A novel approach to exploit indigenous mining algal-microbes in a photo-rotating biological contactor for heavy metal removal from acid mine drainage

Sanaz Orandi[1], David M. Lewis[1], Afsar Eslami [2], Ali Mohebbi[3]

[1] *Micro-algae Engineering Research Group, School of Chemical Engineering, University of Adelaide, North Terrace, South Australia, 5005, sanaz.orandi@adelaide.edu.au*
[2] *Water & Environment Research, R&D centre, National Iranian Copper Industries Company, Rafsanjan, Iran, eslami@nicico.com*
[3] *International Centre for Science, High Technology & Environmental Sciences, Mahan, Kerman, Iran, amohebbi2002@yahoo.com*

Abstract

Extremophilic acidic microbial biofilms, that thrive in acidic mine drainages (AMD), are well known as natural metal-resistant biosorbents for removing heavy metals through active and passive cellular functions. To benefit from the resistant and cleansing nature of these microorganisms and to develop a system applicable to mining sites, an indigenous AMD biofilm was immobilized in a laboratory scale photo-rotating biological contactor (PRBC) to investigate its heavy metal removal potential from AMD. The microbial consortium used for biofilm development contained filamentous green micro-algae, bacteria, fungi and yeasts, collected from AMD at the Sarcheshmeh copper mine in Iran. The PRBC was operated with synthesised AMD representing the multi-ion and acidic composition of the wastewater (composing 18 elements with a pH of 3.5±0.3) from which the microbial consortium was collected. Light, nutrient dosing and rotational speeds were optimised to encourage biofilm development. Electron microscopy was used to monitor the development of the algal-microbial biofilm on the PRBC discs over a 60 day batch mode operation. The PRBC was then run continuously with a 24 h hydraulic residence time (HRT) over a ten week period. The weekly average of water analysis results demonstrated the ability for the algal-microbial biofilm to remove 15-50% of various metals in the order of Cu> Ni> Mn> Zn> Sb> Cr> Co> Al. These results clearly indicate the significant potential for indigenous AMD biofilm to be exploited within a PRBC for AMD treatment.

Keywords: acid mine drainage, micro-algae, biofilm, photo-rotating biological contactor, biotreatment

Introduction

Extremophilic indigenous microorganisms, including micro-algae, fungi and bacteria, have adapted to survive under the hostile conditions of AMD (Lottermoser 2010). These microbes thrive in extensive biofilms along acidic drainages, attached to substrates (Aguilera et al. 2008). Coexistence and synergistic relationships among the multi-species microbial community of the biofilm protects them from environmental stresses and enables them to survive under the extreme conditions found in AMD (Aguilera et al. 2010). The significant role of these microorganisms in AMD is their natural cleansing ability for removing heavy metals through functions including bioaccumulation, biosorption and biomineralisation (Das et al. 2009). A judicious consortium of live metal resistant cells can ensure better removal through the previously mentioned functions and also continuous metabolic metal uptake after physical adsorption (Malik 2004).

A number of studies reported the metal removal capacity of live microorganisms in a batch or continuous systems using different types of reactors included rotary systems such as Rotating Biological Contactor (RBC) (Bayramoglu et al. 2006; Travieso et al. 2002; Costley and Wallis 2001a,b). RBC facilitates the immobilisation of microorganisms as attached biofilm on the support media (Rodgers and Zhan 2003). High interfacial areas generated by the discs, simple and practical design and operation, low land occupancy, low energy consumption, low cost of operation and maintenance, and high reaction rate and treatment efficiency are the principal advantages of using RBC (Rodgers and Zhan 2003). RBCs have been evaluated in the mining industry for the removal of contaminants such as ferrous iron, cyanide and its various species, oxalate, and selenium from mining effluents (Kapoor et al. 2004). However, the literature on the application of RBC for biotreatment of heavy metals is still limited and more work is required before this opportunity can be fully exploited by the mining industry. Additionally, limited research has been carried out on the utilization of indigenous AMD microorganisms in RBC to remove heavy metals and treat AMD.

The reported study is a novel approach to exploit indigenous AMD microorganisms as an effective and environmentally friendly biosorbent for removing a variety of elements, included heavy metals, from a multi-ion synthetic AMD (Syn-AMD). To immobilise the algal-microbial consortium, a laboratory scale photo-rotating biological contactor (PRBC) was used to develop an algal-microbial biofilm. This paper presents the efficiency of indigenous AMD biofilm in a PRBC for primary or secondary treatment of AMD at mine sites under acidic conditions.

Materials and methods

Photo-rotating biological contactor

A single stage laboratory scale PRBC was constructed as explained previously in Orandi et al. (2012a). Sixteen Poly Vinyl Chloride (PVC) discs, 0.25 m diameter each, were roughened with 5-grit grade sandpaper and mounted on a horizontal shaft with a spacing of 20 mm. The shaft was mounted in a Plexiglas® trough so that the discs were 40% submerged. The shaft was coupled to a motor and operated with a speed controller which was used to maintain the rotational speed at 2 rpm during biofilm establishment and at 5 rpm during the treatment period. A feed tank was connected to the trough to introduce a Syn-AMD through an inlet. A volume of 15 L was required for 40% immersion of the discs in the trough. An outlet was placed on the trough to maintain this working volume during continuous operation with a 24 h hydraulic residence time (HRT) over a ten week period. The Syn-AMD was supplied to the system at 10 mL/min using a MasterFlex peristaltic pump. Eight tubular cool white fluorescent lamps were installed inside a semi-cylindrical cover on top of the PRBC, giving 756 μmol m^{-2} s^{-1} illumination required for algal biofilm growth and maintenance.

Synthetic AMD and elemental analysis

The PRBC was operated with a Syn-AMD, composed of nutrients (NO_3 and PO_4) and 18 elements including Cu, Mn, Zn; and Sb, Ni, Cr, Co, Al, at high (20-80 mg/L) and lower concentrations (0.005-2.5 mg/L), respectively. The calculated value of each parameter was based on the analysis of AMD from the Sarcheshmeh copper mine, as described in Orandi et al. (2012b). During continuous operation, elements were analysed in triplicate at weekly intervals using inductively coupled plasma-mass spectroscopy (ICP-MS) to evaluate the potential of the system for metal removal from the Syn-AMD over an extended period. Sampling, preservation and analytical methods were carried out according to standard methods (Orandi et al. 2012b).

Microbial biosorbents

An indigenous AMD microbial consortium (1.5 L with 0.1 g/L dry weight), collected from AMD at Sarcheshmeh copper mine (Orandi et al. 2007), was used as microbial biosorbent in this study. The microbial consortium dominated by filamentous green micro-algae and containing bacteria and fungi developed an algal-microbial biofilm in the PRBC over a 60 day batch operation. The continuous operation and biotreatment investigations commenced when the averaged dried weight of biofilm in the PRBC increased up to 60 g. Biofilm structure was monitored by scanning electron microscopy (SEM) (Orandi et al. 2012a).

Results and discussion

After the development of an indigenous mining algal-microbial biofilm, the PRBC was operated continuously for ten weeks to evaluate the efficiency of the system for removing metals (Cu, Mn, Zn, Ni, Sb, Cr, Co, Al) from a multi-ion Syn-AMD. The weekly removal percentage for each element is presented in Fig. 1.

The results showed the maximum removal for Cu and Ni varied between 40-50% of the initial concentrations in the influent, over the experiment. Among the elements with higher initial concentrations, Mn and Zn were removed at the lower ranges, between 30-40% and 25-35%, respectively. The mechanism of metal biosorption is a complicated process and depends on a variety of factors including the ambient/environmental conditions such as pH, the initial concentration of metal ions and biomass, and competition of ions for binding sites (Das et al. 2009).

The pH of the Syn-AMD in PRBC was maintained at 3.5 ± 0.3 during continuous operation which is typically the average pH of AMD. pH is reported to be a critical variable that influences biosorption (Costley and Wallis 2001b). Under low pH condition, due to the presence of hydrogen ions which compete successfully with other cations for binding sites, many potential metal binding sites are occupied, resulting in poor metal biosorption results (Das et al. 2009). The initial metal concentration and ion competition for binding sites also affect the metal selectivity of the biofilm from a multi-ion solution (Bayramoglu et al. 2006). In this study Cu and Ni uptake did not appear to be affected by the presence of other metal ions.

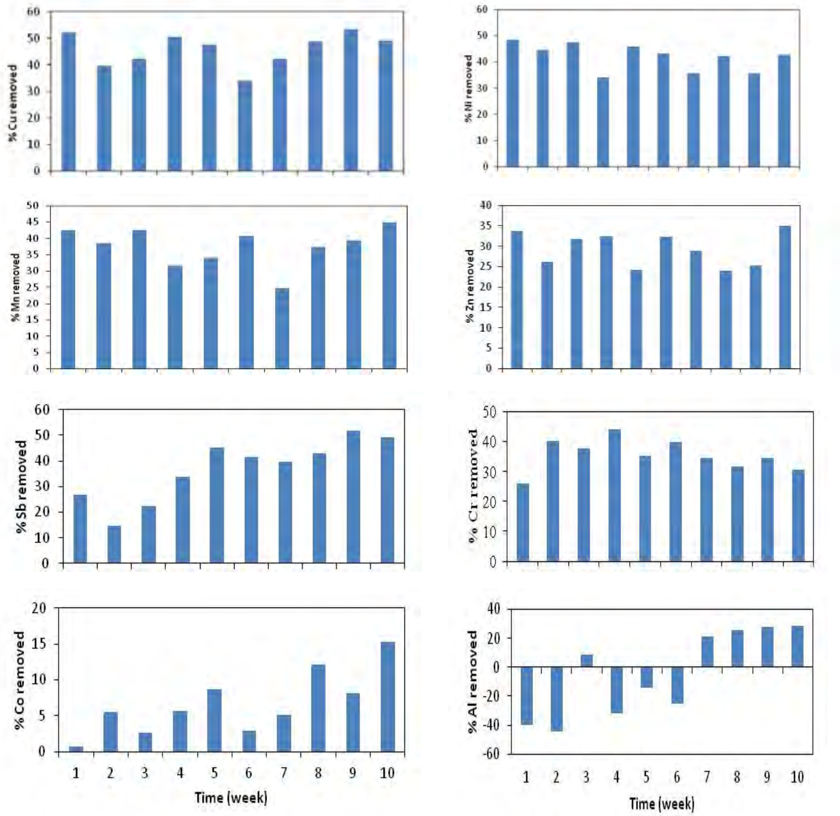

***Figure 1** Weekly removal efficiency of the PRBC during continuous operation for the elements: Cu, Ni, Mn, Zn, Sb, Cr, Co, and Al.*

These metals out-competed other ions for available adsorption sites. Many studies reported that Cu was preferentially adsorbed in the presence of other ions (Costley and Wallis 2001a,b). In the reported study, the indigenous algal-microbial consortium thrived in the AMDs that contained more than 80 mg/L of copper. The establishment of this copper-selective population could favour multi-layered adsorption of Cu and the removal efficiency was not adversely affected by the presence of other ions. The removal percent of Mn was similar to Cu and Ni. The higher initial concentrations of Cu and Mn in the influent were attributed to a higher removal rate. Ni and Mn are considered recalcitrant pollutants as they cannot be removed by chemical treatment in traditional systems and many microorganisms have a relatively low binding capacity for them (Malik, 2004). The 45-50% removal for Ni and Mn in the current system under acidic conditions highlights the effectiveness of using an indigenous algal microbial biofilm in the PRBC. The removal of Zn was less than other metals with higher initial concentration, namely Cu, Ni and Mn. A similar study showed Zn removal can be adversely affected by the presence of competing ions (Costely and Wallis 2001a,b).

The metal biosorption process by living cells is a two-step process. In the dominant first step, metal ions are adsorbed to the surface of cells by interactions between metals and functional groups displayed on the surface of cells (Das et al. 2008). The second step is related to the active uptake of metals coinciding with the metabolic function of live cells. The relatively stable and high removal of Cu, Ni and Mn in the reported study could be due to the well-known metabolic requirements of these elements for micro-algae. Cu, Zn, Mn, Co and Ni are of the essential micronutrients for growth and metabolism of all aquatic micro-algae (Malik 2004).

Among the metals with lower initial concentrations in the influent, the removal efficiency of Sb was low during the first few weeks. However, removal increased up to 50% after the first four weeks and remained between 40-50% during the final 6 weeks of the experiment. This was attributed to multi-layer metal adsorption on cell walls. The removal efficiency for Cr also increased from 25% to 45% over the first four weeks. However, the removal capacity varied between 30-40% over the later weeks. Co and Al were not readily assimilated. Co removal gradually increased up to 15% from its initial concentration. Al initially accumulated in the system (as shown by a negative percentage). However, a consistent removal pattern of aluminium (~25%) was observed during the later weeks in the experiment. The lower removal of these ions could be due to the lower initial concentrations and biofilm selectivity for these elements in the synthesized AMD. Additionally, some of elements such as Al and Co do not have a significant role in metabolic function and the toxic effect of these elements can adversely affect cellular function (Costley and Wallis 2001b). Consequently cells may exhibit resistance mechanisms to enable them to withstand high concentrations of such metals, and hence exhibit low sorption capacities.

These results illustrate the selectivity of the algal-microbial biofilm to remove certain elements, where the double charged ions were removed in the order of Cu>Ni>Mn>Zn and the trace metals were removed in the order of Sb>Cr>Co>Al. This study demonstrates the potential for a PRBC inoculated with indigenous AMD microorganisms to remove multiple metals at varying concentrations, which is not practical with traditional chemical precipitation processes usually adopted in the mining industry.

Conclusions

An indigenous microbial consortium, dominated with filamentous green micro-algae, was collected from AMD at Sarcheshmeh copper mine, Iran. The microbial consortium developed an algal-microbial biofilm in a laboratory scale PRBC, over a batch period. The PRBC was then operated continuously with a Syn-AMD over 10 weeks to evaluate the heavy metal removal capacity of the biofilm. The heavy metals were removed up to 50% of their initial concentration in the order of Cu> Ni> Mn> Zn> Sb> Cr; under low pH of 3.5. Co and Al were also removed up to 15% and 25%, respectively. The results demonstrated the potential of indigenous AMD algal-microbial biofilms and PRBCs to be exploited for heavy metal removal from

AMD. Future work is required to evaluate the biofilm potential to remove heavy metals from natural AMDs at pilot scale using various configurations of the PRBC (e.g. sequential) to determine the commercial potential for this technology to be used in the mining industry.

Acknowledgements

This work was financially supported by the R&D centre at the Sarcheshmeh copper mine in Iran and GHD Pty Ltd in Adelaide, South Australia. Special thanks go to Saeid Ghasemi for his cooperation to undertake work at the mine site, to Mohammad Reza Nikouei for his great assistance in the field and to John Ewers and Joanne Princi for their cooperation at GHD. Additionally, the authors would like to thank Jason Jeffrey Hiorns, Jason Peak and Michael Jung for building the PRBC in the Chemical Engineering workshop, The University of Adelaide.

References

Aguilera A, Gonzalez-Toril E, Souza-Egipsy V, Amaral-Zettler L, Zettler E, Amils R (2010) Phototrophic Biofilms from Rio Tinto, an Extreme Acidic Environment, The Prokaryotic Component. Microbial Mats 14: 469-481

Aguilera A, Souza-Egipsy V, Martin-Uriz PS, Amils R (2008) Extracellular matrix assembly in extreme acidic eukaryotic biofilms and their possible implications in heavy metal adsorption. Aquatic Toxicology 88 (4): 257–266

Bayramoglu G, Tuzun I, Celik G, Yilmaz M, Arica MY (2006) Biosorption of mercury, cadmium and lead ions from aqueous system by microalgae *Chlamydomonas reinhardtii* immobilised in alginate beads. International Journal of Mineral Processing 81:35-43

Costley SC, Wallis FM (2001a) Bioremediation of heavy metals in a synthetic wastewater using a rotating biological contactor. Water Research 35: 3715-3723

Costley SC, Wallis FM (2001b) Treatment of heavy metal-polluted wastewaters using the biofilms of a multistage rotating biological contactor. World Journal of Microbiology and Biotechnology 17: 71-78

Das BK, Roy A, Singh S, Bhattacharya J (2009) Eukaryotes in acidic mine drainage environments: potential applications in bioremediation. Reviews in Environmental Science and Biotechnology 8: 257-274

Das N, Vimala R, Karthika P (2008) Biosorption of heavy metals-An overview. Indian Journal of Biotechnology 7: 159-169

Kapoor A, Dinardo O, Kuiper A (2004) Biological oxidation of ferrous ions under acidic conditions using rotating biological contactor, Journal of Environmental Engineering and Science 3: 311-318

Malik A (2004) Metal bioremediation through growing cells. Environment International 30: 261-278

Orandi S, Yaghubpur A, Sahraei H (2007) Influence of acid mine drainage on aquatic life at Sar Cheshmeh copper mine. Geochimica Et Cosmochimica Acta 71(15): A742

Orandi S. Lewis DM, Moheimani N (2012a) Biofilm establishment and heavy metal removal capacity of an indigenous mining algal-microbial consortium in a photo-rotating biological contactor. Journal of Industerial Microbiology and Biotechnology (in press)

Orandi S, Lewis DM (2012b) Synthesising acid mine drainage to maintain and exploit indigenous mining micro-algae and microbial assemblies for biotreatment investigations. Environmental Science and Pollution Research (in press)

Travieso L, Pellon A, Benitez F, Sanchez E, Borja R, O'Farrill N, Weiland P (2002) BIOALGA reactor: preliminary studies for heavy metals removal. Biochemical Engineering Journal 12: 87-91