We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Biological Cr(VI) Reduction: Microbial Diversity, Kinetics and Biotechnological Solutions to Pollution

Evans M. N. Chirwa and Pulane E. Molokwane
University of Pretoria,
South Africa

1. Introduction

The reduction of Cr(VI) to Cr(III) in the environment is beneficial to ecosystems since Cr(VI) is highly toxic and mobile in aquatic systems, whereas Cr(III) is less mobile, readily forms insoluble precipitates and is about 1000 times less toxic than Cr(VI) (Mertz, 1974; NAS, 1974). Similar reactions have been used lately in reducing uranium-6 (U(VI)) to the less mobile tetravalent form U(IV) for possible application in areas around nuclear waste repositories (Chabalala & Chirwa, 2010).

Biological Cr(VI) reduction is limited by its toxicity to the organisms that reduce it. In certain groups of bacteria, the Cr(VI) reduction capability may be transferred across species. Such a possibility was demonstrated in a study by Bopp & Ehrlich (1988) where Cr(VI) reduction genes were transferred on plasmids across different serotypes of *Pseudomonas fluorescens*. In 1992-1993, Wang and Shen (1993) evaluated Cr(VI) reduction activity in a transformed *Escherichia* species formerly known as B1. *E. coli* B1 is metabolically diverse and was demonstrated to function well in a multi-pollutant environment. For example, B1, later designated ATCC 33456, was able to grow on metabolites formed during degradation of aromatic compounds and reduce Cr(VI) to Cr(III) in the process (Chirwa & Wang, 2000). Successful simultaneous removal of Cr(VI) together with organic co-pollutants demonstrated the feasibility of treating pollutants in real-life where Cr(VI) is discharged together with a variety of toxic organic copollutants.

In later years, various isolates of Cr(VI) reducing bacteria have been isolated from different sites around the world showing that the Cr(VI) reducing capability of microorganisms is ubiquitous in nature (Ganguli & Tripathi, 2002; Zakaria *et al.*, 2007, Molokwane *et al.*, 2008). Several organisms have shown adaptability to Cr(VI) exposure by either acquiring resistance to Cr(VI) toxicity or by participating in the detoxification of the environment for their own survival through the conversion of Cr(VI) to the less toxic Cr(III). This chapter evaluates the prospects of application of the biological remediation against Cr(VI) pollution and recent improvements on the fundamental process.

2. Background

Chromium has been used extensively in industrial processes such as leather tanning, electroplating, negative and film making, paints and pigments processing, and wood

preservation (Beszedits, 1988). Additionally, chromium has been used as a metallurgical additive in alloys (such as stainless steel) and metal ceramics. Chromium plating has been widely used to give steel a polished silvery mirror coating. The radiant metal is now used in metallurgy to impart corrosion resistance. Its ornamental uses include the production of emerald green (glass) and synthetic rubies. Due to its heat resistant properties, chromium is included in brick moulds and nuclear reactor vessels (Dakiky *et al*, 2002).

Through the above and many other industrial uses, a large amount of chromium (approximately 4,500 kg/d) is discharged into the environment making it the most voluminous metallic pollutant on earth. Almost all chromium inputs to the natural systems originate from human activities. Only 0.001% is attributed to natural geologic processes (Merian, 1984).
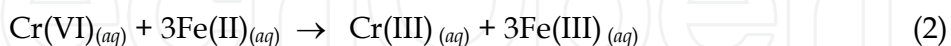
Chromium from the anthropogenic sources is discharged into the environment mainly as hexavalent chromium [Cr(VI)]. Cr(VI) – unlike Cr(III) – is a severe contaminant with high solubility and mobility in aquatic systems. Cr(VI) is a known carcinogen classified by the U.S.EPA as a Group A human carcinogen based on its chronic and subchronic effects (Federal Register, 2004). It is for this reason that most remediation efforts target the removal of Cr(VI) primarily.

Chromium is conventionally treated by transforming Cr(VI) to Cr(III) at low pH through the following reduction-oxidation (redox) reaction:

$$Cr_2O_7{}^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O + 1.33v \ (E^0) \tag{1}$$
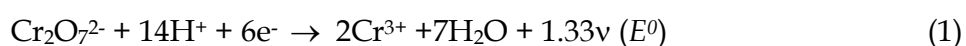
(Garrel & Christ, 1965), followed by precipitation as chromium hydroxide (Cr(OH)$_3$(s)) at a higher pH. Because of the difference in electric potential between the two states, substantial amounts of energy are needed to overcome the activation energy for the reduction process to occur. It is therefore assumed that spontaneous reduction of Cr(VI) to Cr(III) never occurs in natural aquatic systems at ambient pH and temperature.

The redox reaction of Cr(VI) to Cr(III) requires the presence of another redox couple to donate the three necessary electrons. Sets of common Cr(VI) reducing couples in natural waters include H_2O/O_2, Mn(II)/Mn(IV), $NO_2{}^-/NO_3{}^-$, Fe(II)/Fe(III), $S^{2-}/SO_4{}^{2-}$, and CH_4/CO_2. Compounds such as pyrite (FeS_2) and iron sulphide (FeS) can serve as reducing agents for Cr(VI). Iron sulphide (FeS) is ubiquitous in reducing environments such as saturated soils, sediments, and sludge zones of secondary clarifiers in sewage treatment plants. Cr(VI) reduction by iron sulphides leaves a complex precipitate in solution:

$$Cr(VI)_{(aq)} + 3Fe(II)_{(aq)} \rightarrow Cr(III)_{(aq)} + 3Fe(III)_{(aq)} \tag{2}$$

$$x Cr(III) + (1-x)Fe(III) + 3H_2O \rightarrow (Cr_xFe_{1-x})(OH)_{3(s)} + 3H^+ \tag{3}$$

where x may vary from 0 to 1 (Eary & Rai, 1988). The precipitate $(Cr_xFe_{1-x})(OH)_3(s)$ is innocuous and unaesthetic, and therefore must be removed from treated water before discharging into the environment. In practice, the removal of byproducts of Cr(VI) reduction such as the Fe-OH complexes may be very difficult and expensive. The final process may require a system operated at low pH ranges (<2.0) for the removal of Fe-OH compounds followed by operation at a much higher pH range (8-9.5) for the removal of the Cr(III) precipitate (CrOH$_3$(s)) (Eary & Rai, 1988).

Chemical treatment can be performed *ex situ* or *in situ*. However, chemical agents to be applied *in situ* must be selected carefully to avoid 'unintended' contamination of the

treatment area. The primary problem associated with chemical treatment is the nonspecific nature of the chemical reagents. Oxidizing/reducing agents added to the matrix to treat one metal could transform other metals in the system into mobile and more toxic forms (NAS, 1974). Additionally, the long-term stability of reaction products is of concern since changes in soil and water chemistry might create conditions favoring the remobilization of previously reduced toxic species.

3. Biological Cr(VI) reduction and removal

Microbial Cr(VI) reduction was first reported in the late 1970s when Romanenko and Koren'Kov (1977) observed Cr(VI) reduction capability in *Pseudomonas* species grown under anaerobic conditions. Since then, several researchers have isolated new microorganisms that catalyse Cr(VI) reduction to Cr(V) or Cr(III) under varying conditions (Shen & Wang, 1993; Chirwa & Wang, 1997a; Ackerley *et al.*, 2004; Zakaria *et al.*, 2007). Other researchers have also observed Cr(VI) reduction in consortium cultures isolated from the environment (Chirwa and Wang, 2000; Chen and Gu, 2005). Cr(VI) reduction is shown to be cometabolic (not participating in energy conservation) in certain species of bacteria, but is predominantly dissimilatory/respiratory under anaerobic conditions (Ishibashi *et al.*, 1990). In the latter process, Cr(VI) serves as a terminal electron acceptor in the membrane electron-transport respiratory pathway, a process resulting in energy conservation for growth and cell maintenance (Horitsu *et al.*, 1987).

Most micro-organisms are sensitive to Cr(VI), but some microbial species are resistant and can tolerate high levels of chromate. In bacteria, Cr(VI) resistance is mostly plasmid borne whereas Cr(VI) reductase genes have been found both on plasmids and on the main chromosome. Different resistance strategies have been identified, including:

- extraction of chromate via the transmebrane sulphate shuttle (Brown *et al.*, 2006; Hu *et al.*, 2005);
- counteracting chromate-induced oxidative stress by activating enzymes involved in ROS scavenging (catalase, superoxide dismutase) (Ackerley *et al.*, 2004);
- specialised repair of DNA damage by SOS response enzymes (RecA, RecG, RuvAB) (Hu *et al.*, 2005);
- regulation of iron uptake, which may serve to sequester iron in order to prevent the generation of highly reactive hydroxyl radicals via the Fenton reaction (Brown *et al.*, 2006); and
- extracellular reduction of Cr(VI) to Cr(III) which is then removed easily by reacting with functional groups of bacterial cell surfaces (Ngwenya & Chirwa, 2011).
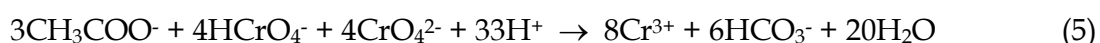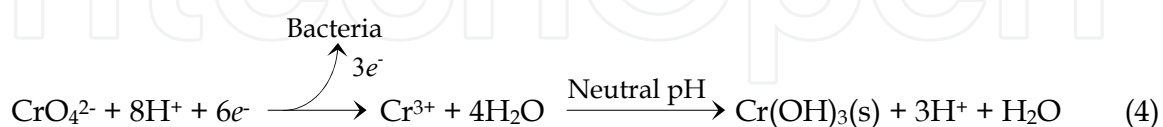
In a few cases, Cr(VI) resistance has been associated with the regulation of uptake mechanisms such as the sulphate uptake shuttle system. Because of its structural similarity to sulphate (SO_4^{2-}), CrO_4^{2-} in some species crosses the cell membrane via the sulphate transport system (Cervantes *et al.*, 2001). After crossing the membrane, CrO_4^{2-} is reduced to Cr^{3+} which interferes with DNA replication resulting in increased rate of transcription errors in the cell's DNA. Additionally, Cr^{3+} may alter the structure and activity of enzymes by reacting with their carboxyl and thiol groups (Cervantes *et al.*, 2001).

Among the resistance mechanisms listed above, the extracellular reduction of Cr(VI) may be utilised in environmental engineering. Although the process is facilitated by bacteria for

their own survival, this process can be used to lower the concentration of Cr(VI) in a contaminated environment.

4. Biological treatment option

Cr(VI) reduction by microorganisms often results in consumption of large amounts of proton as reducing equivalents which results in the elevation of the background pH. The increased pH facilitates the precipitation of the reduced chromium as chromium hydroxide, $Cr(OH)_3(s)$ as shown in Equations 4 & 5 below:

$$CrO_4^{2-} + 8H^+ + 6e^- \xrightarrow{\text{Bacteria} \atop 3e^-} Cr^{3+} + 4H_2O \xrightarrow{\text{Neutral pH}} Cr(OH)_3(s) + 3H^+ + H_2O \qquad (4)$$

$$3CH_3COO^- + 4HCrO_4^- + 4CrO_4^{2-} + 33H^+ \rightarrow 8Cr^{3+} + 6HCO_3^- + 20H_2O \qquad (5)$$

Equation 4 illustrates the general biological Cr(VI) reduction reaction in Cr(VI) reducing bacteria (CRB) reconstructed from redox half reactions whereas Equation 5 illustrates a typical reaction under anaerobic conditions using acetic acid as a carbon source and electron donor. Other fatty acid byproducts of hydrolysis can also serve as electron donors for Cr(VI) reduction (Chirwa & Wang, 2000). The obvious advantage of the above process is that it eliminates the need for addition of chemicals in the precipitation stage of the process.

Several carbon sources and reactor configurations have been evaluated. The performance of microbial cultures in treating Cr(VI) is limited mainly by the toxicity effects and the Cr(VI) reduction capacity of the cells (Shen & Wang, 1994). The latter has been demonstrated in several species of bacteria (Shen *et al.*, 1996; Chirwa & Wang, 2000). The problem of limited Cr(VI) capacity in cells is circumvented by using either continuous-flow or biofilm processes, both of which facilitate continuous replenishment of killed or inactivated cells in the system (Nkhalambayausi-Chirwa & Wang, 2005).

5. Cr(VI) reducing microorganisms

The advent of molecular biology has made possible the identification and characterisation of several Cr(VI) reducing species from the environment. Previously, researchers could only identify microbial species that can be cultured using standard broth and agar media. We soon realise that several species of bacteria are not able to grow on standard culturing and growth media and others depend on complex interrelationships with other organisms in a microbial community. Recently, genetic sequencing of 16S rDNA genes and metagenomic techniques have been used to supplement the conventional methods of species identification and characterisation (Jukes & Cantor, 1969). This allows identification of both culturable and unculturable organisms in environmental samples. It also helps uncover species that have not been identified before. Examples of identified Cr(VI) reducing bacteria and their growth conditions are shown in Table 1.

Table 1 illustrates the whole range of species and growth conditions for Cr(VI) reducing organisms. Most of the bacterial species shown in Table 1 were isolated from chromium (VI) contaminated environments (i.e. sediments, wastewater treatment plants, soil etc). Although earlier isolates grew mostly on aliphatic carbon sources, later studies have shown diversity in the preferred carbon sources and electron donors. For example, some consortium cultures

Name of Species	Isolation Conditions/C-Sources	References
Achromobacter sp. Str.Ch1	Anaerobic / Luria Broth; glucose-lactate	Zhu *et al.*, 2008
Agrobacterium radiobacter EPS-916	Aerobic-Anaerobic / glucose-mineral salts medium	Llovera *et al.*, 1993
Bacillus megaterium TKW3	Aerobic / nutrient broth-minimal salt medium-glucose, maltose, and mannitol	Cheung & Gu, 2006
Bacillus sp.	Aerobic/ Vogel-Bonner (VB) broth-citric acid; D-glucose	Chirwa & Wang, 1997a;
Bacillus sp. ES 29	Aerobic / Luria-Bertani (LB) medium	Camargo *et al.*, 2003
Bacillus subtilis	Aerobic / Minimal medium - trisodium citrate and dehydrate glucose	Garbisu *et al.*, 1998
Bacillus drentesis, Bacillus thuringiensis	Aerobic/Luria Betani Broth	Molokwane & Chirwa, 2009
Deinococcus radiodurans R1	Anaerobic / Basal Medium, Lactate, Acetate, Pyruvate, Succinate	Frederickson *et al.*, 2000
Enterobacter cloacae HO1 *strain*	Anaerobic / KSC medium-Sodium acetate	Wang *et al.*, 1989
Escherichia coli ATCC 33456	Aerobic-Anaerobic / Nutrient broth medium; glucose, acetate, propionate, glycerol and glycine	Shen & Wang, 1993
Enterobacter sp	Aerobic/Luria Betani Broth	Molokwane & Chirwa, 2009
Lysinibacilus sphaericus	Aerobic/Luria Betani Broth	Molokwane & Chirwa, 2009
Ochrobactrum sp.	Aerobic / glucose	Zhiguo *et al.*, 2009
Pantoea agglomerans SP1	Anaerobic / acetate	Francis *et al.*, 2000
Pseudomonas fluorescens	Aerobic-Anaerobic / Glucose-Acetate-Pyruvate-Lactate-Succinate	Bopp *et al.*, 1983
Pseudomonas fluorescens LB300	Aerobic / Vogel-Bonner broth	Bopp & Ehrlich, 1988
Pseudomonas putida MK1	Anaerobic / Luria-Bertani -citric acid- Tris-acetic acid	Park *et al.*, 2000
Providencia sp.	Aerobic-Anaerobic / Luria broth (tryptone-yeast extract)	Thacker *et al.*, 2006
Shewanella alga (BrYMT) ATCC 55627	Aerobic-Anaerobic / M9 broth- Glucose	Guha *et al.*, 2001
Shewanella putrefaciens MR-1	Anaerobic / lactate- fumarate	Myers *et al.*, 2000

Table 1. Identified Cr(VI) reducing bacteria.

were shown to grow in the absence of organic carbon sources – utilising only bicarbonate (HCO_3^-) as the carbon source (Molokwane & Chirwa, 2009). The table also illustrates that Cr(VI) reducing microorganisms are ubiquitous in nature. They thrive on a range of carbon sources and are found in almost all possible environments. This in itself shows the feasibility of the biological treatment process as it could be adapted to a wide range of effluent and environmental conditions.

6. Proposed Cr(VI) reduction mechanisms

As stated earlier, Cr(VI) reduction may be cometabolic (not participating in energy conservation) in certain species of bacteria, but could be predominantly dissimilatory/ respiratory under anaerobic conditions in certain species. In the latter process, Cr(VI) serves as a terminal electron acceptor in the membrane electron-transport respiratory pathway, a process resulting in energy conservation for growth and cell maintenance (Horitsu *et al.*, 1987; Ishibashi *et al.*, 1990). In the dissimilatory/respiratory process, electrons are donated from the electron donor to Cr(VI) via NADH (Chirwa & Wang, 1997a).

6.1 Cr(VI) reduction by cytoplasmic enzymes
Although it is proven that specialised Cr(VI) reducing enzymes (reductases) exist inside the Cr(VI) reducing bacterial cells, several components of the cell's protoplasm also reduce Cr(VI). Components such as NADH (NADPH in some species), flavoproteins, and other hemeproteins readily reduce Cr(VI) to Cr(III) (Ackerley *et al.*, 2004). It is therefore expected that the cytoplasm fraction of disrupted cells from most organisms will reduce Cr(VI). Such a reduction process is not metabolically linked but will directly affect the cell since most of the intracellular proteins catalyse a one-electron reduction from Cr(VI) to Cr(V) which also generates harmful reactive-oxygen species (ROS) that cause damage to DNA.

6.2 Cr(VI) reduction by soluble reductase
Of special interest are the Cr(VI) reducing enzymes that are produced deliberately by the cell and exported into the media to reduce Cr(VI). Since protein excretion is an energy intensive process, most of these enzymes are produced constitutively, i.e., they are produced only when Cr(VI) is detected in solution and are therefore highly regulated (Chueng & Gu, 2007). The evidence of extracellular Cr(VI) reduction has been presented by a few researchers using a mass balance of Cr(VI) and its reduced species in media and cells (Shen & Wang, 1993; Chirwa and Wang, 1997b).

In a cellular mass balance evaluation by Chirwa & Wang (1997b), Cr(III) uptake by pelleted cells after centrifugation of a sample of *Pseudomonas fluorescens* LB300 was determined to be only 5% of the initial added Cr as Cr(VI). Cr(III) accumulation in the pelleted cells was determined by measuring the difference in Cr(III) level in solution before and after washing the cells three times in 1.0 N HCl. Results showed that less than 5.0% of Cr(III) was retained in the pelleted cells after 24 hours (0.36 ± 0.04 mg Cr(III)/L in pelleted cells; 8.55 ± 0.22 mg Cr(III)/L in supernatant; 8.62 ± 0.34 mg Cr(III)/L in culture medium). Similar results were obtained earlier by Shen & Wang (1993) with batch cultures of *Escherichia coli* ATCC 33456 in which only about 2.0% of Cr(III) transformed from Cr(VI) remained in the cell pellets.

Extracellular Cr(VI) reduction is beneficial to the organism in that the cell does not require transport mechanisms to carry the chromate and dichromate into the cell and to export the Cr^{3+} into the medium. Both Cr^{6+} and Cr^{3+} react easily with DNA, the presence of which can

result in DNA damage and increased rates of mutations. Extracellular reduction of Cr(VI), thus, protects the cell from the DNA damaging effects of Cr(VI). It may be due to this reason that certain species of bacteria have adapted the extracellular Cr(VI) reduction process for survival in Cr(VI) contaminated environments.

From an engineering perspective, using cells that reduce Cr(VI) externally is specifically beneficial since the cells can be separated easily from an expired medium and reused in the reactor system. If Cr(VI) is reduced internally, the resulting Cr(III) will tend to accumulate inside the cell, thus it will be difficult to recover reduced Cr or regenerate the cells.

6.3 Membrane pathway

Microorganisms are known to have evolved biochemical pathways for degrading or transforming toxic compounds from their immediate environment either simply for survival or to derive energy by using the toxic compounds as electron donors or electron sinks. The biotransformation pathways commonly take advantage of the advanced and well conserved membrane electron transport respiratory apparatus within the organisms (Dickerson, 1980). For example, the redox reactions involving some of the metallic pollutants are coupled to the electron transport through electron carriers in the cytoplasmic membrane and the flux of protons through the ATP-synthase. The proton flux and production of ATP through the ATP-synthase generates the required energy equivalents for use in cellular metabolism (Lloyd, 2003).

In other studies, two pathways of Cr(VI) reduction are suggested for gram-negative bacteria (Figure 1). The first mechanism suggests Cr(VI) reduction mediated by a soluble reductase with NADH serving as the electron donor either by necessity (Horitsu et al., 1987) or for maximum activity (Ishibash et al., 1990). The NADH-dehydrogenase pathway is expected to predominate under aerobic conditions. In the second mechanism, Cr(VI) acts as an electron acceptor in a process mediated by a membrane-bound Cr(VI) reductase activity (Horitsu et al., 1987).
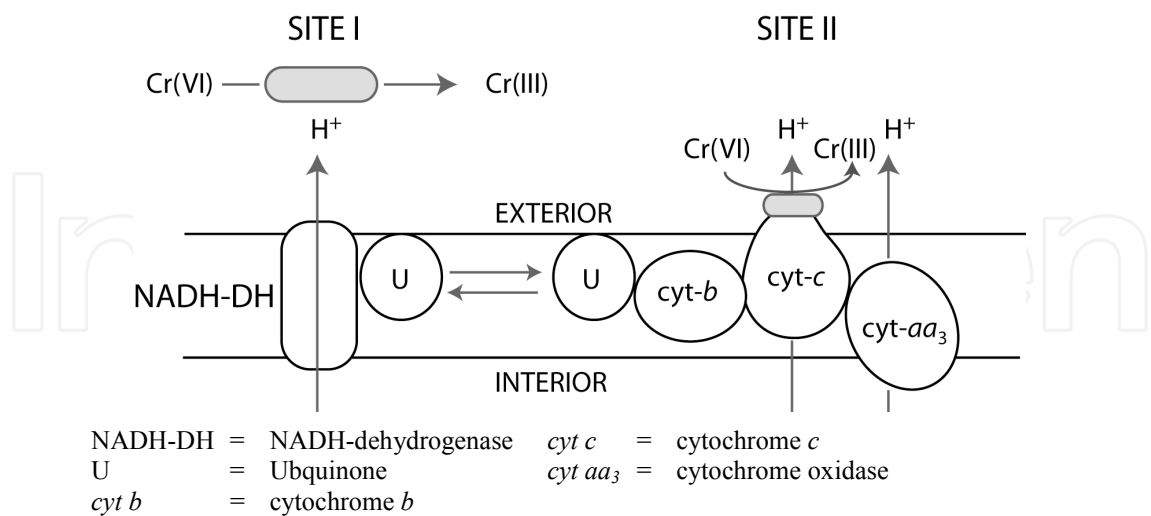

NADH-DH	=	NADH-dehydrogenase	cyt c = cytochrome c
U	=	Ubquinone	cyt aa₃ = cytochrome oxidase
cyt b	=	cytochrome b	

Fig. 1. The conceptual electron transport pathway through the inner cell membrane.

Although the overall reduction of Cr(VI) to Cr(III) ($CrO_4^{2-} \rightarrow Cr^{3+}$) is thermodynamically favorable, this reaction is limited by reaction kinetics under physiological conditions (Garrels & Christ, 1965). The kinetics of Cr(VI) reduction can be improved by coupling Cr(VI) reduction to other energy yielding reactions such as oxidation of organic compounds.

Metabolically linked Cr(VI) reduction associated with the oxidation of NADH was demonstrated in anaerobic cultures of *E. coli* ATCC 33456 under Cr(VI) concentrations below the toxic inhibition threshold (Chirwa & Wang 2000; Nkhalambayausi-Chirwa & Wang, 2001). Under such conditions, Cr(VI) may be used as the principle electron sink and energy is conserved for cell growth and maintenance.

Observations of Cr(VI) reduction under aerobic conditions suggest a cometabolic process where transport of electrons to Cr(VI) does not yield conserved energy for metabolism. In such systems, Cr(VI) is reduced at the expense of metabolic activity in the cells. This was demonstrated using a cumulative mass balance analysis for a continuous-flow biofilm system where cell growth was disrupted during high Cr(VI) loading, but the metabolic activity resumed after Cr(VI) loading was lowered below the toxicity threshold of 10 mg/L (Figure 2). The observed optimum Cr(VI) reduction efficiency just before system
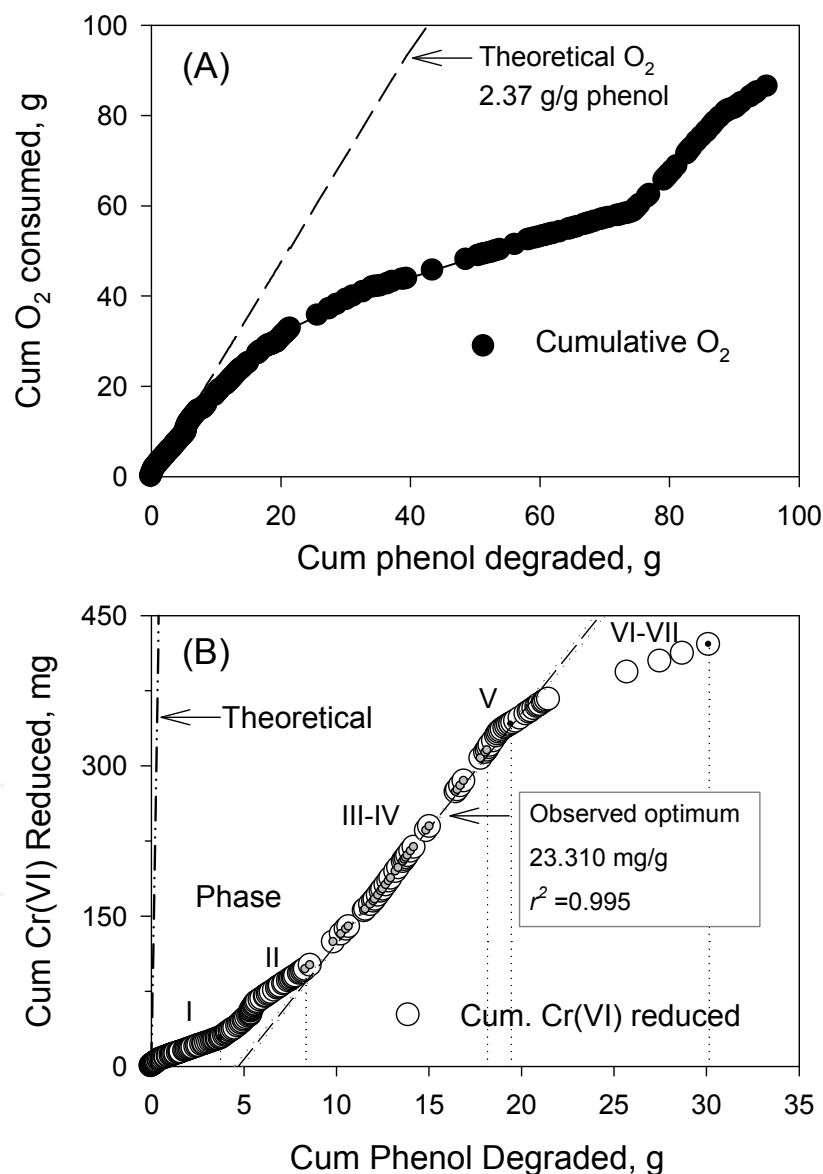

Fig. 2. Cumulative mass balance showing delayed Cr(VI) reduction in a coculture of *Pseudomonas putida* DMP-1 and *Escherichia coli* ATCC 33456 under high loading conditions (Phase III-V). (After Nkhalambayausi-Chirwa and Wang, 2001).

overloading suggested that electrons may be diverted from other biological activities towards Cr(VI) reductase until all Cr(VI) was reduced. If Cr(VI) is still not completely reduced after the cells have sacrificed the maximum number of reducing equivalents to Cr(VI) reduction, then biological activity is completely compromised and the cells may die.

6.4 Genetic regulation

The pioneering work on microbial Cr(VI) reduction was conducted by Romanenko & Koren'Kov (1977) using an unidentified species of *Pseudomonas fluorescens* from Cr(VI) contaminated sediments. Further work revealed that Cr(VI) reduction can either be plasmid borne as was the case with several Pseudomonas species (Bopp and Ehrlich, 1988; Bopp *et al.*, 1983) or located on the chromosomal DNA as is the case with several Bacilli and Enterobacteriaceae (Lu & Krumholz, 2007). Earlier studies also showed that elements carried on the plasmid DNA are transposable across species. This was demonstrated by the creation of *Escherichia coli* ATCC 33456 by transferring the plasmid carrying the Cr(VI) reducing genes from *Pseudomonas fluorescens* LB300 (Shen & Wang, 1993).

So far, only one protein, ChrR, has been demonstrated to receive electrons directly from NADH to achieve Cr(VI) reduction. The protein was purified using classical biochemical techniques from *Pseudomonas putida* (Park *et al.*, 2000) and the resulting homogeneous enzyme successfully catalysed the reduction of chromate. N-terminal and internal amino acid sequence determination of the enzyme allowed the design of appropriate primers to clone the *chrR* gene into *Escherichia coli* (Park *et al.*, 2002). BLAST searching of protein databases with the derived ChrR amino acid sequence revealed a conserved family of proteins whose members are present in a wide range of organisms. Over 40 of these homologs, including the predicted product of a previously uncharacterized open reading frame (*yieF*) from *Escherichia coli*, showed 30% amino acid identity with ChrR. The ChrR and YieF homologs were shown to contain the characteristic signature of the NADH_dh2 family of proteins, which consists of bacterial and eukaryotic NAD(P)H oxidoreductases (Lu & Krumholz, 2007).

The regulation of Cr(VI) reduction in an operon structure was observed in *Bacillus cereus* SJ1 and *Bacillus thuringiensis* strain 97-27 in which the Cr(VI) reduction genes were demonstrated to be upward regulated by the promoter *chrI* which in turn regulated the Cr(VI) resistance gene *chrA1* and arsenic resistance genes *arsR* and *arsB* (He *et al.*, 2010) (Figure 3).

From the observations by He *et al.* (2010) the *chrA1* gene encoding ChrA protein showed the highest amino acid identity (97%) with a homologous protein annotated as chromate transporter in *Bacillus thuringiensis* serovar konkukian str. 97-27. Interestingly, the *chrA1* gene is located downstream of the potential transcriptional regulator gene *chrI*. The region of *chrA1* and *chrI* also contains several putative coding sequences (CDSs) encoding homologs of Tn7-like transposition proteins and a resolvase that is potentially involved in horizontal gene transfer events (Figure 3). ChrI is assumed to control a 26 kb region with a relatively low GC content in *B. thuringiensis* 97-27 (32.8%) which is lower than the average GC content of 35.4% in a corresponding ChrI regulated region in *B. cereus* SJ1.

In both Bacilli, the Chr Operon is interlaced with the arsenic resistance genes including the regulatory genes for the arsenic resistance operon repressor ArsR, arsenic resistance protein

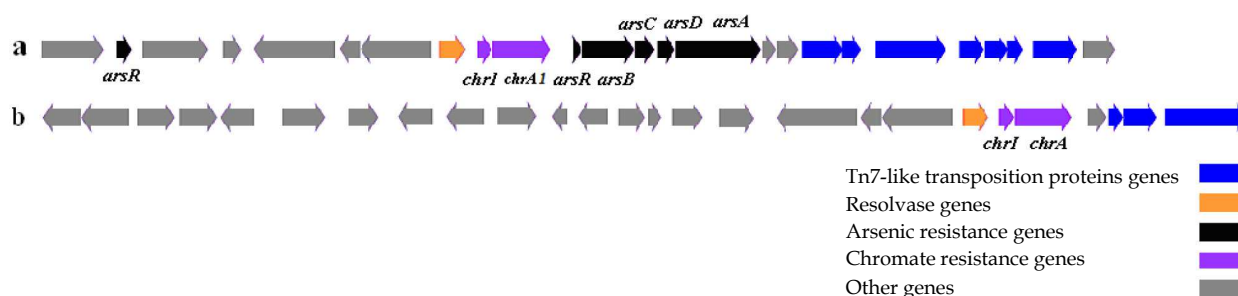ArsB, arsenate reductase ArsC, arsenic chaperon ArsD and arsenic pump ATPase ArsA (He *et al.*, 2010).

Fig. 3. Comparison of genetic determinants of chromate resistance and chromate reduction between (a) *Bacillus cereus* SJ1 and (b) *Bacillus thuringiensis* serovar konkukian str. 97-27. (After He *et al.*, 2010).

7. Cr(VI) removal from solution - biosorptive processes

In previous studies, it was demonstrated that some species of bacteria possess adsorptive properties that facilitate removal of metal species from aquatic solutions. These adsorptive properties are dependent on the distribution of reactive functional groups on the cell wall surfaces of bacteria, such as; carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups (Parmar *et al.*, 2000). Available information is mostly based on studies conducted under aerobic conditions. There is limited information on microbial adsorptive behaviour under oxygen stressed conditions and toxic environments.

The only available information is on the adsorptive ability of sulphate reducing bacteria for toxic metals including radionuclides (Bruhn *et al.*, 2009). However, as yet, there is lack of knowledge on the nature of the surface reactive groups on SRB cell surfaces that account for its high metal adsorption ability.

In a recent study, Ngwenya & Chirwa (2011) investigated the chemical nature of the cell surfaces of a sulphate reducing bacteria consortium and its interaction with mono- and divalent cations under anaerobic conditions. The study utilized a surface complexation modelling approach to predict the trends of the adsorption of the cationic species.

In the above study, the distribution of functional groups and adsorption reactions on SRB cell surfaces were characterised using a combination of Gram potentiometric titrations, FTIR, and surface complexation modelling. Four types of binding sites were identified: site 1 corresponding to carboxylic acid functional groups (pK$_a$ = 4-5); the near-neutral site 2 corresponding to phosphates (pK_a =6-7), and sites 3 and 4 corresponding to basic sites and phenolic sites (pK$_a$ = 8-12). The most abundant proton binding sites belonged to site 4 (hydroxyl/amine group) and accounted for about 40% of the total concentration of binding sites for the consortium. The effect of ionic strength was also evident from the metal ion adsorption studies. A decrease in metal adsorption was observed at higher ionic strengths. These results promise feasibility of application for recovery of adsorbed metallic species for reuse and regeneration of the cells.

Since the bacteria cell walls show an adsorptive capacity for cationic species, reduction of the oxyanionic chromate (CrO_4^{2-}) to Cr(III), which exists in solution at lower pH as Cr^{3+}, could be necessary for effective removal Cr(VI) from solution. In spite of the effectiveness of biosorption in removing Cr(VI), past studies have strongly supported precipitation as the primary removal mechanism of reduced chromium (Shen & Wang, 1993; Chirwa & Wang, 1997b).

8. Biofilm systems

Microorganisms in nature and in reactor systems rarely grow as separate cells. The microorganisms form complex communities either in the form of agglomerations called flocs or as biofilm on the surfaces of inanimate objects and other organisms. The performance of a microbial culture is not only a function of its capability to degrade or transform a pollutant but also the configuration of the community in which it resides. There are complex interrelationships that occur within the microstructure that affect the availability of substrates, symbiotic existence through toxicity shielding of more sustainable species, and transfer of metabolites to organisms that could otherwise not grow on the only available primary substrate in the bulk liquid.

Nkhalambayausi-Chirwa and Wang in 2001 took advantage of the complex structure of the micriobial biofilm to improve the performance of *Escherichia coli* ATCC 33456 in reducing Cr(VI). The microorganisms in the biofilm could benefit from the spatial and physiological heterogeneity within the biofilm community (Stoodley *et al.*, 1999). In this case, phenol degrading species and Cr(VI) reducing species were grown together in a biofilm reactor such that *E. coli* utilised the anaerobic conditions in deeper layers of the biofilm for growth and Cr(VI) reduction whereas *P. putida* degraded the primary carbon source (phenol) into organic acid metabolites (Nkhalambayausi-Chirwa & Wang, 2001). In so doing, *P. putida* detoxified the environment for *E. coli* and provided secondary carbon and energy sources for *E. coli*.

The operational model of the biofilm system is shown in Figure 4 with the dissolved species represented as C_B, P_B, and U_B in the bulk liquid and $C_{(t,x)}$, $(P_{(t,x)})$, and $U_{(t,x)}$ in the biofilm zone, where C = Cr(VI) concentration (mg/L), P = phenol concentration (mg/L), and U = metabolites concentration (mg/L). The generic representation of dissolved species concentration in the biofilm is given by, $\mathbf{y}_{(t,x)}$. The particulate matter in the reactor consisted of *P. putida* (X_P), *E. coli* (X_E), and inert biomass (X_I). The subscript B in the biomass terms indicates unattached biomass in the bulk liquid.
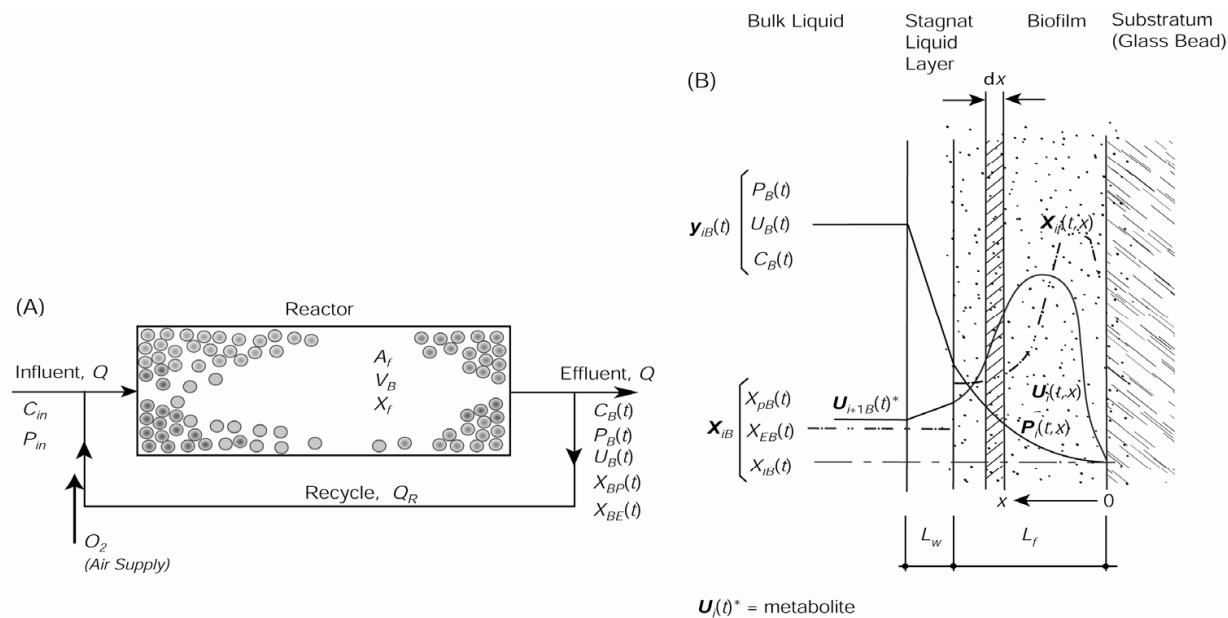

Fig. 4. Conceptual mixed-culture biofilm model for (a) control volume space, and (b) biofilm environment.

In the above model, the primary carbon source (phenol), Cr(VI), and O₂ diffuse into the biofilm where they are taken up by living organisms. A stagnant liquid layer of thickness L_w inherently resists the transport of the dissolved species into the biofilm resulting in generation of a concentration gradient towards the liquid/biofilm interface. Since phenol is toxic to the *E. coli* species used in the study, *P. putida* out-competed *E. coli* in the outer layers of the biofilm. And since *P. putida* is obligately anaerobic, it was out-competed by *E. coli* as O₂ became scarce deeper in the biofilm.

The above system only illustrates the complex nature of the interdependent systems in a near natural environment. Some of these processes can be engineered but some may be lost during the implementation of bioremediation process. During the process of developing the above described coculture, many pairs of aromatic compound degrading species and Cr(VI) reducing species of bacteria were tested but, in most cases, one of the species could be out competed due to susceptibility to toxicity or slow growth.

9. Diffusion/Reaction model

The removal of the dissolved species and cell growth is represented by a set of diffusion-reaction partial differential equations (PDEs) with the conversion reactions occurring mainly inside the biofilm. The PDEs represent a mass balance across an infinitesimal biofilm section (δz) parallel to the substratum surface (Figure 4b) as follows:

$$\frac{\partial(\hat{\mathbf{u}})}{\partial t} = \varepsilon(t) \cdot \frac{\partial(\mathbf{j}_{\hat{\mathbf{u}}})}{\partial z} + \mathbf{r}_{\hat{\mathbf{u}}f} \tag{6}$$

$$\frac{\partial(\hat{\mathbf{x}})}{\partial t} = \varepsilon(t) \cdot \frac{\partial(\mathbf{j}_{\hat{\mathbf{x}}})}{\partial z} + \mathbf{r}_{\hat{\mathbf{x}}f} \tag{7}$$

where: $\mathbf{j}_{\hat{\mathbf{x}}} = D_{w\hat{\mathbf{x}}} \, \partial(\hat{\mathbf{x}})/\partial z$, mass flux rate of biomass ($ML^{-2}T^{-1}$), $\mathbf{r}_{\hat{\mathbf{u}}f}$ = the vector of removal rates of dissolved species in the biofilm ($ML^{-3}T^{-1}$), $\mathbf{r}_{\hat{\mathbf{x}}f}$ = the vector of biomass production rates in the biofilm zone ($ML^{-3}T^{-1}$), and ε = is a biofilm porosity constant (V_{fvoids}/V_{ftotal}). The movement of cells across the biofilm is induced by physical displacement due to growth whereas dissolved species are transported by diffusion. Thus, the values of the j terms for cells are expected to be lower (by orders of magnitude) than the j terms for dissolved species in the biofilm.

The outer and inner boundary conditions for dissolved species $\hat{\mathbf{u}}$ and biomass $\hat{\mathbf{x}}$ are defined by:

$$\mathbf{j}_{\hat{\mathbf{u}}} = k_{L\hat{\mathbf{u}}} \cdot \left(\hat{\mathbf{u}}_B(t) - \hat{\mathbf{u}}_{fs}(t, L_f) \right), \quad z = L_f, \quad \text{outer boundary} \tag{8}$$

$$\mathbf{j}_{\hat{\mathbf{X}}} = \hat{\lambda}(u) \cdot \hat{\mathbf{x}}_f \cdot L_f, \quad z = L_f, \quad \text{outer boundary} \tag{9}$$

$$\mathbf{j}_{\hat{\mathbf{u}}} = 0, \quad z = 0, \quad \text{inner boundary} \tag{10}$$

$$\mathbf{j}_{\hat{\mathbf{X}}} = 0, \quad z = 0, \quad \text{inner boundary} \tag{11}$$

where $k_{L\hat{u}} = D_{w\hat{u}}/L_w$, is the mass transfer rate coefficient (L^2T^{-1}), and $\hat{u}_{fs}$ = dissolved species concentration at the liquid/biofilm interface (ML^{-3}).

The above equations were simulated successfully using optimised reaction rate parameters from batch studies and dynamic parameters from the continuous flow biofilm reactor systems (Nkhalambayausi-Chirwa & Wang, 2005) (Figure 5). The dynamic parameters were estimated from the data obtained from the operation of the reactor at 24 hours hydraulic retention time (HRT) (Phase I-VI). The rest of the phases (VII-XVIII) were simulated using the optimised parameters. The results showed a high predictive accuracy as the model accurately tracked the trends in effluent concentrations for both the electron donor (phenol) and the electron sink (Cr^{6+}).
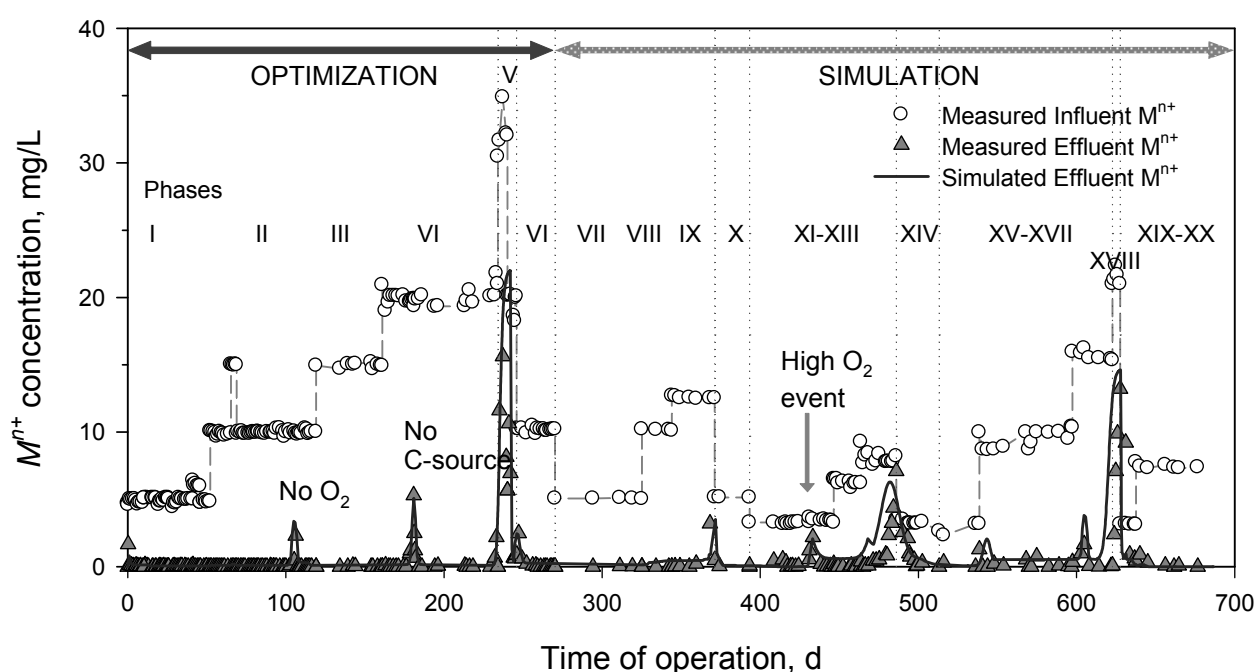
Fig. 5. Simulation of Cr(VI) (M^{6+}) removal in a coculture biofilm system under different HRTs: 24 h (Phase I-VI); 11.7 h (Phase VII-X); 6 h (Phase XI-XIV); 17.9 h (Phase XV-XVIII).

10. *In situ* barrier systems

Several types of treatment walls have been tested in the attenuation of the movement of metals in groundwater. Trench materials have been investigated including zeolite, hydroxyapatite, elemental iron, and limestone (Vidic & Pohland, 1996). Elemental iron has been tested for chromium (VI) reduction and other inorganic contaminants (Powell *et al.*, 1995) and limestone for lead precipitation and adsorption (Evanko & Dzombak, 1997). Biological Permeable Reactive Barriers (BPRBs) use microorganisms as reactants rather than chemical reactants to removal pollutants. Specific application of BPRBs removal of Cr(VI) in groundwater has not been attempted. This has been both due to the unavailability of microorganisms capable of growing under the nutrient deficient groundwater conditions and lack of information on the fate of the reduced chromium species in the barrier.

Recently, the group at University of Pretoria has evaluated the Cr(VI) reduction performance of several anaerobic species of bacteria in microcosm systems simulating groundwater conditions (Molokwane & Chirwa, 2009). The microorganisms were isolated locally to avoid the dilemma of using imported bacteria which is difficult to get and in most instances not allowed by law.

10.1 *In situ* barrier concept

Permeable reactive barriers are an emerging alternative to traditional pump-and-treat systems for groundwater remediation. Such barriers are typically constructed from highly impermeable emplacements of materials such as grouts, slurries, or sheet pilings to form a subsurface "wall." Permeable reactive barriers are created by intercepting a plume of contaminated groundwater with a permeable reactive material (Figure 6). For physical chemical processes such as described above, the reactive materials need to be replenished or replaced after a certain time of operation, a process which is extremely expensive and in some cases not practical. Using microorganisms as the main reactants aims at achieving a self-replenishing system since the bacteria can regenerate themselves. For biodegradable compounds that can be mineralized to CO_2 and H_2O such as petrochemical pollutants, this works perfectly. Unfortunately, metals can only be converted from one form to another such that the converted form may be trapped in the barrier material until measures are taken to remobilise the pollutant to clean the barrier.
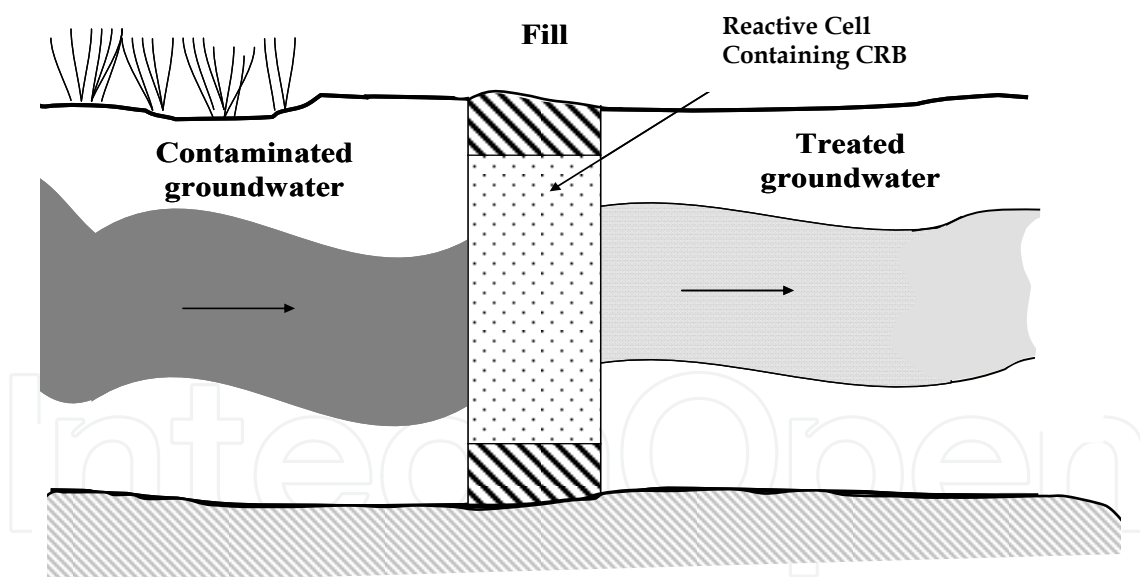

Fig. 6. Elevated view of a permeable reactive barrier configuration for groundwater treatment.

10.2 Application to Cr(VI) and toxic metal removal

As stated earlier, no full scale applications of BPRBs for treating Cr(VI) have been attempted thus far. The group at the University of Pretoria has been evaluating several remediation scenarios for *in situ* treatment of Cr(VI) in groundwater environments. One possibility is *in situ* bioinoculation in which the Cr(VI) reducing mixed-culture of bacteria is injected into the

aquifer and allowed to acclimate to the new conditions. This facilitates *in situ* selection for adaptable organisms. In order for the organisms to flourish in the new environment, the prevailing conditions in the environment must just be suitable for the organisms and this is difficult to predict in advance.

A more futuristic approach is the *in situ* molecular augmentation in which transposable elements carrying the metal reducing genes could be introduced into the environment to be taken up by native bacteria in the environment. Upon assimilation of the foreign genetic elements, the native bacteria could then become competent in neutralising the targeted pollutant(s). In this way, importation of foreign bacteria across ecosystems could be avoided. Genetic carriers such as transposons and plasmids have been used in the experiments to evaluate this process by shuttling genetic information for toxic metal remediation into native species that are already best suited to the target environment. Several species of bacteria are capable of picking up and retaining circular fragments of DNA called Broad-Host-Range Plasmids which may be engineered to carry specific genes for the degradation of xenobiotic compounds and transformation of toxic metals (Vincze and Bowra, 2006).

A similar process can be applied using genetically engineered linear DNA called transposons. Although studies have been conducted using these techniques in laboratory microcosms, the application in actual environments has not been attempted (Hill *et al.*, 1994). In the future, it is foreseeable that these methods will find wide application for the new varieties of recalcitrant pollutants being discharged into the environment from several sources.

10.3 Microcosm performance

Cores from an actual contaminated site were set up in the laboratory as microcosm reactors as shown in Figure 7. Contaminant loading was simulated by gravity feeding as is the case in open aquifers a representative Cr(VI) polluted site in Brits (North West Province, South Africa). The experimental systems were installed and operated as packed-bed reactors. All microcosm reactors were operated under a feed concentration of 40 mg/L, representing the observed concentration at the actual site (Brits). 1 mL samples drawn from the influent and effluent were centrifuged at 6000 rpm (2820 × g) for 10 minutes to remove soil particles followed by analysis for Cr(VI) and total Cr as described below.

The microcosm reactors were operated without any added organic carbon sources in the feed solution and no minerals apart from those already found in the soil. Since the system was being developed for possible application in the groundwater environment, introduction of potentially polluting organic carbon sources is not desirable. Autotrophic organisms in the soil are thus expected to use bicarbonate (HCO_3^-) as carbon source and nutrients from soil and decaying vegetation overlying the soil. Efforts are under way to characterise the composition of the organic matter coming from the soil using TOC, DIC, and GC/MS analysis.

The experiments consisted of two non-sterile reactors (R1 and R4) containing native bacteria from the soil, two sterile reactors (R2 and R5) sterilized by autoclaving at 121°C for 30 min., and two consortium inoculated non-sterile reactors (R3 and R6) containing bacteria from dried activated sludge and native soil bacteria. All reactors were operated under a feed concentration of 40 mg/L.
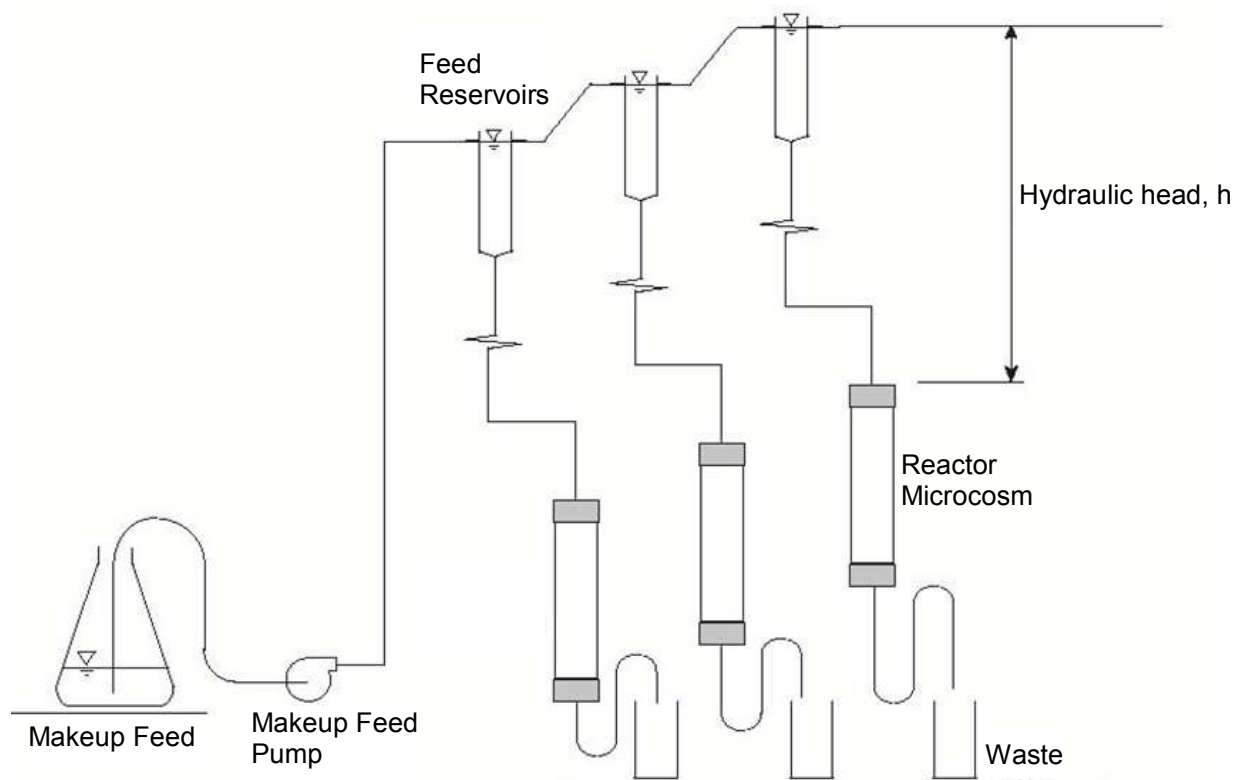
Fig. 7. Experimental setup of the gravity-fed microcosm reactor system.

Columns that experienced severe short-circuiting (R4 and R5) were discontinued. Only reactors R1, R2, R3, and R6 were fully tested. Since the cores were extracted from approximately the same depth at the site, the resistance to flow was almost the same with higher flow rates observed in Reactors 1 and 3.

Data collected showed that one of the columns inoculated with Cr(VI) reducing bacteria (R6) achieved near complete removal of Cr(VI), however, the effectiveness of removal was relatively low at a higher hydraulic loading rate (data not shown). Chromium removal of approximately 95% was observed in the slow feeding reactor R6 (flow rate, Q = 0.310 cm^3/hr) (Figure 8). The removal rate was lower, approximately 80%, in the column with a higher flow rate of 0.608 cm^3/hr (R3). No Cr(VI) removal was observed in the sterilised and in the non-inoculated (native bacteria) controls. The performance of the reactors under different loading conditions is summarised in Table 2.

These experiments clearly show that it is possible to introduce microbial cultures into the environment in a controlled way to achieve Cr(VI) reduction in flowing water. The results do not show how the reduced Cr species, suspected to be predominantly Cr^{3+}, could be remobilised and extracted from the barrier zone once it starts affecting the hydraulic conductivity of the barrier.

10.4 Microbial culture analysis
10.4.1 Characteristics of initial consortium

The robustness of the barrier system was evaluated by monitoring the survival of microorganisms from the Cr(VI) reducing inoculum in the microcosm simulating the aquifer environment. The original inoculum was obtained from dry sludge from sand drying beds
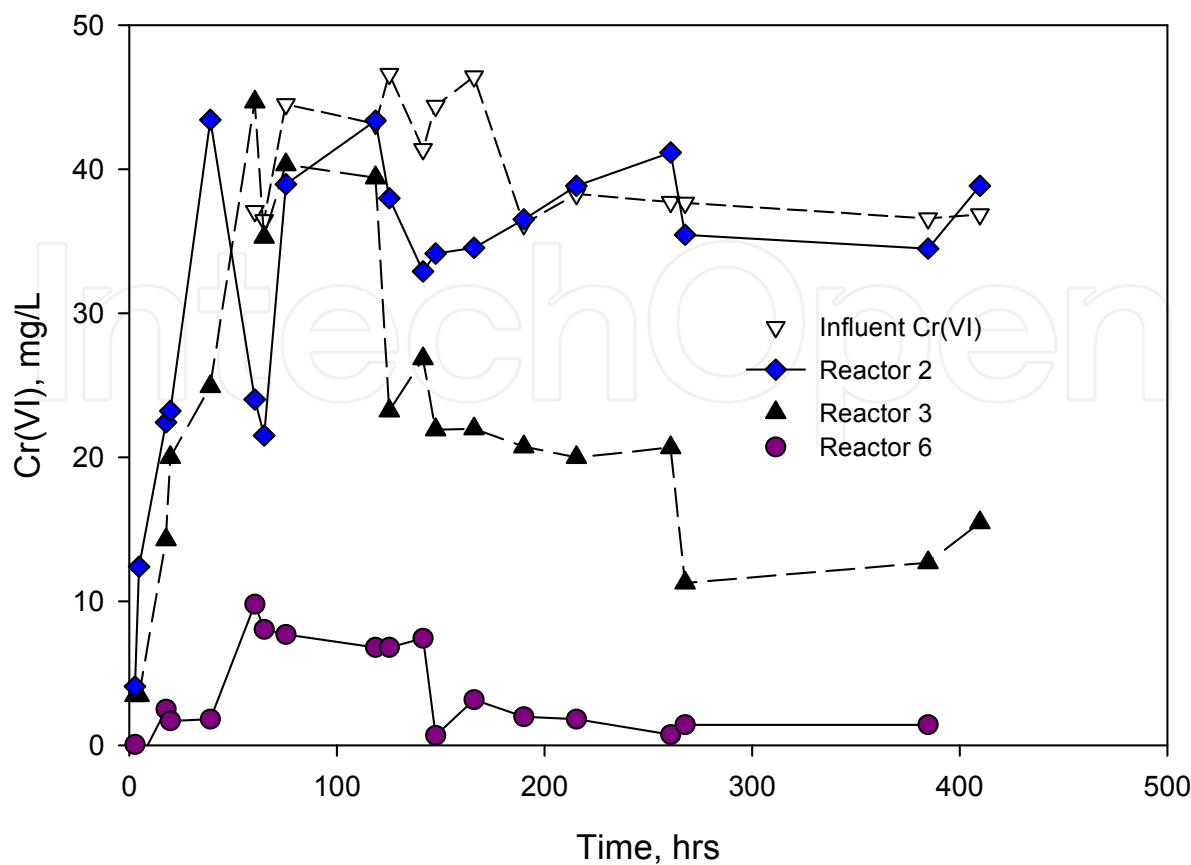
Fig. 8. Cr(VI) reduction in microcosm reactors: Reactor 2 = sterilised column, Reactor 3 = inoculated non-sterile reactor, and Reactor 6 = inoculated non-sterile reactor.

at Brits Sewerage Works (Brits, SA). The sludge bacteria used to inoculate enrichment cultures under microaerobic conditions (under 100 mg/L Cr(VI)) and the colonies isolated based on morphology were further purified and analysed. The predominant species under these enrichment conditions were the Gram-positive *Bacilli* mainly due to inhibition of anaerobic species by oxygen in the sample. Partial sequences of 16S rRNA matched the *Bacillus* groups – *Bacillus cereus* ATCC 10987, *Bacillus cereus* 213 16S, *Bacillus thuringiensis* (serovar finitimus), *Bacillus mycoides* – and two Microbacterium group – *Microbacterium foliorum* and *Microbacterium sp*. S15-M4 (Table 3). A phylogenetic tree was constructed for the species from purified cultures grown under aerobic conditions based on a basic BLAST search of rRNA sequences in the NCBI database (Figure 9).

Reactor Number and Type	Effluent Cr(VI) Conc. mg/l	Effluent Cr(III) Conc. mg/l	Cr(VI) Removal %
Native-soil R1	39.0 ± 2.0	0.0 ± 0.0	0.0 ± 0.0
Non inoculated R2	37.8 ± 1.5	0.0 ± 0.0	0.0 ± 0.0
Inoculated R3	6.7 ± 0.8	1.5 ± 0.4	80 ± 3.6
Inoculated R6	1.9 ± 0.3	3.2 ± 1.1	95.3 ± 1.4

Table 2. Performance of gravity-fed microcosm reactors operated under an influent Cr(VI) concentration of 40 mg/L (0.310 cm^3/h).
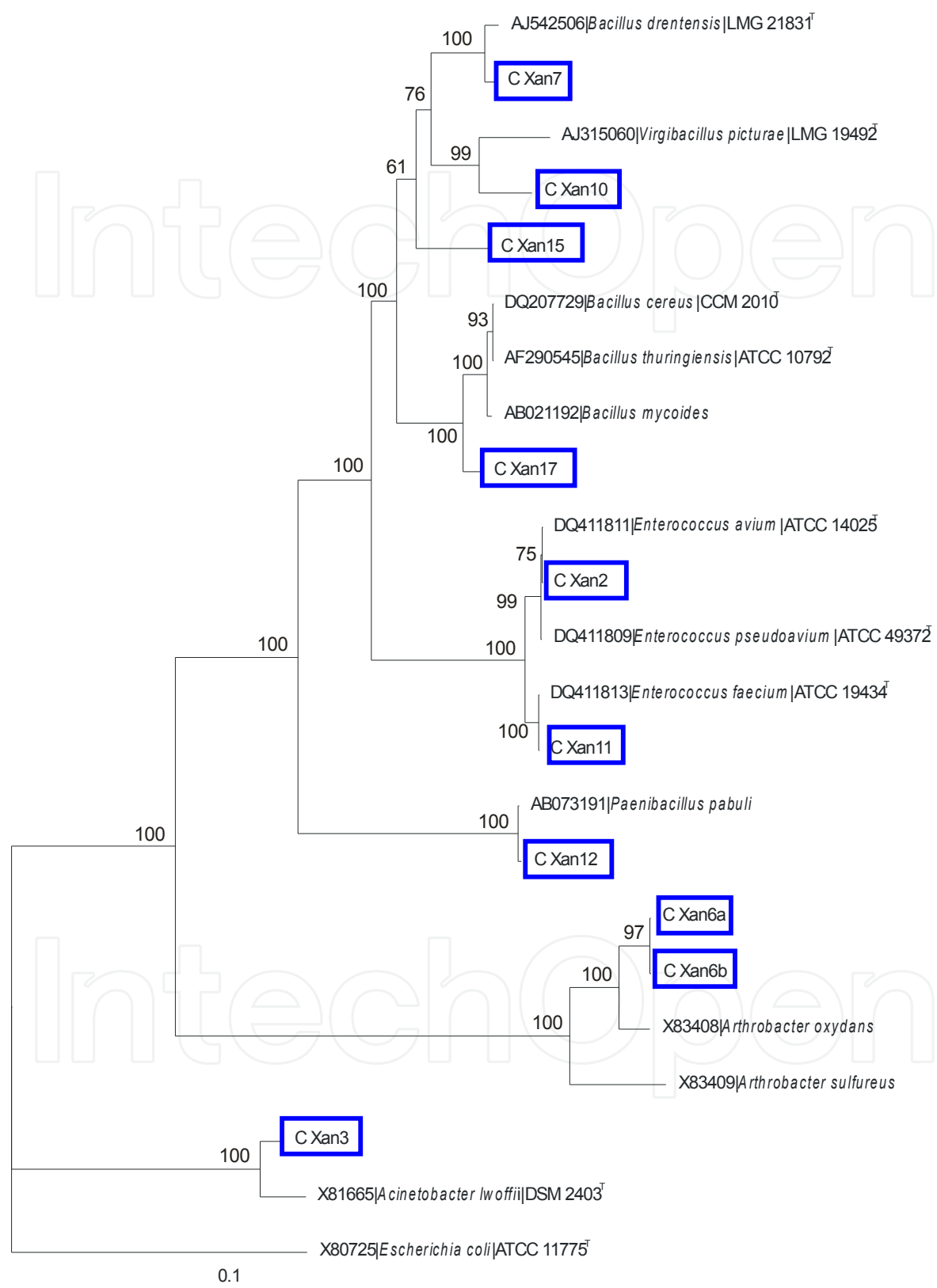
Fig. 9. Phylogenetic tree of species from Brits dry sludge reflecting microbial diversity under anaerobic conditions.

Inoculation culture Consortium		Culture in Reactor 3 and 6 at end of experiment	
Type	**Predominant species**	**Type**	**Predominant species**
X1	*Bacillus cereus* 213 16S, *Bacillus thuringiensis* 16S	A	*Pantoea* or *Enterobacter sp.*
X2	*Bacillus cereus* ATCC 10987, *Bacillus thuringiensis* str. Al Hakam	B	*Bacillus sp.* possibly *Bacillus thuringiensis/ cereus* group
X3	*Bacillus cereus* ATCC 10987, *Bacillus thuringiensis* str. Al Hakam	C	*Pantoea* or *Enterobacter sp.*
X4	*Bacillus mycoides* BGSC 6A13 16S. *Bacillus thuringiensis serovar finitimus* BGSC 4B2 16S	D	*Lysinibacillus sphaericus* strain BG-B111, *Bacillus sp.* G1DM-64, *Bacillus sphaericus*
X5	*Bacillus mycoides* BGSC 6A13 16S. *Bacillus thuringiensis serovar finitimus* BGSC 4B2 16S	E	*Bacillus sp.* possibly *Bacillus thuringiensis/ cereus* group
X6	*Bacillus mycoides* BGSC 6A13 16S. *Bacillus thuringiensis serovar finitimus* BGSC 4B2 16S	F	*Bacillus sp.* possibly *Bacillus thuringiensis/ cereus* group
X7	*Bacillus mycoide* BGSC 6A13 16S. *Bacillus thuringiensis serovar finitimus* BGSC 4B2 16S	G	*Bacillus cereus* strain ZB

Table 3. Microbial culture changes after operation of the microcosms reactors for 15 days under an influent Cr(VI) concentration of 40 mg/L.

10.4.2 Characterisation of microcosm bacteria (after 15 days)

After operating the reactors under oxygen stressed conditions in the presence of other soil bacteria, a community shift was expected. In reactors R3 and R6, the soil contained a wide range of soil dwelling species of bacteria as well as the newly introduced bacteria from the sand drying bed sludge. The microbial dynamics monitored by the 16S rRNA fingerprinting showed a decrease in culturable species after exposure to Cr(VI) as shown in Tables 3. Only the *B. cereus* and *B. thirungiensis* serotypes persisted either due to resilience against toxicity or adaptation to the changing conditions in the reactor. The *Lysinibacillus* group is also a well known sludge bacteria. Both Bacilli (*B. cereus* and *B. thuringiensis*) and the *Lysinibacillus* species contain well known Cr(VI) reducing serotypes such as *Bacillus* K1 (Shen *et al.*, 1996), *Bacillus cereus*, *Bacillus thirungiensis* (Camargo *et al.*, 2003), and *Lysinibacillus sphaericus* AND 303 (Pal *et al.*, 2005).

10.4.3 Culture composition at the end of experiment

Several species from the original sludge culture disappeared from the consortium after operating the microcosm reactors for 17-20 days. Instead, other species not originally observed appeared in the reactors (Figure 10). Some species in the samples also showed associations with gram-negative species belonging to the Enterococcus and Escherichia groups. These results confirmed the adaptability of the cultures at the community level. Linked with the performance data, the results suggest that more competent species were selected after a long time of exposure to Cr(VI).
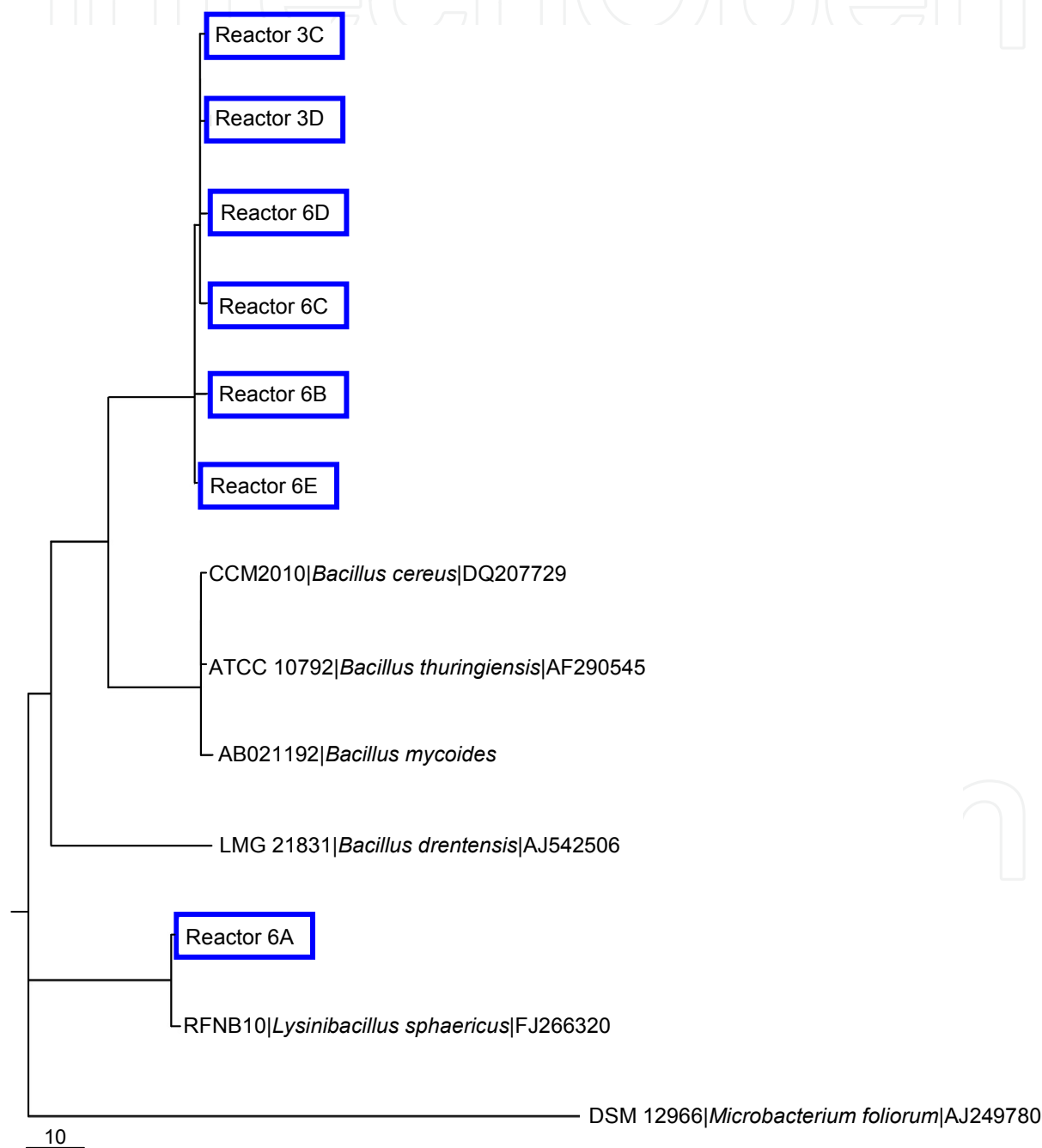
Fig. 10. Phylogenetic tree of species from microcosm reactors after operation under 40 mg/L influent Cr(VI) concentration for 17-20 days.

11. Conclusion

Since the first Cr(VI) reducing bacteria were isolated in the 1970's, a lot of progress has been made in isolating and developing higher performing cultures adapted to various environments. New research using genetic tools has yielded new cultures and new understanding of the Cr(VI) reduction process both at the molecular level [through genetic studies] and at culture community level [through genomics and proteomics]. Pure and mixed cultures of bacteria have been applied successfully in treating industrial effluents containing high levels of Cr(VI). However, application of biological systems in the remediation of contaminated environments still faces a challenge. Although culture performance under natural conditions has been evaluated using laboratory microcosms, more research is still required to elucidate the fate and possibility of recovery of artificial microbial barriers. The question of the fate of reduced Cr species and what to do about the foreseeable blockage by hydroxide species remains unanswered. In order for the *in situ* bioremediation technology to work for Cr(VI) and other toxic heavy metals, a solution must be found for feasible recovery of the barrier zones involving remobilisation of reduced Cr species.

12. Acknowledgement

13. References

Ackerley, D.F.; Gonzalez, C.F.; Park, C.H.; Blake R.; Keyhan, M. & Matin A. (2004). Chromate-Reducing Properties of Soluble Flavoproteins from *Pseudomonas putida* and *Escherichia coli. Applied And Environmental Microbiology*, Vol.20, No.2, (February 2004), p. 873-882, ISSN 0099-2240.

Beszedits, S. (1988). Chromium Removal from Industrial Wastewaters, In: *Chromium in the Natural and Human Environments*, pp. 232-263, Nriagu, O. & Nieboer E. (Eds.), John Wiley, ISBN 978-0471856436, New York, New York, USA.

Bopp, L.H.; Chakrabarty, A.M. & Ehrlich, H.L. (1983). Chromate Resistance Plasmid in *Pseudomonas fluorescens. Journal of Bacteriology*, Vol.155, No.3, (September 1983), pp. 1105-1109, ISSN 0021-9193.

Bopp, L.H. & Ehrlich, H.L. (1988). Chromate Resistance and Reduction in *Pseudomonas fluorescens* Strain LB300. *Archives of Microbiology*, Vol.150, No.5, (September 1988), pp. 426-431, ISSN 0302-8933.

Brown, S.D.; Thompson, M.R.; Verberkmoes, N.C.; Chourey, K.; Shah, M.; Zhou, J.Z.; Hettich, R.L. & Thompson, D.K. (2006). Molecular Dynamics of the *Shewanella oneidensis* Response to Chromate Stress. *Molecular Cell Proteomics*, Vol.5, No.3, (March 2006), pp. 1054-1071, ISSN 535-9476.

Bruhn, D.F.; Frank, S.M.; Roberto, F.F.; Pinhero, P.J. & Johnson, S.G. (2009). Microbial Biofilm Growth on Irradiated, Spent Nuclear Fuel Cladding. *Journal of Nuclear Materials*, Vol.384, No.2, (February 2009), pp. 140-145, ISSN 0022-3115.

Camargo, F.A.O.; Bento, F.M.; Okeke, B.C. & Frankenberger, W.T. (2003). Chromate Reduction by Chromium-Resistant Bacteria Isolated from Soils Contaminated with Dichromate. *Journal of Environmental Quality*, Vol.32, No.4, (July 2003), pp. 1228-1233, ISSN 0047-2425.

Cervantes, C.; Campos-Garcia, J.; Devars, S.; Gutierrez-Corona, F.; Loza-Tavera, H.; Torres-Guzman, J.C. & Moreno-Sanchez, R. (2001). Interactions of Chromium with Microorganisms and Plants. *FEMS Microbiology Review*, Vol.25, No.3, (May 2001), pp. 335-347, ISSN 0168-6445.

Chabalala, S. & Chirwa, E.M.N. (2010). Uranium(VI) Reduction and Removal by High Performing Purified Anaerobic Cultures from Mine Soil. *Chemosphere*, Vol.78, No.1, (January 2010), pp. 52-55, ISSN 0045-6535.

Chen, Y. & Gu, G. (2005). Preliminary studies on continuous chromium(VI) biological removal from wastewater by anaerobic-aerobic activated sludge process. *Bioresource Technology*, Vol.96, No.15, (October 2005), pp. 1713-1721, ISSN 0960-8524.

Chirwa, E.M.N. & Wang, Y.-T. (1997a). Hexavalent Chromium Reduction by *Bacillus sp.* in a Packed-Bed Bioreactor. *Environmental Science and Technology*, Vol.31, No.5, (May 1997), pp. 1446-1451, ISSN 0013-936X.

Chirwa, E.M.N. & Wang, Y.-T., (1997b). Chromium(VI) Reduction by *Pseudomonas fluorescens* LB300 in Fixed-Film Bioreactor. *Journal of Environmental Engineering*, Vo.123, No.8, (August 1997), pp. 760–766, ISSN 0733-9372.

Chirwa, E.N. & Wang, Y.-T. (2000). Simultaneous Cr(VI) Reduction and Phenol Degradation in an Anaerobic Consortium of Bacteria. *Water Research*, Vol.34, No.8, (August 2000), pp. 2376-2384, ISSN 0043-1354.

Cheung, K.H. & Gu, J.D. (2007). Mechanism of Hexavalent Chromium Detoxification by Microorganisms and Bioremediation Application Potential: A Review. *International Biodeterioration and Biodegradation*, Vol.59, No.1, (January 2007), pp. 8-15, ISSN 0964-8305.

Dakiky, M.; Khamis, M.; Manassra, A. & Mer'eb, M. (2002). Selective Adsorption of Cr(VI) in Industrial Waste Water Using Low-Cost Abundantly Available Adsorbents. *Advances in Environmental Research*, Vol.6, No.4, (October 2002), pp. 533–540, ISSN 1093-0191.

Dickerson, R.E. (1980). Cytochrome c and the Evolution of Energy Metabolism. *Scientific American*, Vol.242, No.1, (January 1980), pp. 136-153, ISSN 0036-8733.

Eary, L.E. & Rai, D. (1988). Chromate Removal from Aqueous Wastes by Reduction with Ferrous Ion. *Environmental Science and Technology*, Vol.22, No.8, (August 1988), pp. 972-977, ISSN 0013-936X.

Evanko, C.R. & Dzombak, D.A. (1997). Remediation of Metals-Contaminated Soils and Groundwater. Technology Evaluation Report. Ground-Water Remediation Technologies Analysis Center, Pittsburgh, Pennsylvania, USA. Available online <http://www.clu-in.org/download/toolkit/metals.pdf>

Federal Register, (2004). Occupational Safety and Health Administration. Occupational Exposure to Hexavalent Chromium. *69 Federal Register 59404*. October 4, 2004.

Francis, C.A.; Obraztsova, A.Y. & Tebo, B.M. (2000). Dissimilatory Metal Reduction by the Facultative Anaerobe *Pantoea agglomerans* SP1. *Applied and Environmental Microbiology*, Vol.66, No.2, (February 2000), pp. 543-548, ISSN 0099-2240.

Ganguli, A. & Tripathi, A.K. (2002). Bioremediation of Toxic Chromium from Electroplating Effluent by Chromate-Reducing *Pseudomonas aeruginosa* A2 Chr in Two Bioreactors. *Applied Microbiology and Biotechnology*, Vol.58, No.3, (March 2002), pp. 416–420, ISSN 0175-7598.

Garbisu, C.; Alkorta, I.; Llama, M.J. & Serra, J.L. (1998). Aerobic Chromate Reduction by *Bacillus subtilis*. *Biodegradation*, Vol.9, No.2, (March 1998), pp. 133-141, ISSN 0923-9820.

Garrels, R.M. & Christ, C.L. (1965). In *Solutions, Minerals and Equilibria*, pp. 403-435. Harper and Row Publishers, ISBN 978-0867201482, New York, New York, USA.

Guha, H.; Jayachandran, K. & Maurrasse, F. (2001). Kinetics of Chromium (VI) Reduction by a Type Strain *Shewanella alga* under Different Growth Conditions. *Environmental Pollution*, Vol.115, No.2, (December 2001), pp. 209-218, ISSN 0269-7491.

He, M.; Li, X.; Guo, L.; Miller, S.J.; Rensing, C. & Wang, G. (2010). Characterization and Genomic Analysis of Chromate Resistant and Reducing *Bacillus cereus* Strain SJ1. *BMC Microbiology*, Vol.10:221, (2010), pp. 1-10. Available online < http://www.biomedcentral.com/1471-2180/10/221>

Hill, K.E.; Fry, J.C. & Weightman, A.J. (1994). Gene Transfer in the Aquatic Environment: Persistence and Mobilization of the Catabolic Recombinant Plasmid pDlO in the Epilithon. *Microbiology*, Vol.140, No.7, (July 1994), pp. 1555-1563, ISSN 1350-0872.

Horitsu, H.; Futo, S.; Miyazawa, Y.; Ogai, S. & Kawai, K. (1987). Enzymatic Reduction of Hexavalent Chromium by Hexavalent Tolerant *Pseudomonas ambigua* G-1, *Agricultural and Biological Chemistry*, Vo.51, No.9, (September 1987), pp. 2417-2420, ISSN 0002-1369.

Ishibashi, Y.; Cervantes, C. & Silver, S. (1990). Chromium Reduction in *Pseudomonas putida*. *Applied and Environmental Microbiology*, Vol.56, No.7, (July 1990), pp. 2268-2270, ISSN 0099-2240.

Jukes, T.H. & Cantor, C.R. (1969). Evolution of Protein Molecules, In: *Mammalian Protein Metabolism*, pp. 21-123, Munro, H.N. (Ed.), Academic Press, ISBN 9780125106047, New York, New York, USA.

Li, X. & Krumholz, L.R. (2007). Regulation of Arsenate Resistance in *Desulfovibrio desulfuricans* G20 by an *arsRBCC* Operon and an *arsC* Gene. *Journal of Bacteriology*, Vol.189, No.10, (May 2007), pp. 3705-3711, ISSN 0021-9193.

Li, X. & Krumholz, L.R. (2009). Thioredoxin Is Involved in U(VI) and Cr(VI) Reduction in *Desulfovibrio desulfuricans* G20. *Journal of Bacteriology*, Vol.191, No.15, (August 2009), pp. 4924-4933, ISSN 0021-9193.

Llovera, S.; Bonet, R.; Simon-Pujol, M. & Congregado, F. (1993). Chromate Reduction by Resting Cells of *Agrobacterium radiobacter* EPS-916. *Applied and Environmental Microbiology*, Vol.59, No.10, (October 1993), pp. 3516-3518, ISSN 0099-2240.

Lloyd, J.R. (2003). Microbial Reduction of Metals and Radionuclides. *FEMS Microbiology Reviews*, Vol.27, No.2-3, (2003), (June 2003), pp. 411-425, ISSN 0168-6445.

Merian, E. (1984). Introduction on Environmental Chemistry and Global Cycles of Arsenic, Beryllium, Cadmium, Chromium, Cobalt, Nickel, Selenium, and Their Derivatives. *Toxicological and Environmental Chemistry*, Vol.8, (1984), pp. 9-38, ISSN 0277-2248.

Mertz, W. (1974). Chromium as a Dietary Essential for Man, In: *Trace Elements Metabolism*, ed. Hoekstra, W. G., Suttie, J. W., Ganther, K. E., and Mertz, (Eds.), *Proceedings of the Second International Symposium of Trace Elements Metabolism in Animals*, pp. 185-198, University Park Press, Baltimore, Maryland, USA.

Molokwane, P.E. & Chirwa, E.M.N. (2009) Microbial Culture Dynamics and Chromium (Vi) Removal in Packed-Column Microcosm Reactors. *Water Science and Technology*, Vol.60, No.2, (July 2009), pp. 381-388, ISSN 0273-1223.

Molokwane, P.E.; Nkhalambayausi-Chirwa, E.M. & Meli, K.C. (2008). Chromium (VI) Reduction in Activated Sludge Bacteria Exposed to High Chromium Loading: Brits Culture (South Africa). *Water Research*, Vol.42, No.17, (October 2008), pp. 4538-4548, ISSN 0043-1354.

Myers, C.R.; Carstens, B.P.; Antholine, W.E. & Myers, J.M. (2000). Chromium(VI) Reductase Activity is Associated with the Cytoplasmic Membrane of Anaerobically Grown *Shewanella putrefaciens* MR-1. *Journal of Applied Microbiology*, Vol.88, No.1, (January 2000), pp. 98-106, ISSN 1364-5072.

NAS (1974). The Relation of Selected Trace Elements to Health and Disease, In: *Geochemistry and the Environment - I.*, pp. 533. US National Academy of Engineering, Washington DC, USA.

Ngwenya N. and Chirwa E.M.N. (2011). Biological Removal of Cationic Fission Products from Nuclear Wastewater. *Water Science and Technology*, Vol.63, No.1, (January 2011), pp. 124-128, ISSN 0273-1223.

Nkhalambayausi-Chirwa, E.M. & Wang, Y.-T. (2001). Simultaneous Chromium(VI) Reduction and Phenol Degradation in a Fixed-Film Coculture Bioreactor: Reactor Performance. *Water Research*, Vol.35, No.8, (August 2001), pp. 1921-1932, ISSN 0043-1354.

Nkhalambayausi-Chirwa, E.M. & Wang, Y.-T. (2005). Modeling Cr(VI) Reduction and Phenol Degradation in a Coculture Biofilm Reactor. *ASCE Journal of Environmental Engineering*, Vol.131, No.11, (November 2005), pp. 1495-1506, ISSN 0733-9372.

Ohtake, H.; Fujii, E. & Toda, K. (1987). Reduction of toxic chromate in an industrial effluent by use of a chromate-reducing strain of *Enterobacter cloacae*. *Environmental Technology Letters*, Vo.11, No.7, (July 1990), pp. 663-668, ISSN 0143-2060.

Pal, A.; Sumana Dutta, S. & Paul, A.K. (2005). Reduction of Hexavalent Chromium By Cell-Free Extract of *Bacillus sphaericus* AND 303 Isolated from Serpentine Soil. *Current Microbiology*, Vol.51, No.5, (November 2005), 327-330, ISSN 0343-8651.

Park, C.H., Keyhan, M., Wielinga, B., Fendorf, S. & Matin, A. (2000). Purification to Homogeneity and Characterization of a Novel *Pseudomonas putida* Chromate Reductase. *Applied and Environmental Microbiology*, Vol.66, No.5, (May 2000), pp. 1788-1795, ISSN 0099-2240.

Park, C. H.; Gonzalez, C. F.; Ackerley, D. F.; Keyhan, M. & Matin, A. (2002). Molecular Engineering of Soluble Bacterial Proteins with Chromate Reductase Activity, In: *Remediation and Beneficial Reuse of Contaminated Sediments*, pp. 103–111., Hinchee, R.E. Porta, A. & Pellei, M. (Eds.), Batelle Press, ISBN 978-1574771299, Columbus, Ohio, USA.

Parmar, N.; Warren, L.A.; Roden, E.E. & Ferris, F.G. (2000). Solid Phase Capture of Strontium by The Iron Reducing Bacteria *Shewanella alga* Strain BrY. *Chemical Geology*, Vol.169, No.3-4, (September 2000), pp. 281–288, ISSN 0009-2541.

Romanenko, V.I. & Koren'kov, V.N. (1977). A Pure Culture of Bacteria Utilizing Chromate and Dichromate as Hydrogen Acceptors in Growth under Anaerobic Conditions. *Mikrobiologiya*, Vol.46, pp. 414-417, ISSN 0026-3656.

Shen, H. & Wang, Y.T. (1993). Characterization of Enzymatic Reduction of Hexavalent Chromium by *Escherichia coli* ATCC 33456. *Applied and Environmental Microbiology*, Vol.59, No.11, (November 1993), pp. 3771-3777, ISSN 0099-2240.

Shen, H. & Wang Y T. (1994). Modeling Hexavalent Chromium Reduction in *Escherichia coli* ATCC 33456. *Biotechnology and Bioengineering*, Vol.43, No.4, (April 1994), pp. 293-300, ISSN 0006-3592.

Shen, H., Pritchard, P.H. & Sewell, G.W. (1996). Microbial Reduction of Cr(VI) during Anaerobic Degradation of Benzoate. *Environmental Science and Technology*, Vol.30, No.5, (April 1996), pp. 1667-1674, ISSN 0013-936X.

Stoodley, P.; DeBeer D.; Boyle, J.D. & Lappin-Scott H.M. (1999). Evolving Perspectives of Biofilm Structure. *Biofouling*, Vol.14, No.1, (January 1999), pp. 75-94, ISSN 0892-7014.

Thacker, U.; Parikh, R.; Shouche, Y. & Madamwar, D. (2006). Hexavalent Chromium Reduction by *Providencia sp.*. *Process Biochemistry*, Vol.41, No.6, (June 2006), pp. 1332-1337, ISSN 1359-5113.

Vidic, R.D. & Pohland, F.G. (1996). Treatment Walls, *Technology Evaluation Report TE-96-01*, Ground-Water Remediation Technologies Analysis Center, Pittsburgh, PA, USA. Available online <http://www.clu-in.org/download/remed/tmt_wall.pdf>

Vincze, E. & Bowra, S. (2006). Transformation of Rhizobia with Broad-Host-Range Plasmids by Using a Freeze-Thaw Method. *Applied and Environmental Microbiology*, Vol.72, No.3, (March 2006), pp. 2290–2293, ISSN 0099-2240.

Wang, P.C.; Mori, T.; Komoril, K.; Sasatsu, M.; Toda, K. & Ohtake, H. (1989). Isolation and Characterization of an Enterobacter cloacae Strain that Reduces Hexavalent Chromium under Anaerobic Conditions. *Applied and Environmental Microbiology*, Vol.55, No.7, (July 1989), pp. 1665–1669, ISSN 0099-2240.

Zakaria, Z.A.; Zakaria, Z.; Surif, S. & Ahmad, W.A. (2007). Biological Detoxification of Cr(VI) using Wood-Husk Immobilized *Acinetobacter haemolyticus*. *Journal of Hazardous Materials*, Vol.148, No.1-2, (September 2007), pp. 164-171, ISSN 0304-3894.

Zhiguo, H.; Fengling, G.; Tao, S.; Yuehua, H. & Chao, H. (2009). Isolation and Characterization of a Cr(VI)-Reduction *Ochrobactrum sp.* strain CSCr-3 from Chromium Landfill. *Journal of Hazardous Materials*, Vol.163, No.2-3, (April 2009), pp. 869-873, ISSN 0304-3894.

Zhu, W.; Chai, L.; Ma, Z.; Wang, Y.; Xiao, H. & Zhao, K. (2008). Anaerobic Reduction of Hexavalent Chromium by Bacterial Cells of *Achromobacter sp.* Strain Ch1. *Microbiological Research*, Vol.163, No.6, (November 2008), pp. 616-623, ISSN 0944-5013.

Biodiversity

Edited by Dr. Adriano Sofo

ISBN 978-953-307-715-4

Hard cover, 138 pages

Publisher InTech

Published online 10, October, 2011

Published in print edition October, 2011

Biodiversity is strongly affected by the rapid and accelerating changes in the global climate, which largely stem from human activity. Anthropogenic activities are causing highly influential impacts on species persistence. The sustained environmental change wildlife is experiencing may surpass the capacity of developmental, genetic, and demographic mechanisms that populations have developed to deal with these alterations. How biodiversity is perceived and maintained affects ecosystem functioning as well as how the goods and services that ecosystems provide to humans can be used. Recognizing biodiversity is essential to preserve wildlife. Furthermore, the measure, management and protection of ecosystem biodiversity requires different and innovative approaches. For all these reasons, the aim of the present book is to give an up-to-date overview of the studies on biodiversity at all levels, in order to better understand the dynamics and the mechanisms at the basis of the richness of life forms both in terrestrial (including agro-ecosystems) and marine environments.

INTECH

open science | open minds