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Remobilization of Trivalent Chromium and the Regeneration of In Situ Permeable Reactive Barriers during Operation

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Chromium exists largely in two oxidation states, namely hexavalent chromium (Cr(VI)) which is carcinogenic and mutagenic to living organisms including humans, and tetravalent chromium (Cr(III)) which is known to be 1,000 times less toxic than Cr(VI). It is therefore desirable in most cases to reduce Cr(VI) to Cr(III). Various studies have been conducted on the Cr(VI) reduction process either *in situ* or *ex situ*. Among the suggested treatment technologies, *in situ* bioremediation using permeable reactive barrier as seen as the most attractive option because it can be implemented with the least impact to the environment. In this study, we evaluated the short-term operation of a Cr(VI) reducing barrier and regeneration of the biological reactive barrier to achieve continuous long-term operation. It was observed from the study that the chromium hydroxide ($Cr(OH)_3$) produced precipitated and thus affected the porosity and hydraulic conductivity of the barrier system. It was therefore proposed to implement a regeneration process involving remobilization of precipitated $Cr(OH)_3$ using a dilute acid (0.1 M HCl). Lowering the pH of these introduced harsh conditions which necessitated the evaluation of a possible culture shift during the regeneration phase. Microbial culture composition was evaluated using a 16S RNA finger printing method. The microbial barrier was initially inoculated with indigenous bacterial species from dried sludge. The possible microbial culture community shift in the system was evaluated using 16S rRNA fingerprinting of colonies observed from samples collected after operating the system for seven days. Phylogenic results confirmed that, after the microbial barrier system operation for seven days, the well-known Cr(VI) reducers *Pseudomonas plecoglossiccida, Acinetobacter haemolyticus* and *Comamonas testosterone* remained predominant in the culture community. The microbial barrier system successfully immobilized Cr(VI) at feed concentrations as high as 50 mg/l Cr(VI). Continuous operation of a barrier based on this technology will depend on a development of a pumping system which will ensure successful remobilisation of the metal precipitate for recovery during the regeneration process.

1. Introduction

Chromium in the environment exists mainly in two forms: trivalent chromium (Cr(III)) which is insoluble and less mobile (Molokwane and Chirwa., 2009) and hexavalent chromium (Cr(VI)) which is highly soluble and mobile in water. Cr(VI) is very toxic and carcinogenic such that it is listed as a Class A carcinogen by the U.S. EPA (Federal Register, 2004). In biological system Cr(VI) acts as a carcinogenic, mutagen and teratogen. In plants concentrations as low as 0.5 mg/L in solution and 5 mg/L in soils can inhibit seed germination (Fendorf, 1995). Cr(III) on the other hand is not toxic to living organisms as it is necessary in animal nutrition (Bartlett and James, 1988). Chromium the element was first isolated by the French chemist Nicolas-Louis Vauquelin in 1797 from a sample of a very beautiful orange-red material (Jacobs and Testa, 2005). Among the main exporters of chromite ore in the world are South Africa, Kazakhstan and Zimbabwe. Exports from these countries account for 97 % of the world wide chromite ore production (Bachmann et al., 2010). In the environment chromium is released by industrial processes such as paint and pigment production, leather tanning, wood preservation, rubber and steel production. This contaminant ends up in the groundwater system and thus leads to pollution. The contamination of sites in South Africa is made worse by the existence of abandoned and closing mining or process operation (Molokwane et al., 2008). Stewart et al. (1998) highlighted three strategies for remediating chromium

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contamination in the environment, these being, by excavation and landfilling the excavated materials, by *ex-situ* processes such as land farming or *in situ* processes such as soil flushing and bioventing. Another *in situ* strategy utilizing ferrous iron ($Fe2^+$), sulfides or microorganisms using organisms such as *Pseudomonas plecoglossicida, Comamonas testosterone* and *Acinetobacter haemolyticus* has also gained popularity.

Permeable reactive barriers have been developed and demonstrated to be effective for the treatment of dissolved metal, acid-mine nutrients and dissolved nutrients. In recent years, the biological remediation barriers have been used more successfully in treating toxic organic compounds in water which can be degraded completely to water and carbon dioxide using specially selected organisms (Liu et al., 2006). Bacteria used in the barriers protect themselves from the toxic substances in environment by transforming toxic compounds through oxidation, reduction, or methylation into more volatile, less toxic, or readily precipitating forms. It is for this reason that a permeable reactive barrier embedded with microorganisms is gaining popularity due to the high removal efficiency the overall reduced cost; microorganisms can be target specific and its simplicity. This technology is efficient, however, the end product of the Cr(VI) reduction process is a precipitate metal chromium hydroxide ($Cr(OH)_3(s)$) which is immobilized in the pores of the barrier medium and can later cause clogging.

In this study, we investigate a process for mobilizing the precipitate and the removing the accumulating heavy metal content in the barrier. The mobilization process is intended to simulate a controlled regeneration run during the operation of a permeable reactive barrier system.

2. Material and methods

2.1 Source of Cr(VI) reducing organisms

A consortium of Cr(VI) reducing bacteria was sourced from sand drying beds at the Brits Wastewater Treatment Works (North West Province, South Africa). The bacteria was enriched in medium containing Cr(VI). Batch studies were then conducted to determine the Cr(VI) reducing capability of the consortium. The enriched culture achieved 97 % Cr(VI) removed after incubation for 24 h in batches cultures prepared from 20 mg/L and 50 mg/L Cr(VI).

2.2 Mesocosm Reactor Studies

A 123 X 52 X 50 (LXBXH) tank was constructed from plexiglass® (Evonik Rohm GmbH, Essen, Germany) reinforced by steel bars. Nine sampling ports of 30 cm in length and 11 mm in diameter glass tubing where inserted during the packing of the reactor. A barrier embedded with a consortium of bacteria was placed 10 cm from the influent side of the reactor. Three sampling port were placed upstream of the barrier to evaluate the conditions of chromium before the water enters the barrier zone and six sampling ports where placed after the barrier to assess the performance of the barrier.

2.3 Reactor Startup

Basal mineral media was fed into the reactor for two weeks to acclimatise the bacteria to the environment in the reactor. An initial concentration of 20 mg/L of Cr(VI) this was done for 19 days. A higher concentration of 50 mg/L was then fed to the reactor for the next 21 days. After 6 weeks of operation, 0.1 M of hydrochloric acid was fed to the reactor to dissolve the precipitate followed by an electrokinetic process to mobilise chromium species to a collection point.

2.4 Analytical methods

A UV/VIS spectrophotometer (WPA, Light Wave II, Labotech, South Africa) was used at the wavelength of λ = 540 nm (10 mm light path) to measure the Cr(VI) concentration after acidification of 0.2 mL of the sample with 1 N N_2SO_4 and reaction with 1,5-diphenyl carbazide to produce a purple colour (APHA, 2005). Total Cr was measured at a wavelength of 359.9 nm using a Varian AA – 1275 Series Flame Atomic Adsorption Spectrophotometer (AAS) (Varian, Palo Alto, CA (USA)) equipped with a 3 mA chromium hollow cathode lamp. Cr(III) was determined as the difference between total Cr and Cr(VI) concentration.

2.5 Microbial Diversity Analysis

In order to prepare for 16S rRNA sequence identification, microbial cultures were grown on agar plates to develop colonies and the colonies were first classified based on morphology. LB and plate count (PC) agar was used for colony development. Phylogenetic characterization of cells was performed on individual colonies of bacteria from grown aerobically and anaerobically from soil samples extracted from the mesocosm reactor. Genomic DNA was extracted from the pure cultures grown from individual colonies using QIAGEN DNeasy kit (QIAGEN Ltd, West Sussex, UK). The 16S rRNA genes of isolates were amplified by reserve transcriptase-polymerase chain reaction (RT-PCR) using primers pA and pH1 (Primer

pA corresponds to position 8-27; Primer pH to position 1541-1522 of the 16S gene) (Molokwane et al., 2009). An internal primer pD was used for sequencing (corresponding to position 519-536 of the 16S gene). The resulting sequences were matched to known bacteria in the GenBank using a basic BLAST search of the National Centre for Biotechnology Information (NCBI, Bethesda, MD).

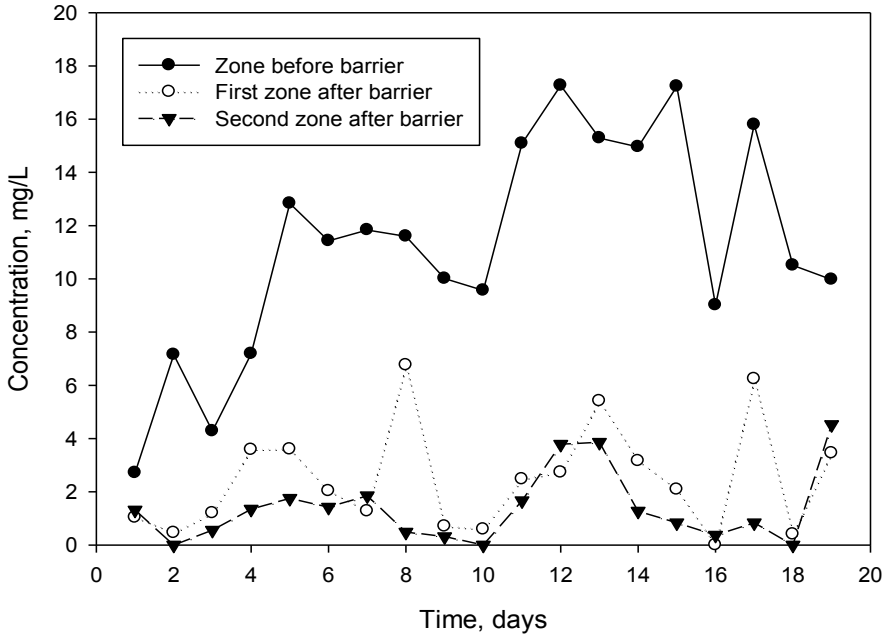

Figure 1: Biological barrier reduction at 20 ppm

3. Results and discussion

3.1 Mesocosm Performance

Figures 1 and 2 show the Cr(VI) reduction in the culture inoculated barrier system. These are the results from the first 19 days of operation with an influent concentration loading of 20 mg/L (Figure 1) and 50 mg/L (Figure 2). The column labeled “zone before barrier” shows average values of readings from ports 1, 2 and 3. These results represent control conditions of operational conditions in the non-inoculated aquifer environment. The column marked “zone after barrier” contains average values for data collected from ports 4, 5 and 6, which is the zone immediately after the barrier and the “second zone after the barrier” represents results from ports 7, 8 and 9 far afield. Tables 1 and 2 show the last 6 days of operation under 20 mg/L and 50 mg/L Cr(VI) influent feed concentration. Results from the operation of the zone under different Cr(VI) loading conditions are summarised in Tables 1 and 2. These results in the Tables depict operation under quasi steady-state conditions which was used to determine when to begin the barrier clean-up step.

Table 1: Cr(VI) reduction in the barrier operated with an influent Cr(VI) concentration of 20 mg/L

Time (days)	Zone before barrier	First zone after barrier	Second zone after barrier
14	15	3.2	1.3
15	17.2	2.1	0.8
16	9.0	0	0.4
17	15.8	6.2	0.8
18	10.5	0.4	0
19	10.0	3.5	4.5

Table 2: Cr(VI) reduction in the barrier operated with an influent Cr(VI) concentration of 50 mg/L

Time (days)	Zone before barrier	First zone after barrier	Second zone after barrier
14	50	37.2	17.2
15	49.0	35.1	14.2
16	49.8	35.8	14.5
17	50.0	39.4	17.1
18	47.4	37.3	14.3
19	44.6	31.9	14.0

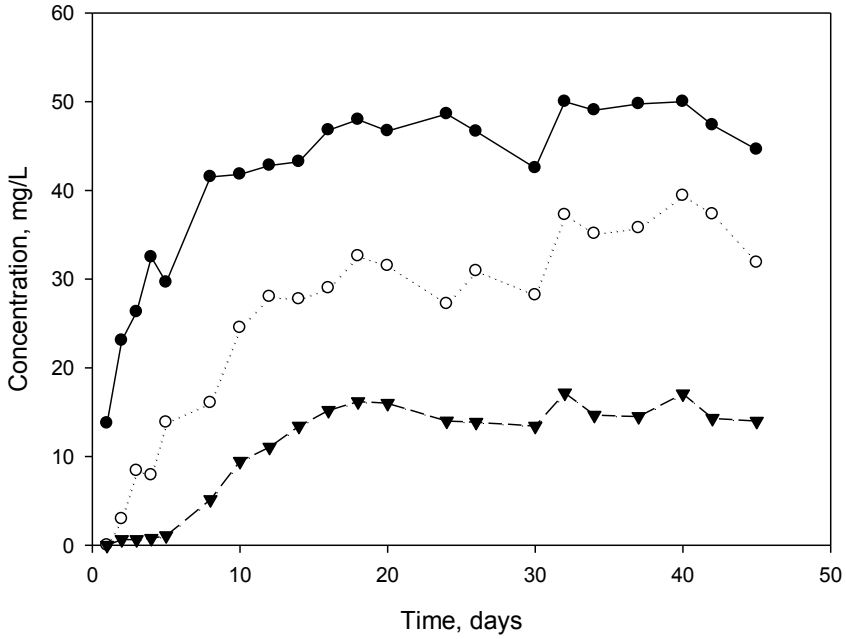

Figure 2: Biological barrier reduction at 50 ppm

The results indicate that the microorganisms are indeed chromium reducing and chromium resistant. Figure 1 shows the reduction of Cr(VI) at the initial concentration of 20 mg/L. On day two 100 % of Cr(VI) was reduced by the microorganisms, this trend of reduction went on till the end of the experiment. Overall performance was in the range 75-100 %. At the concentration of 50 mg/L (Figure 2) the highest Cr(VI) reduction rate was observed during the first 5 days. Up to 100 % Cr(VI) removal was achieved during the first 5 days after which a decline in Cr(VI) reduction capacity was observed. The latter decline in Cr(VI) reducing capacity was attributed to decreasing capacity of the barrier which could be experiencing a decreasing pore volume capacity due to accumulation of precipitates. The removal efficiency continued decreasing until the experiment was terminated at 45 days with a removal efficiency of 75 %.

Figure 3 shows the phylogenetic tree from the reactor, some chromium reducing organisms where identified namely: *Pseudomonas mosselii*, *Pseudomonas plecoglossicida* and *Pseudomonas oryzihabitans* which are from the *Pseudomonas putida* group. Poornima (2010) and other researchers have reported Cr(VI) reducing activity in several strains of *P. putida*. Other Cr(VI) reducing Bacilli were also evident at the beginning of the experiment including *Bacillus thuringiensis* and *Bacillus cereus*.

After 3 weeks of operation $Cr(OH)_3$ precipitate was observed in the reactor. This increased as at 10 weeks 80-90 % of the reactor was covered with the precipitate. In order to effectively remove $Cr(OH)_3$ that is formed at the pH 5.5 and higher (Jeyasingh and Philip, 2004) an acid needs to be added to decrease the pH. 0.1 M HCl was added to the reactor. In this study, all the green chromium hydroxide precipitate disappeared after feeding a dilute solution containing 0.1 M HCl for a period of 3 weeks.

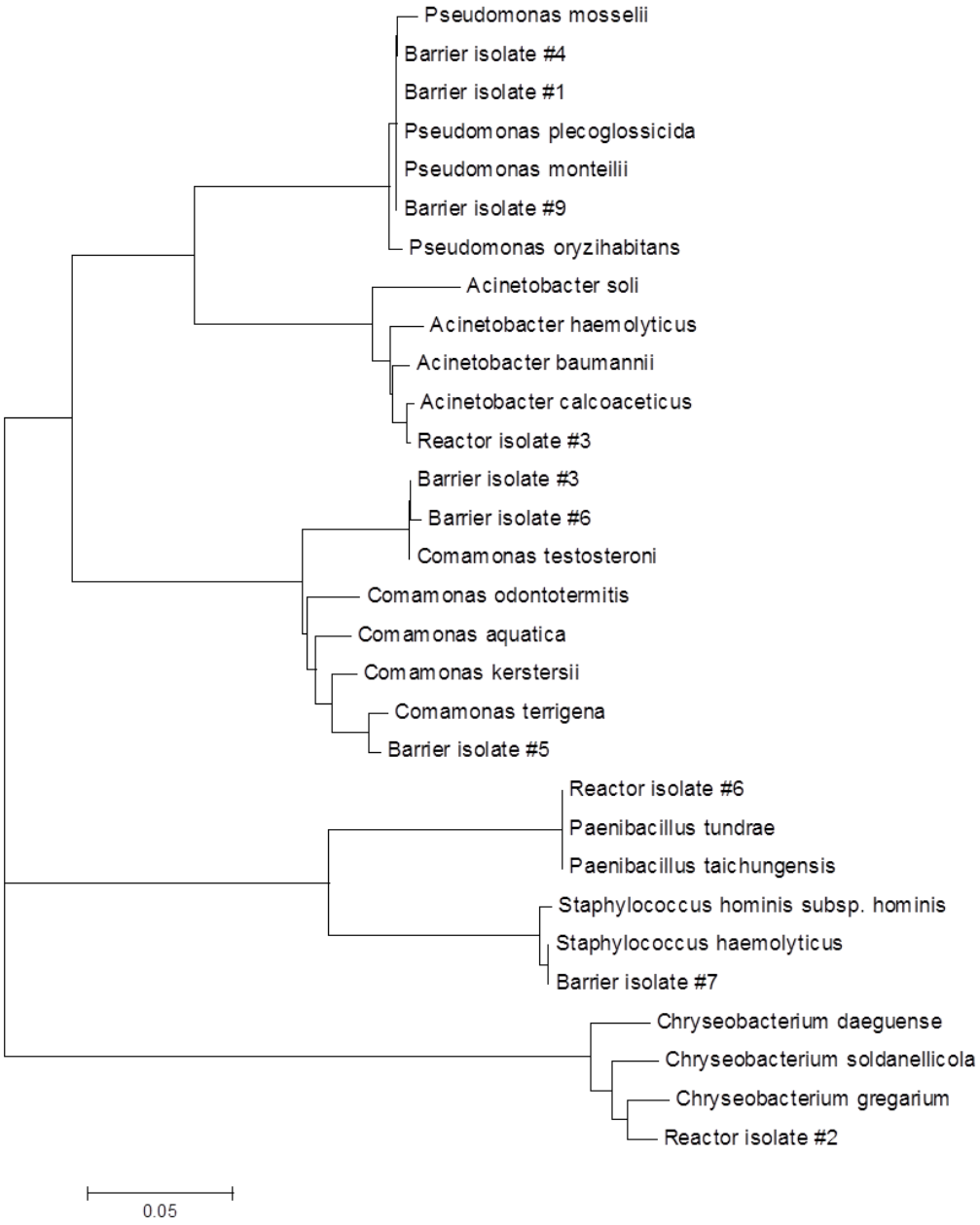

Figure 3: Phylogenetic tree from BLAST search

4. Conclusions

Biological reactive barrier as an in situ remediation technology is fast becoming a popular technology. More than 95 % of Cr (VI) was efficiently reduced to Cr(III) and the precipitate $Cr(OH)_3$ was resolubilise to Cr (III) to prevent it from being re-oxidised back to Cr(VI) which is harmful for the environment. Hydrochloric acid has proven to be efficient as solubilising the precipitate because at the end of the experiment all the precipitate in the reactor had been solubilised. This can be concluded that $Cr(OH)_3$ had been solubilised to a less toxic and mobile Cr^{3+}. This proves that hydrochloric acid can be used to remobilise the precipitate. The remobilised trivalent chromium can be used for ornamental and automotive parts plating. Further research needs to be conducted to find the effects of acids to the groundwater system.

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