Diversity and Performance of Sulphate-Reducing Bacteria in Acid Mine Drainage Remediation Systems

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Abstract

Microbial diversity in acid mine drainage from eMalahleni, Mpumalanga, South Africa. Enrichment of SRB improves its performance in sulphate reduction. Microbial community shows synergy between SRB *Proteobacteria* and facultative *Bacilli*. Sulphate reduction of 85% and cadmium reduction of 98% were observed within 7 days of continuous operational mode. The microbial community showed wider substrates utilisation.

Keywords

Acid mine drainage • Sulphate-reducing bacteria • Heavy metal removal • Microbial diversity

1 Introduction

One of the perils of increasing industrial activity is the current increase in wastewater generation, which poses a threat to both human and aquatic environments. Mining is an example of such activities that has been on the rise, especially in developing nations due to its contribution to the economy of those countries. Mining activities do not utilise a considerable quantity of potable water compared to other industries, yet it is the largest producer of toxic wastewater (Corcoran 2010). Over a century, mining has contributed to the well-being of South Africans; hence, the country has many abandoned mine sites that are the primary source of several environmental and health problems (Mhlongo and Amponsah-Dacosta 2016). There are about 5906 abandoned mine sites in South Africa, due to the fact that mining operations cannot be relocated, generating approximately 6 billion tons of mine wastewater (Auditor-General 2009). The extraction of pyrite (sulphide bearing minerals) during mining activities exposes them to the atmosphere which leads to a chain of complex geochemical reactions that produce metal-laden acid mine drainage (AMD) and residual sulphate. Biological treatment with sulphate-reducing bacteria (SRB) is a recognised technology for the treatment of AMD. The ability of the SRB to remediate AMD and thus produce sulphide and bicarbonate in the presence of a suitable electron donor and carbon source aids the treatment of AMD. Culture-dependent and culture-independent approaches have been used to study the microbial diversity of air, soil, water and wastewater (Kamika and Momba 2014). The culture-independent technique has the advantage of direct analysis and classification of microbial populations in a specific environmental sample (Handelsman 2004; Riesenfeld et al. 2004). This study aims at, firstly, profiling the microbial community of SRB in AMD collected from coal mine in Mpumalanga, South Africa, and secondly, to determine the performance of the SRB consortium in a continuously stirred tank reactor (CSTR) containing AMD.

2 Materials and Methods

Acid mine drainage (AMD) samples were collected as wastewater from a coal mining site in Mpumalanga Province, South Africa, using standard sampling procedure (EPA 2007). The samples were filtered using 45-μm cellulose acetate filters and stored in a polyethylene bottle

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at 4 °C. The physicochemical characteristics of AMD sample at a temperature of 20 °C had high redox potential (Eh = 229.5 mV) and low pH (2.98) including turbidity of 145 NTU and electrical conductivity of 7.84 mS/cm. A volume (100 mL) of AMD was inoculated in a sterile 500-mL bioreactor containing sterilised 400 mL modified Postgate medium B (Postgate 1984). The constituents of Postgate medium B were (g/L): KH_2PO_4 0.5; NH_4Cl 1.0; Na_2SO_4 1.0; $CaCl_2 \cdot 2H_2O$ 0.1; $MgSO_4$ 2.0; yeast extract 1.0; ascorbic acid 0.1; thioglycolic acid 0.1; $FeSO_4 \cdot 7H_2O$ 0.5; NaCl 26; sodium lactate 5 mL; and pH 7–7.5. The bioreactor was incubated anaerobically at 35 °C for 7 days until the colour of the medium changed to black-grey, which indicated a positive growth of the SRB (Ghazy et al. 2011). After 7 days of anaerobic incubation, 100 mL of inoculum containing numerous isolates was transferred into 400 mL sterile Postgate medium B in a new sterilised 500-mL bioreactor. The procedure was repeated thrice. All reagents were analytical grade.

The genomic DNA of the SRB sample was extracted and sequencing was done using 341F (5′—CCTACGG GNGGCWGCAG—3′) and 785R (5′—GACTACHVGGG-TATCTAATCC—3′) targeting V3—V4 of the 16S rRNA genes. The culturable SRB were cultured on nutrient agar supplemented with cycloheximide for bacteria growth, and rose bengal and potato dextrose agar supplemented with chloramphenicol and penicillin-streptomycin, respectively, for fungi growth. The identities of the pure strains and SRB consortium, together with the associated enzymes, were further confirmed in a series of biochemical reactions carried out on a VITEK® 2 Compact 30 system (BioMérieux, France) using the colorimetric reagent cards: BCL (Gram-positive spore-forming *Bacilli*), GN (Gram-negative), GP (Gram-positive) and YST (yeast and yeast-like organisms) as described previously (Akinpelu et al. 2017).

The anaerobic experiments were conducted in a 1-L working volume glass reactor equipped with an overhead stirrer fitted with a two-blade propeller for continuous mixing at 250 rpm. The bioreactors containing 800 mL Postgate medium B were initiated with 10% inoculum at 35 °C and pH around 7 for 21 days with 70% of the medium being drawn weekly and replaced with fresh Postgate medium B. Sodium bromoethanesulphonate (3.2 g/L) was added to the culture during enrichment (21 days) to avoid methanogenic activity. After establishing viable microbial population, fresh wastewater—AMD (10% v/v)—was introduced to the reactors operated in continuous mode. The reactors were kept in the continuous mode for 7 days and sampled at predetermined intervals. The reactors were left in a static batch mode for the next 14 days and then sampled.

3 Results and Discussion

According to the Illumina MiSeq analysis, a total read count of 133, 191 sequences of high quality was obtained from the AMD sample and assigned to different phyla. Overall, 11 phyla with 16 classes were identified, of which phyla *Firmicutes* (75.11%) and unknown microorganisms (24.61%) were the most abundant followed by *Proteobacteria* (0.25%), *Actinobacteria* (0.02%) and other low-abundance phyla. This study confirmed the dominance of *Firmicutes* and *Proteobacteria* in the microbial community of the AMD as was previously reported (Teng et al. 2017; Méndez-García et al. 2015; Kamika and Momba 2014). The biochemical results concurred with the metagenomics analyses, showing dominance of *Bacilli* in the microbial community; however, most of the species identified in VITEK® are facultative organisms such as *Bacillus smithii*, *B. cereus* and *B. thuringiensis*, as well as *B. subtilis* which grows strictly under anaerobic condition using nitrate as electron acceptor (Nakano and Zuber 1998; Hoffmann et al. 1995). The microbial community showed a wider range of substrate utilisation as expected. Notable amongst the substrates utilised were pyruvate, D-glucose, urea, acetate, and DL-lactate, amongst others.

In this study, the bioreactor was operated at temperature 35 ± 2 °C for 42 days, which included an enrichment stage and start-up pH of 7–7.5. On the 22nd day, raw heavy metal-laden AMD containing 8080 mg/L was supplied to the system. There was a gradual increase in sulphate reduction up to 1195 mg/L on the 7th day in continuous mode which was accompanied by microbial proliferation. This was indicative of a high performance by the SRB consortium, considering the initial sulphate concentration used in the system. The high residual sulphate concentration could be attributed to the low rate of reduction as well as higher concentrations of heavy metals in the raw AMD used. Earlier, Jong and Parry (2003) had shown that higher metal concentration inhibits SRB activities in sulphate reduction due to their toxicity and thus reduces SRB metabolism. In addition, a 50% reduction in SRB removal efficiency was caused by high copper concentration (Song et al. 1998). A drop in pH and an increase in redox potential (Eh) were observed at commencement of the continuous operational mode probably due to the introduction of highly acidic with high Eh of the raw AMD. After day 2 of operating in a continuous mode, there were a steady increase in the pH and a decrease in Eh, an indication of the SRB' adaptation to the new conditions. A similar increase in pH and a reduction in Eh were observed when AMD was treated in an up-flow anaerobic sludge blanket bioreactor and a packed bed bioreactor (Najib et al. 2017; Dev et al. 2016; Jong and Parry 2003).

Heavy metals were removed in the form of metal sulphide precipitates. Except Mg^{2+}, Zn^{2+}, As^{3+} and Cr^{3+} which showed removal percentages of 55, 58, 66 and 69%, respectively, all other metals being removed above 70%. It was reported that Al^{3+}, Cu^{2+}, Ni^{2+}, Fe^{2+} and Pb^{2+} are precipitated at pH below 7 but are completely precipitated at pH above 9.5 (Kurniawan et al. 2006; Aubé and Zinck 2003). Cd^{2+} showed the highest removal efficiency (98%) followed by Al^{3+} (97%). The high metal removal was due to the pH being below 7 and the presence of facultative heavy metal tolerant *B. cereus* which was identified in the SRB consortium. Some research has reported a complete removal of heavy metals in the range 80–90% of sulphate reduction operation (Chang et al. 2000; Drury 1999). Therefore, the high sulphate reduction (85%) and incomplete heavy metal removal in this study can be improved by optimising the process parameters such as pH, initial sulphate concentration and hydraulic retention time (HRT) while maintaining kinetically favourable conditions for the growth of the SRB.

4 Conclusion

The microbial profile showed that the phylum *Proteobacteria* was the predominant phylum constituting the SRB consortium with facultative members of phylum *Firmicutes*, being determined to be helpful in the removal of heavy metal in the form of precipitation. It was evident that enrichment time (21 days) of the SRB played a major role in the high sulphate reduction and heavy metal precipitation, including the ability of the SRB being able to utilise a wider substrate range as energy sources with the production of several aminopeptidases which act as biocatalyst in raw AMD treatment. This study can thus be helpful in the design of an effective bioprocess for the treatment of sulphate and heavy metal-laden AMD.

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