcanol Geotherm Res*, vol. 97, pp. 407-423.

Xu, Y., Schoonen, M.A.A., Nordstrom, D.K., Cunningham, K.M. & Ball, J.W. 1998. Sulfur geochemistry of hydrothermal waters in Yellowstone National Park: I. the origin of thiosulfate in hot spring waters. *Geochim Cosmochim Acta*, vol. 62, pp. 3729-3743.

Yamashita, T., Ookawa, N., Ishida, M., Kanamori, H., Sasaki, H., Katayose, Y. & Yokoyama, H. 2016. A novel open-type biosensor for the in-situ monitoring of biochemical oxygen demand in an aerobic environment. *Sci Rep*, vol. 6, pp. 1-9.

Yang, S., Jia, B. & Liu, H. 2009. Effects of the Pt loading side and cathode-biofilm on the performance of a membrane-less and single-chamber microbial fuel cell. *Bioresour Technol*, vol. 100, pp. 1197-1202.

Yanilkin, V.V., Nastapova, N.V., Nasretdinova, G.R., Fazleeva, R.R. & Osin, Y.N. 2016. Molecular oxygen as a mediator in the electrosynthesis of gold nanoparticles in DMF. *Electrochem Commun*, vol. 69, pp. 36-40.

Yarzábal, A., Brasseur, G. & Bonnefoy, V. 2002. Cytochromes c of *Acidithiobacillus ferrooxidans*. *FEMS Microbiol Lett*, vol. 209, pp. 189-195.

Yeruva, D.K., Velvizhi, G. & Mohan, S.V. 2016. Coupling of aerobic/anoxic and bioelectrogenic processes for treatment of pharmaceutical wastewater associated with bioelectricity generation. *Renewable Energy*, vol. 98, pp. 171-177.

Yi, H., Nevin, K.P., Kim, B., Franks, A.E., Klimes, A., Tender, L.M. & Lovley, D.R. 2009. Selection of a variant of *Geobacter sulfurreducens* with enhanced capacity for current production in microbial fuel cells. *Biosens Bioelectron*, vol. 24, pp. 3498-3503.

You, S., Zhao, Q., Zhang, J., Jiang, J. & Zhao, S. 2006. A microbial fuel cell using permanganate as the cathodic electron acceptor. *J Power Sources*, vol. 162, pp. 1409-1415.

Young, P.L. 1997. The longevity of minewater pollution: a basis for decision-making. *Sci Total Environ*, vol. 194, pp. 457-466.

Yu, E.H., Wang, X., Krewer, U., Li, L. & Scott, K. 2012. Direct oxidation alkaline fuel cells: from materials to systems. *Energy Environ Sci*, vol. 5, pp. 5668-5680.

Yu, C., Liang, Z., Das, A. & Hu, Z. 2011. Nitrogen removal from wastewater using membrane aerated microbial fuel cell techniques. *Water Res*, vol. 45, pp. 1157-1164.

Yu, X. & Pickup, P.G. 2008. Recent advances in direct formic acid fuel cells (DFAFC). *J Power Sources*, vol. 182, pp. 124-132.

Yuan, X.Z., Meng, Y.T., Zeng, G.M., Fang, Y.Y. & Shi, J.G. 2008. Evaluation of tea-derived biosurfactant on removing heavy metal ions from dilute wastewater by ion flotation. *Colloids Surf Physicochem Eng Aspects*, vol. 317, pp. 256-261.

Yuan, X., Wang, H., Colin Sun, J. & Zhang, J. 2007. AC impedance technique in PEM fuel cell diagnosis—A review. *Int J Hydrogen Energy*, vol. 32, pp. 4365-4380.

Yue, Y., Wu, F., Choi, H., Shaver, C., Sanguino, M., Staffel, J. & Liang, H. 2017. Electrochemical synthesis and hydrophilicity of micro-pored aluminum foil. *Surf Coat Technol*, vol. 309, pp. 523-530.

Zaharieva, I., Chernev, P., Risch, M., Klingan, K., Kohlhoff, M., Fischer, A. & Dau, H. 2012. Electrosynthesis, functional, and structural characterization of a water-oxidizing manganese oxide. *Energy Environ Sci*, vol. 5, pp. 7081-7089.

Zawodzinski, T., Minteer, S. & Brisard, G. 2006. Physical and analytical electrochemistry: The fundamental core of electrochemistry. *Electrochem Soc Interface*, vol. 15, pp. 62-65.

Zhang, B., Feng, C., Ni, J., Zhang, J. & Huang, W. 2012. Simultaneous reduction of vanadium (V) and chromium (VI) with enhanced energy recovery based on microbial fuel cell technology. *J Power Sources*, vol. 204, pp. 34-39.

Zhang, T., Tu, Z., Lu, G., Duan, X., Yi, X., Guo, C. & Dang, Z. 2017. Removal of heavy metals from acid mine drainage using chicken eggshells in column mode. *J Environ Manage*, vol. 188, pp. 1-8.

Zhang, Y. & Angelidaki, I. 2015. Recovery of ammonia and sulfate from waste streams and bioenergy production via bipolar bioelectrodialysis. *Water Res*, vol. 85, pp. 177-184.

Zhao, F., Rahunen, N., Varcoe, J.R., Chandra, A., Avignone-Rossa, C., Thumser, A.E. & Slade, R.C.T. 2008. Activated carbon cloth as anode for sulfate removal in a Microbial Fuel Cell. *Environ Sci Technol*, vol. 42, pp. 4971-4976.

Zhao, F., Rahunen, N., Varcoe, J.R., Roberts, A.J., Avignone-Rossa, C., Thumser, A.E. & Slade, R.C.T. 2009. Factors affecting the performance of microbial fuel cells for sulfur pollutants removal. *Biosens Bioelectron*, vol. 24, pp. 1931-1936.

Zhen, G., Kobayashi, T., Lu, X. & Xu, K. 2015. Understanding methane bioelectrosynthesis from carbon dioxide in a two-chamber microbial electrolysis cells (MECs) containing a carbon biocathode. *Bioresour Technol*, vol. 186, pp. 141-148.

Zou, G., Papirio, S., Ylinen, A., Di Capua, F., Lakaniemi, A. & Puhakka, J. 2014. Fluidized-bed denitrification for mine waters. Part II: effects of Ni and Co. *Biodegradation*, vol. 25, pp. 417-423.

Zou, G. 2015, Biological nitrogen removal from acidic, heavy metal containing waters, Tampere University of Technology*. Doctoral thesis.*

Zou, G., Papirio, S., Lai, X., Wu, Z., Zou, L., Puhakka, J.A. & Ruan, R. 2015. Column leaching of low-grade sulfide ore from Zijinshan copper mine. *Int J Miner Process*, vol. 139, pp. 11-16.

Tampereen teknillinen yliopisto
PL 527
33101 Tampere

Tampere University of Technology
P.O.B. 527
FI-33101 Tampere, Finland

ISBN 978-952-15-3984-8
ISSN 1459-2045