Investigation of algal-microbial biofilms for acid mine drainage treatment

Sanaz Orandi

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PANEL OF SUPERVISORS

Principal Supervisor

A/Prof. David M. Lewis

Ph.D. (University of Adelaide)

School of Chemical Engineering

The University of Adelaide

Email: david.lewis@adelaide.edu.au

Phone: +61 8 83135503

Fax: +61 8 83134373

Cooperative Supervisors

Dr. Navid R. Moheimani

Ph.D. (Murdoch University)

Algae R&D Center

School of Biological Sciences & Biotechnology

Murdoch University

Email: n.moheimani@murdoch.edu.au

Phone: +61 8 93602682

Fax: +61 8 9360 6651

A/Prof. Peter J. Ashman

Ph.D. (University of Sydney)

School of Chemical Engineering

The University of Adelaide

Email: peter.ashman@adelaide.edu.au

Phone: +61 8 83135072

Fax: +61 8 83134373

Declaration for a thesis that contains publications

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where application, any partner institution responsible for the joint-award of this degree.

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THESIS BY PUBLICATION

This doctoral thesis is submitted as a portfolio of peer-reviewed publications according to the "PhD *Rules & Specifications for Thesis*" of the University of Adelaide. The journals in which these papers were published or accepted are closely related to the research field of this work. The citation information is listed and the journals ranked in the order of impact factor in reference to their scientific significance.

Journal Title	Impact Factor
Journal of Industrial Microbiology and Biotechnology	2.46
Journal of Environmental Science and Pollution Research	2.87
Journal of Applied Microbiology and Biotechnology	3.425
Journal of Environment International	5.2
Applied and Environmental Microbiology	3.8

The thesis is composed of the following papers.

1- Orandi, S., Lewis, D. M., Moheimani, N. R. (2012) Biofilm establishment and heavy metal removal capacity of an indigenous mining algal-microbial consortium in a photo-rotating biological contactor, Journal of Industrial Microbiology and Biotechnology. DOI 10.1007/s10295-012-1142-9

2- Orandi, S., Lewis, D. M. (2012) Synthesising acid mine drainage to maintain and exploit indigenous mining micro-algae and microbial assemblies for biotreatment investigations, Journal of Environmental Science and Pollution Research. DOI 10.1007/s11356-012-1006-x

3- Orandi, S., Lewis, D. M. (2012) Biosorption of heavy metals in a photo-rotating biological contactor - a batch process study, Journal of Applied Microbiology and Biotechnology, DOI 10.1007/s00253-012-4316-5

4- Orandi, S., Lewis, D.M. (2012) Metal/metalloids removal from acid mine drainage by indigenous mine biofilm in a photo-rotating biological contactor - a continous process study, Journal of Environment International, under review (Reference number: Ref. No.: ENVINT-D-12-00791

5- Orandi, S., Amini, J., Moheimani, N.R., Lewis, D. M. (2012) Biodiversity of an indigenous algal-microbial biofilm in acid mine drainage, from field to laboratory investigations. Journal of Applied and Environmental Microbiology (submitted, 31 August, 2012).

The following outcomes were resulted from this thesis included:

1- Orandi, S., Lewis, D. M., Eslami, A, Mohebbi, A (2012) A novel approach to exploit indigenous mining algal-microbes in a rotating biological contactor for the removal of heavy metals from acid mine drainage, Annual Conference International Mine Water Association (IMWA) 2012, Bunbury, Western Australia. (Oral presentation, Received best student presentation award, see full paper in Appendix A)

2- Orandi, S., Cheang, C., Moheimani, N., Lewis, D. M. (2012) Extremophilic micro-algae as biosorbent candidates for phyto-remediation of mining wastewaters, 8th Asian-Pacific Conference on Algal Biotechnology, Adelaide, Australia. (Poster, Received best poster presentation award)

3- Orandi, S., Lewis, D. M. (2011) *Heavy metal removal with indigenous micro-algae isolated from mining wastewater*, Bioprocessing Network annual conference, Adelaide, South Australia. (Poster, Received Young Investigator Award).

4- Orandi, S., Lewis, D. M., Moheimani, N. (2011) A novel approach to develop and maintain an algal biofilm derived from an indigenous mining microbial consortium in a

rotating biological contactor, 4th Congress of the International Society for Applied Phycology, Halifax, Canada. (Poster)

Additionally, this work was presented in the 3 min thesis (3MT) completion held at the University of Adelaide and won the runner up prize.

If I want to summarise my thesis in one sentence,

I would say:

The God-given environment not only serves us with treasures to sell, but also offer balms to put on remaining scars

Sanaz Orandi

March 2013

توانا بود هر که دانا بود

به نام خداوند خورشید و ماه — که دل را به نامش خرد داد راه

جز او را مدان کردگار سپهر — فروزنده ی ماه و ناهید و مهر

به دانش گرای و بدو شو بلند — چو خواهی از بد نیابی گزند

ز دانش دریغ نیازی بجوی — وگر چند سختیت آید به روی

ز نادان بنالد دل سنگ و کوه — از ایرا ندارد برکس شکوه

توانا بود هر که دانا بود — ز دانش دل پیر برنا بود

حکیم ابوالقاسم فردوسی

Acknowledgement

A few years ago, when I was asked about my most ambitious wish, I would respond “getting my PhD, building upon my Master’s findings”. However, to achieve this goal I had to go through the toughest time in my life.

I would like to acknowledge all the people who had a significant role in my achievements as without their help I would not have been able to get to this stage.

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Cassandra Cheng, Ali Mirsepahi. A big thank to Amir Ahmad Forghani for his great analytical and editorial assistance.

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Thank to my son, Benyamin. Since his birth, he has been patient with a busy mom who was committed to her studies. I am sure he will be forever relieved that my PhD is completed!

Thank to my parents for encouragement to choose studying and learning as the most joyful and satisfying reward in life. Thanks for looking after my Husband during my three year absence. I apologise to you for having to continuously defend my decision to undertake my PhD and raise my Son in Australia. Mom, you always wished all your children to become a Doctor; sorry it took so long as I was your first child but last to become a Doctor ☺

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Sanaz Orandi

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ABSTRACT

Mining wastewaters, typically acid mine drainage (AMD) have been listed as one of the most severe types of contaminated surface waters containing heavy metals (e.g. Cu, Zn, Cr, Pb) and toxic metalloids (e.g. As, Cd, Sb). AMD convey these elements into water bodies and threaten aquatic life and human health, consequently. AMD is required to be treated before discharge to the environment, particularly in arid areas with scarce water resources. To date, neutralisation and evaporation have been commonly used at mine sites to decrease the elemental contents of contaminated surface waters. However, these techniques are expensive or ineffective for removing recalcitrant elements e.g. Mn; and produce large volumes of contaminated sludge. In recent decades, the exploitation of microorganisms for treating wastewaters, particularly municipal wastewaters, has significantly improved water treatment technologies, and are referred to as biotreatment/bioremediation. High efficiency, cost effectiveness and sustainability are associated with biotreatment. The application of biotreatment has been investigated for AMD treatment and documented extensively. However, the results are limited and not comprehensive enough for an applicable system to be deployed in mine sites. The main objective of my PhD research was to establish and develop an effective and sustainable AMD biotreatment system for removing metals/metalloids, applicable for mine sites. The indigenous mine microorganisms were used as biosorbents in a biotreatment system, obtained from AMD resources at Sarcheshmeh copper mine, Iran. The microbial sample contained mainly filamentous and unicellular green micro-algae, *Klebsomidium* sp. and *Chlamydomonsae* sp.; bacteria, *Acidithiobacillus ferroxidance*, *Leptospirillum ferroxidans* and *Pseudomonas* sp.; and fungi, *Aspergillus* sp. and *Penicillium* sp. The AMD, from which the indigenous microbial consortium was collected, was analysed to quantify its cation and anion (including nutrients PO_4^{-3} and NO_3^-) contents. The analysis data was used to synthesise a multi-ion AMD composed of 25 components (cations and anions at concentrations 0.005-100 mg/L), high sulphate (>1000 mg/L) and low pH (~3). The indigenous microbial assembly was maintained in synthetic AMD (Syn-AMD) *in vitro*. For the biotreatment investigations, a laboratory-scale photo-rotating biological contactor (PRBC) was designed and used to immobilise the microbial consortium as an algal-microbial biofilm. The PRBC was initially operated in batch mode, using Syn-AMD and indigenous microbes as PRBC solution and inoculum, respectively.

An algal-microbial biofilm (60g dry weight) was successfully grown on the discs' surfaces in the PRBC after 12 weeks. The PRBC was then operated at both batch and continous modes to investigate the efficiency of the system for removing different elements from the Syn-AMD. Batch systems were conducted in 7-day periods under pH 3 and 5. The batch results showed that the algal-microbial biofilm system was able to reduce the concentration of major elements from 10 to 60 % at pH 3 in the order of Na > Cu > Ca > Mg > Mn > Ni > Zn, whereas higher results (40-70 %) were recorded for these elements at pH 5 in the order of Cu > Mn > Mg > Ca > Ni > Zn > Na. The removal trend for each element contained maximum and minimum removal values that occurred during the experiment. The removal efficiency of the system for trace elements varied extensively between 3 and 80 % under both pH conditions.

The efficiency of the system was also evaluated in continous condition, by introducing Syn-AMD (pH~3) into the PRBC at the flow rate of 10 ml/min and hydraulic retention time of 24 h. The operation of PRBC within a 28-day period showed similar removal efficiency (10-60%) compared with the batch operation, for most of elements. The chemical composition of treated water was examined daily within 28 days and the results revealed absorption (7 days) and desorption periods occurring alternatively. The increase and decrease of pH by 0.5 and 0.2 were recorded at the same time of absorption and desorption periods, which was attributed to mobilisation and immobilisation mechanisms occurring in the algal-microbial biofilm.

The system was operated for a further 10 weeks continuously and the results demonstrated the average weekly removal for major elements from 20 to 50% in the order of Cu> Mg> Ni> Na> Mn> Ca> Zn whereas for trace elements varied broadly between 10 and 80 %. Scanning electron microscopy (SEM) analysis illustrated the accumulation of heavy metals in/on the biofilm. Biofilm analysis also revealed the presence of different elements up to 10% of the dried biomass.

The results demonstrated the effectiveness and sustainability of indigenous environmental friendly algal-microbial biofilm to be exploited for removing most of elements from AMD. The results offer a potentially sustainable approach for the primary treatment of AMD at mine sites.

CONTENTS