POLITECNICO DI MILANO
DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING
DOCTORAL PROGRAMME IN ENVIRONMENTAL AND INFRASTRUCTURE ENGINEERING

IN SITU TECHNOLOGIES FOR THE TREATMENT OF HEXAVALENT CHROMIUM

Doctoral Dissertation of:
Andrea Filippo Mastorgio

Supervisor:
Eng. Elena Sezenna

Tutor:
Prof. Sabrina Saponaro

The Chair of the Doctoral Program:
Prof. Riccardo Barzaghi

31st Cycle – 2015/2019

RINGRAZIAMENTI

Un saluto al gruppo bonifiche del Politecnico di Milano, orgogliosamente, potrò raccontare di averne fatto parte in questi anni; il grazie di cuore per il raggiungimento di questo obiettivo a tutti coloro che hanno lavorato con me in questo progetto e, soprattutto, a Sabrina, Elena e Gabriele.

Grazie di tutto agli amici che mi hanno sostenuto, ed un pensiero alla mia famiglia per un futuro sereno.

ABSTRACT

Chromium is one of the most frequently used metal contaminants. Its hexavalent form Cr(VI), which is exploited in many industrial activities, is a known human carcinogen and, being water-soluble in the full pH range, represents a major threat to groundwater resources. Many polluted sites show extensive Cr(VI) plumes in groundwater.

The traditional approach to Cr(VI) remediation by contaminated soil excavation and off-site disposal and groundwater remediation through the “Pump and Treat" method is still the selected option at most sites, even new in situ technologies have been developed in recent decades up to full scale and nowadays accounts for many field applications. These technologies include chemical-physical removal processes through electrokinetic and soil flushing, chemical reduction with iron or sulfur-based reagents, or bio-induced reduction with registered brand reagents. Due to the complex behavior of chromium in the environment and to its interaction with numerous chemical species in the subsurface, careful evaluations of site-specific conditions and maybe specific tests are required for selecting the best approach to site remediation.

In the first part of this thesis, a thorough review of existing literature on the environmental behavior of chromium and available technologies made it possible to identify significant scenarios of Cr(VI) contamination and feasible approaches to Cr(VI) remediation in the different contexts, leading to the definition of selection criteria for preliminary assessment of potential exclusion of some technologies. The main factors that influence to the choice include: i) pH (5-7; 7-9), ii) Cr(VI) concentration (below/above 100 mg/kg unsaturated soil or 10 mg/l in groundwater), iii) iron availability in the soil (above/below 1 g_{Fe}/kg) and iv) soil heterogeneity (variation in hydraulic conductivity or intrinsic permeability within or more than 2 orders of magnitude).

The experimental part of the work aimed to investigate in situ bioremediation, and the remedial option with the greatest potential for combining efficiency and sustainability, both in terms of costs and environmental impacts.
Microorganisms, thanks to their adaptability and metabolic versatility, are capable of not relying solely on various toxic compounds for carbon or energy sources, but also of adopting different detoxification strategies in order to adapt and survive in contaminated environments. Biological activity in the subsurface can be stimulated to ensure, proper environmental conditions (e.g. neutral pH, electron acceptors availability, …), by means of external supplies of substrates, nutrients and electron acceptors. Experimental activities investigated:

(a) Bio-induced reduction, relying on the injection of rapidly biodegradable organic substrates to promote reducing conditions into the aquifer, viable for Cr(VI) reduction.

(b) Bioelectrochemical systems (BESs), taking advantage of the ability of several microrganisms to make use of solid electrodes as electron acceptors or donors to carry out their own metabolic reactions.

Bio-induced reduction technology has already been used in full-scale remedial works, traditionally done using expensive trademark registered products. As alternative organic substrates, two different cheap food industry by-products (ultrafiltration permeate of cheese whey and waste from brewing processes) have been investigated in terms of removal of Cr(VI) from groundwater and of the kinetics of the process in lab-scale batch tests. Batch microcosms using 5 and 10 mg/L Cr(VI) initial concentration, two different soils, and 25% or 50% solid/liquid ratios were set up. Important removal of dissolved Cr(VI) was observed at the end of the total incubation time (approximately 40 days). Key factors to Cr(VI) removal in induced bioremediation are the initial concentration of Cr(VI) and the availability of Fe(II) for Cr(III) co-precipitation. Precipitation of the reduced chemical form is not instantaneous; stable redox potentials below -200 mV vs SHE were required to observe Cr(VI) reduction. Higher Cr(VI) abatements were observed in the soil with a higher total heterotrophic bacteria concentration (10^4 vs 10 CFU/$g_{d.w.}$).
The use of inexpensive by-products can be considered as a possible alternative to registered products for Cr(VI) bio-induced reduction, with interesting prospects in terms of limiting costs and environmental impacts. A peculiar limitation with by-products is the difficult definition of the appropriate dose to be used to suit to site-specific conditions.
Doses that are too high can cause negative secondary effects in the aquifer and the accumulation of residues and metabolites, conversely, an insufficient supply produces a stall in Cr(VI) reduction. Furthermore, with bio-induced reduction, chromium precipitates throughout the contaminated area with no concrete chance of recovering the metal, which possibly undergoes partial re-oxidation with time.

BESs is an innovative technology, with only some positive laboratory experiences in soil/sediment treatment reported so far. Most of the available data refers to exposure to electrostatic fields or electrical fields generated by direct current, already exploited in electrokinetic processes.
Most of the experiences available in literature on Cr(VI) treatment with BES have been focused on wastewater treatment combined with energy recovery in Microbial Fuel Cells (MFC). Such systems use Cr(VI) at the cathode as an effective electron acceptor of the electrons resulting from the oxidation of organic substances at the anode. The high chemical reduction potential of Cr(VI), especially at acidic pH, is responsible for energy production. In the case of groundwater, an external power supply is required due to a low concentration of oxidable organic matter and a pH typically around neutral value.
The research activity focused on the design and development of lab-scale BESs to carry out Cr(VI) reduction tests. Lab-scale batch and continuous tests in microbial 3-

electrode cells (M3Cs) were used to investigate Cr(VI) removal in polluted water at 1-2 mg/l Cr(VI). To run these systems an potentiostat was used that had been built in-house, to set the biocathode potential in the range -300 mV and +700 mV vs Standard Hydrogen Electrode (SHE). Abiotic and open-circuit controls were also set up, to discriminate i) purely electrochemical from biological reduction, and ii) compare bioelectrochemical to just biological reduction.
BESs, with properly set electrode potential, showed higher Cr(VI) removal efficiencies in comparison to purely electrochemical or biological processes. The fastest decrease in Cr(VI) concentration was observed in a test with a biocathode poised at -300 mV vs. SHE, that, after only six days' operation, showed a 7% residual chromium concentration. Microbial analysis, performed by 16S rRNA gene sequencing, made it possible to evaluate the selection in the systems of bacterial communities containing electro-active and/or Cr(VI) reducing/resistant bacteria. Known electro-active bacteria (EAB) were able to adapt; autotrophs like *Alcaligenaceae* appear to be favored. *Bradyrhizobiaceae, Trueperaceae* and *Flavobacteriaceae* were selectively enriched on the polarized cathode biofilm; the microbial consortium makes a contribution to high removal of chromium to the biocathode.

INDEX

INTRODUCTION AND PURPOSE OF THE WORK

Cr(VI) is water soluble in the full pH range and extremely toxic to the health of humans and all living organisms because of its mutagenic and carcinogenic properties. The unregulated disposal of Cr(VI) effluents and improper management of Cr(VI)-containing waste have led to widespread chromium contamination of soils and groundwater around the world. In Chapter 1 of this thesis chromium characteristics in natural environment are presented; as a consequence of its mobility in water, groundwater resources are especially vulnerable to Cr(VI) contamination, with levels often shown to exceed the internationally acceptable exposure limit of 50 μg Cr(VI)/l. In Chapter 2 conventional methods for site remediation, "Dig and Dump" (D&D) of unsaturated soil and groundwater Pump and Treat (P&T) are discussed in comparison to innovative in situ strategies. Cr(VI), like any metal, is not removed, but only changes its oxidation state; this requires careful evaluation of the long-term stability of reduced products and possible re-oxidation mechanisms. In particular, chemical reduction based on iron and sulphur reagents, biological reduction, electrokinesis and soil flushing were taken into account. Due to the highly complex behavior of inorganic pollutants in the environment, there are no written "Decision Guides" available for hexavalent chromium to refer to in choosing the potentially most suitable remediation technology in relation to the site-specific conditions.

A promising strategy to be explored for in-situ bioremediation is the application of bioelectrochemical systems (BESs), that consist of an anode, where an oxidation reaction takes place, and a cathode, where a reduction reaction takes place, and at least one or both reactions are microbially mediated. In Chapter 3 peculiarities of this technology are discussed; such as the ability of certain microorganisms to use the electrodes as inexhaustible electron acceptors/donors which make it possible to supply electrons without adding organic substrates for the stimulation of microbial activity.

In Chapter 4 a review provides an analysis of current knowledge and experiences in bioelectrochemical treatment of hexavalent chromium. In literature, the existence of microorganisms resistant to chromium is reported and some of them are also able to reduce it. BESs have been extensively studied and intensively developed especially during the last ten years for wastewater valorisation and treatment; several experimental works investigated hexavalent chromium reduction in Microbial Fuel Cells (MFCs) with bioanodes and either abiotic cathodes or bio-cathodes.

In Chapter 5 significant scenarios are identified to guide one in the selection of the most suitable technology according to the site-specific features. The scenario of

greatest interest is that of soil and groundwater in oxidising conditions, where the chromium remains in hexavalent form if not properly treated. This approach is useful in the preliminary assessment of the remedial options; only full-scale implemented technologies have been introduced in the output sheet, while BES is only discussed due to its potential applicability for on-site remediation.

Research insights have been focused on biological technologies; Chapters 6-8 summarize the experimental activities carried out over the years of study.
Induced bio-reduction (Chapter 6) is based on the injection of organic carbon sources, whose rapid microbial degradation by indigenous heterotrophic microorganisms prompts anaerobic conditions and produces reductants, such as S^{2-}, Fe(II) and fermentation metabolites, able to mediate Cr(VI) reduction and precipitation. Tests were carried out in batch unstirred systems, with two different soils, solid/liquid ratios, initial Cr(VI) concentrations and substrates. The tests aimed to verify the theoretical conditions for Cr(VI) reduction shown by the Pourbaix diagram, in a real soil system. The use of inexpensive byproducts as substrates, that show similar features to the commercial reagents, was shown to be an interesting alternative for Cr(VI) bio-induced reduction with interesting prospects in terms of reduced costs and limited environmental impacts. The performance of in situ Cr(VI) treatments is therefore influenced by the abundance of suitable electron acceptors and donors, as well as carbon sources and nutrients for the microorganisms, often requiring external supplements for a balance. Controlling the supply rates can be a crucial step to avoid unwanted side reactions and the accumulation of undesired substances (e.g. excess substrates or nutrients, fermentation products) with deterioration of soil and water quality.
For bioremediation of Cr(VI) in groundwater, where oxidable organic matter is typically low, BES requiring an external electricity supply represents an alternative. Researches focused on designing and developing lab-scale BESs through the acquisition of materials, development of methods for setting up and monitoring the systems, and carrying out tests for Cr(VI) reduction. Major tasks included an experience with Electro-Synthesis (ES) and Electro-Fermentation (EF) in biocathodic BESs (Chapter 7) using pure culture. Bioelectrochemical production of organics from inorganic carbon in cathodes could be used to reduce Cr(VI) heterotrophically with the aid of a cathode. Furthermore, the feasibility of biocathodes stimulating fermentation from organic waste was evaluated. The purpose for a contaminated aquifer could be the acceleration of a bio-induced process thanks to the presence of a BES.
Cr(VI) reduction (Chapter 8) starts with some preliminary tests, reduction has been observed in BES where a voltage potential was set up between the electrodes. However, in these systems there was little control over how the reactions take place and the real contribution the EAB made on the electrode was not clear. Therefore Cr(VI) reduction was investigated using a BES in a Microbial 3-electrode Cells (M3C) configuration. Attention was paid to the appropriate experimental setup, in terms of required equipment, monitoring and control. The work aimed to determine the effectiveness of a microbial biocathode for Cr(VI) reduction in comparison to a pure microbial and pure electrochemical control, in order to start exploring the possibility

of effective Cr(VI) reduction in natural surface water or groundwater at lower Cr(VI) concentrations than wastewater investigated so far.
To minimize the total energy required and to facilitate the rapid development of an EAB in the first phase of the work, electrodes were acclimated in the anodic chamber of a MFC. A relationship between substrate availability and current production was observed; at the end of the acclimation period, current density in the MFC reached stable peak values, while coulombic efficiency showed a marked increase with time. Families of EAB were identified by 16S rRNA gene sequencing, performed in collaboration with Milano Bicocca University.
Once the EAB had developed, one of the electrodes was transferred to a M3C. To approach real groundwater conditions, no organic substance was added in the working chamber, dosing carbonates as the sole carbon source. Tests for Cr(VI) reduction (initial concentration: 1-2 mg/l) were carried out under anaerobic conditions with the M3C biocathode poised between -300 to +700 mV vs SHE. Some controls were set up in order to distinguish electrochemical from biological removal; a study of proper microbial consortia was performed.
A continuous flow test was also conducted. This system has provided useful information as to the development of research on this technology, due also to the limited number of continuous BES studies for the removal of Cr(VI) reported in literature.

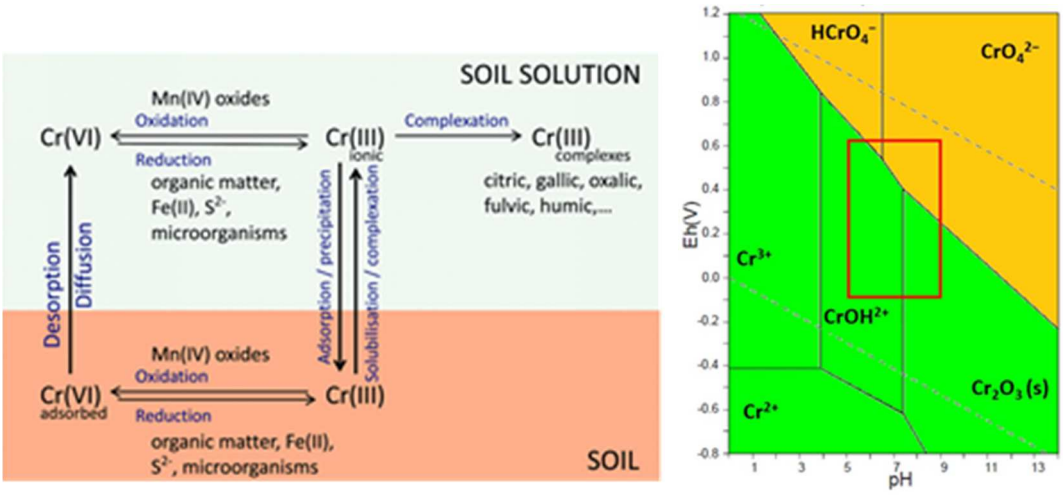
1

CHROMIUM AND CONTAMINATION

1.1 Chromium chemistry and behavior in the environment

Chromium primarily exists in two valence states, trivalent Cr(III) and hexavalent Cr(VI). Cr(III), the most common form of naturally occurring chromium, is relatively stable and has low solubility in aqueous solutions (Erdem et al., 2004; Unceta et al., 2010); thus it is largely immobile in the subsurface at typical environmental conditions (pH 6-9) (Dhal et al. 2013). In aqueous systems, hexavalent chromium can be present in different species (Panagiotakis et al. 2015): primarily as chromic acid [H_2CrO_4] and its salts, hydrogen chromate ion [$(HCrO_4)^-$] and chromate ion [$(CrO_4)^{2-}$]. Cr(VI) is highly soluble in water and therefore mobile; moreover, it is a proven carcinogen (classified as a Class A), mutagen, and teratogen and exhibits acute toxicity to different biological systems (CCME 1999; USEPA 1998b; Guertin et al. 2005; Humphries et al. 2005; US Department of Health 2009; US Department of Health 2012). In relation to these effects, the USEPA has classified Cr(VI) as one of the seventeen most dangerous elements for human health (Marsh and McInerney, 2001; Palmer and Puls, 1994).

The migration of chromium in soils, surface and ground-water and its toxicity is strictly related to its chemical form (Figure 1,1a). The equilibrium of the different chromium species depends on total chromium concentration, oxidation-reduction potential (ORP) and pH of the system (Figure 1.1b) (Palmer and Puls, 1994; Pradhan et al. 2017). Under proper pH and Eh conditions, or in the presence of reducing/oxidizing agents, the prevailing chemical species can change (Accornero et al., 2010). Cr(VI) species are stable under oxidizing conditions, whereas $Cr(OH)_3$ is stable under reducing conditions (James et al. 1997; Massacheleyn et al. 1992). The pH range of interest includes values between 5 (acidic) and 9 (alkaline), which can be considered the possible extremes for soil under natural conditions (US Department of Agriculture 2017); the ORP typically encountered in an aquifer falls in the range −100 and +600 mV (vs. Standard Hydrogen Electrode - SHE) (Ohio EPA 2014).



a) b)

Figure 1.1. Scheme of cycling and transformation of Cr species in soil/soil solution (a); Diagram of Pourbaix showing dominating chromium species in diluted aqueous solutions (in yellow Cr(VI) species, in green Cr(III) species), as a function of Eh (redox potential Eh vs. SHE) and pH (b). The red rectangle encloses the area of natural environmental conditions (Ščančar and Milači, 2014).

With reference to the area within the red box in Figure 1.1b, the prevalence of Cr(VI) species is located in the portion of basic pHs and redox higher than +200 mV. However, the theoretical Pourbaix diagram of Cr had to be properly adjusted to site-specific conditions, taking into account groundwater and soil composition. Cr(VI) can be reduced to the trivalent form by redox reactions involving organic substances in soil (carbohydrates, proteins and humic species), Fe(II), S^{2-} or as part of the metabolism of some microbial species. Reducing species which serve as electron donors facilitates the reduction process (USDE, 2011).
Amongst the most widespread electron donors, Fe(II) assumes special importance (Palmer and Wittbrodt 1991). In aerated soil, with high redox potential, iron has a trivalent form. In asphyxial soil, with low redox potential, Fe(II) ions in solution are abundant, depending also on the chemical composition of the soil, and are prone to react with hexavalent chromium. At pH 5–6, the redox reaction is (Buerge et al. 1997):

$$3Fe^{2+} + HCrO_4^- + 3H_2O \rightarrow 3Fe(OH)_2^+ + Cr(OH)_2^+ \quad (1)$$

At pH > 7, the reduction mechanism of the hexavalent chromium follows the reaction (He 2003):

$$3Fe^{2+} + CrO_4^{2-} + 4H_2O \rightarrow 3Fe^{3+} + Cr^{3+} + 8OH^- \quad (2)$$

The formation of Cr(III) and Fe(III) species results from reactions (1) and (2). Reacting with each other, or with further dissolved Fe(II), means they do not remain in solution, but are removed in the form of hydroxides.

Cr(III) is less toxic and more stable than Cr(VI) in aquatic environments as under natural conditions, at moderately acidic or alkaline pH, it tends to precipitate as chromium hydroxide or oxide ($Cr(OH)_3$ or Cr_2O_3) (Beverskog and Puigdomenech,

1997; Rai et al., 1989). The oxidation of Cr(III) to Cr(VI) can take place in presence of Mn(IV) oxides (Richard and Bourg 1991; Palmer and Puls, 1994). However, the kinetic is slow, as the process is 10 times slower than reduction of Cr(VI) to Cr(III) (Bartlett et al. 1988; Tiwary et al., 2005).

The significant instances of contamination by Cr(VI) are essentially linked to soils/aquifers under oxidising conditions, with intrinsic permeability greater than 10^{-14} m^2 (coarser lithologies of fine silty sands); in fact, at a redox potential of around +500 mV (vs. SHE), the natural reduction of Cr(VI) to Cr(III) is largerly disadvantaged (Sedlazeck et al. 2017). Conditions of this type are typical of glacial/alluvial deposits with low organic substance and of fragmented rocks.

1.2 Toxicity

Toxicity of hexavalent chromium is mainly due to the higher oxidative potential and solubility of its compounds compared to those of trivalent chromium. Cr(VI) adsorbed due to ingestion or inhalation reaches the blood flow and arrives at cells through ionic exchange processes; this behavior is different to that of Cr(III) because it tends towards tie plasma proteins and, in particular, to transferring (De Flora, 2000). Cr(VI) can reach the liver, kidneys, brain and other organs of the human body through the circulatory system. Ions of Cr(VI) within blood tend to create “critic sites” in target cells that are responsible for the creation of tumors.

Cr(VI) is considered one of the most toxic chemicals and is classified as “carcinogenic” by the International Agency for Research on Cancer (IARC). A recent study by the IARC highlights sufficient proof to demonstrate a correlation between exposition to Cr(VI) and lung cancer, but the correlation with stomach cancer cannot be demonstrated with certainty (IARC, 2012); also USEPA (1998a) has classified hexavalent chromium as a “Carcinogenic Substance Class A” due to the decisive proof of its cancer causing capability.

Ingestion of contaminated foods and water, and inhalation of particulates, are the main exposure pathways because Cr(VI) is not volatile; direct contact with products containing Cr(VI) has a minor role, but it can cause irritation or damage to the eyes and skin if Cr(VI) contacts these organs (Unceta et al., 2010). Irritation or damage to the nose, throat and lungs (respiratory tract) may occur if Cr(VI) is inhaled as scattered inflammatory phenomena. Cr(VI) adsorption due to inhalation depends on many factors, such as the dimension of particulates, the oxidative state of chromium and the solubility of the chemical. The ingested hexavalent chromium acts on the oral cavity, the esophagus, the stomach and the intestine, favoring the formation of ulcers.

Workplace exposure to Cr(VI) causes the most serious problems (OSHA, 2009); workers can inhale Cr(VI) as a dust, fume or mist while, among other things, producing chromate pigments, dyes and powders (such as chromic acid and chromium catalysts); working near chrome electroplating; performing hot work and welding on stainless steel, high chrome alloys and chrome-coated metal; and applying and removing

chromate-containing paints and other surface coatings. Continuos exposure to compounds containing Cr(VI) may provoke alteration and mutation to genes as well as increasing the risk of developing tumors (Dayan et al., 2001). Some studies, carried out on foundry workers involved in ferrochrome production, have pointed to a correlation between chronic exposure to Cr(VI) and the incidence of breakdown of DNA parts (Benova et al., 2002, Gambelunghe et al., 2003, Medeiros et al., 2003). Also data from tests on bacteria and animals suggest a correlation between Cr(VI) exposition and genetic mutations (WHO, 2013).

The World Health Organization (WHO) has calculated a "Tolerable Daily Intake" (TDI) of Cr(VI) for oral exposure equal to 0.9 µg of Cr(VI) per kg of body weight (WHO, 2013).
The toxicity of a carcinogenic pollutant is quantified by the "cancer slope factor (CSF)", representing the incremental risk of developing tumors with reference to the assumption of a unitary dose of chemical throughout the entire life. The value supplied by the Italian department ISS (Istituto Superiore Sanità) for assumption of Cr(VI) through inhalation is 42 (mg/kg/d) (ISS-INAIL, 2018). Over the last decade, United States institutions have carried out many studies to evaluate the possible correlation between assumption of Cr(VI) through ingestion and the increase in tongue and stomach cancer; the US Department of Health (2012) has extrapolated data from experimental activity of the national toxicology program, calculating a value of CSF for ingestion equal to 0,5 (mg/kg/d).

1.3 Industrial use and sources of contamination

The main natural source of chromium is chromite ($FeCr_2O_4$), a member of the spinel mineral group. Millions of tons of chromate are mined at global leveland the amount increases yearly (Figure 1.2); about 45% of chromite is mined from South Africa, 16% from Kazakhstan and 15% from India (Dhal et al., 2013).

a

Country	Reserves	Production (In '000 tons)				
		2007	2008	2009	2010	2011
World: Total (rounded)	>480,000	23,900	23,600	18,700	23,700	24,000
India[a]	54,000	4873	3980	3372	3800	3800
Kazakhstan	220,000	3687	3552	3333	3830	3900
South Africa	200,000	9647	9683	6865	10,900	11,000
USA	620	–	–	–	–	–
Finland	–	556	614	247		
Brazil	–	628	700(e)	700(e)		
Russia	–	777	913	416		
Turkey	–	1679	1886	1770		
Zimbabwe	–	614	442	194		
Other countries	NA	1439	1829	1803	5170	5300

b

Figure 1.2: chromite mine in the Sukinda (Orissa) valley (India) (a); chromium global stock and production in the main States (b) (U.S. Geological Survey, 2012).

If not properly managed, extraction processes of minerals may have a huge environmental impacts due to the dispersion of a large amount of chromium. Solid

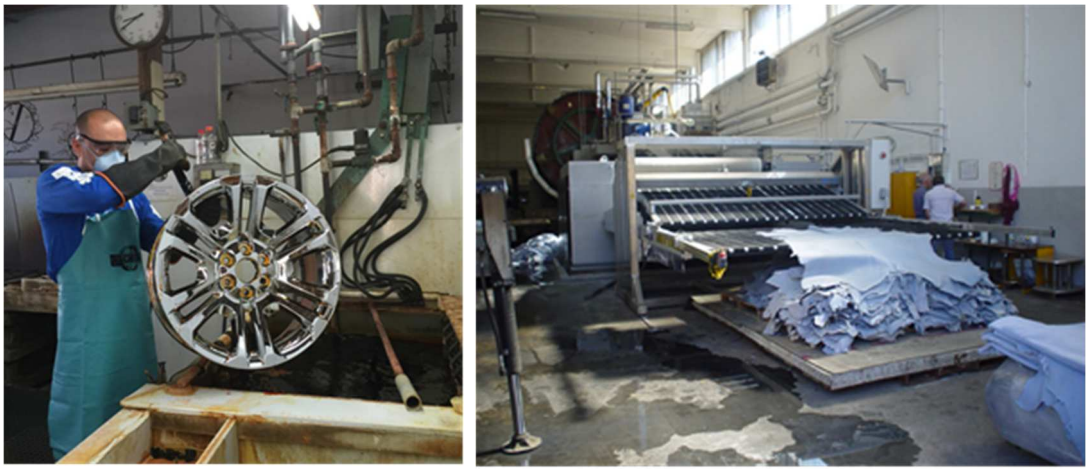
wastes produced by mining operations are often stored outside on permeable soils, in countries which lack environmental legislation, resulting in contamination of water bodies due to the rainfall washout and chromium solubilization (USEPA 2009).

The presence of Cr(VI) in soil and groundwater has also been linked to geogenic processes, namely, weathering of ultramafic and mafic rocks in various areas around the world (Panagiotakis et al. 2015; Lilli et al. 2015; Chrysochoou et al. 2016). Recent reports have established that naturally occurring Cr(VI) is prevalent in groundwater from specific aquifer systems composed of ultramafic rocks in California, Arizona, Mexico, Argentina, Brazil, Italy and Greece (Jafarian and Jafarian, 2017; Kazakis et al., 2017, 2015; Tiwari et al., 2019; Vengosh et al., 2016).

Chromium is an important industrial metal with a wide range of applications (Table 1.1) in metal plating, leather tanning, metal corrosion inhibition, pigment production, refractory materials and wood-preserving industries (Nriagu, 1988; Zayed and Terry, 2003; Apte et al., 2005; Chirwa and Molokwane, 2011; Jadhav and Hocheng, 2012). Wide scale industrial use of hexavalent chromium and its compounds has caused serious environmental pollution, generally relating to accidental or unlawful leakage of waste from production processes or illegal dumping of slags (Guertin et al. 2005). Chromite is used for industrial uses to produce ferrochromite, an alloy containing about 60-70% of chromium or pure metallic chromium (U.S. Geological Survey, 2012).
Ferrochromite is widely used in the manufacturing of stainless steel (Figure 1.3.a); chromium leads to the creation of a surface layer that protects steel from corrosion and oxidation. The chromium content in stainless steel must be higher than 10-12% by weight to avoid gaps in the protective layer.
Chromite is oxidized in the air stream and in molten sodium carbonate (Na_2CO_3) to produce pure metallic chromium. The reaction leads to sodium chromate (Na_2CrO_4) and iron oxide (Fe_2O_3); sodium chromate is used to produce chromium trioxide (or chromium anhydride) (CrO_3), and the compound is used in the galvanic factory to chromate metals (USEPA 1997). Pigments of the chromate group are also commonly used; they are made by chromate ions (CrO_4^{2-}) or bichromate ions ($Cr_2O_7^{2-}$). Chromium is laid down on a metal surface by an electrolytic process to improve its corrosion resistance and to give it a sparkling appearance.
Chromium is also used in the tanning industry to stabilize collagen fibers and to eliminate the putrescibility of animal skins (Figure 1.3b); about 90% of worldwide manufactured skins undergo ~~to~~ a tanning process with chromium because it ensures a better resistance to wear and water and a better appearance compared to the use of vegetable substances (Wang et al., 2007b). The process occurs in acid conditions and may take several hours; tanning with chromium salts is called "*Wet blue*" due to the typical blue coloring that skins take during the manufacturing phases.

Chemical Name		Uses
Chromium Trioxide	Chromic acid, chromia, chromic (VI) acid, chromic trioxide, chromium oxide, chromium (VI) oxide	Most common uses: chromium plating, aluminum anodizing, and chemical intermediate for chromated copper arsenate wood preservatives. Other uses: ceramic glazes, colored glass, metal cleaning, inks, and paints (inorganic pigments).
Lead Chromate Sodium	C.I. pigment Yellow 34, crocoite, lead chromium oxide, plumbous chromate	Decorating china, pigment in industrial paints, rubber and plastics, pigment in oil paints and watercolors, and printing fabrics.
Dichromate	Disodium salt, chromium sodium oxide, dichromic acid, disodium dichromate, sodium bichromate, sodium dichromate	Inks, oxidizing agent in the manufacture of dyes and many other synthetic organic chemicals, electric batteries, manufacture of chromic acid, other chromates and chrome pigments, corrosion inhibiting paints, component of wood preservatives, and colorant for glass.
Zinc Chromate	Zinc salt, chromium zinc oxide, zinc chromium oxide, zinc tetraoxychromate	Priming paints for metals, varnishes and pigments

Table 1.1: Selected Cr(VI) compounds and their uses (OSHA, 2009).



a b

Figure 1.3: chromium plating of a metallic component (a) (*LMchromecorp.com*); manufacturing skins (b) whose blue color is related to the use of chromium salts during tanning phases.

The European Commission has recently issued a number of Directives and Recommendations for environmental protection and the safeguard of workers and consumers that aim at the reduction of chromium use in industrial processes. For example, Directive 2002/95/CE (RoHS5) has forbidden the use of hexavalent chromium for anti-corrosion coatings for some types of electrical and electronic equipment starting from 2006.

The chrome plating systems are equipped with systems for evacuation and treatment of chromic anhydride vapors, a compound with high toxicity and corrosive power (Laxmipriya et al., 2010).
Discharges from tanneries are subjected to treatment processes aimed at recovering the reagents, in particular for trivalent chromium; the treatment process allows -about 80% of the total chromium used to be recovered (Bertoldi, 2007). Hexavalent chromium in industrial wastewater is typically treated by reduction, and subsequent precipitation, to the non-toxic Cr(III) by means of reductants (for instance $FeSO_4$, $Na_2S_2O_5$ and SO_2) (Malaviya and Singh, 2011) or electrochemical processes (including electrocoagulation, electro-reduction, electrodialysis and electrodeionization) (Jin et al., 2015). Generally, these processes are easy to implement and efficient at high or moderate Cr(VI) concentrations. However, the use of reductants produces a large amount of metallic sludge, and these approaches are either ineffective or not cost-effective when applied for trace Cr(VI) treatment. Adsorption on adsorbents, such as activated carbons or zeolites, is also adopted, as this process exhibits several advantages such as simple operation, low cost, and high efficiency (Brungesh et al., 2015; Caputo et al., 1999). Nevertheless, this strategy presents some disadvantages: regeneration of adsorption media is needed and no degradation/detoxification of Cr(VI) to Cr(III) is achieved (Rajeswari et al., 2016). Therefore, operations like the spreading of sludge from tanneries in agriculture lead to huge amounts of chromium in soils despite the treatment processes significantly reducing the chromium concentrations in wastewater.
Environmental effects related to the use of chromium appear more evident given the huge amounts emitted within the atmosphere; about 2.700 tons of chromium were emitted into the air globally during 2010. The most significant sources are related to combustion (about 73% of total emissions) and industrial production (about 23%) (Swietlik et al., 2010).
Combustion of coal to produce electricity is one of the greatest contributors of chromium emissions into the atmosphere; coal contains about 20 ppm of chromium and it transforms into ashes and gases during the combustion process (Shah et al., 2008). Although the gaseous flow is subjected to specific treatment phases, a residual part of chromium escapes from the removal and is emitted into the atmosphere on the fine particulate matter. Likewise, non-negligible fractions of chromium are released into the atmosphere during the thermal disposal processes of residual urban waste.
Most of the chromium emitted into the atmosphere tends to settle on the soil due to its removal from the atmosphere through precipitations.

The unregulated disposal of Cr(VI) effluents and the improper management of Cr(VI)-containing waste (Figure 1.4) have led to widespread chromium contamination of soils and groundwater around the world. Today the main problems are related to the huge contamination of soils as a result of uncontrolled spills over the past decades.

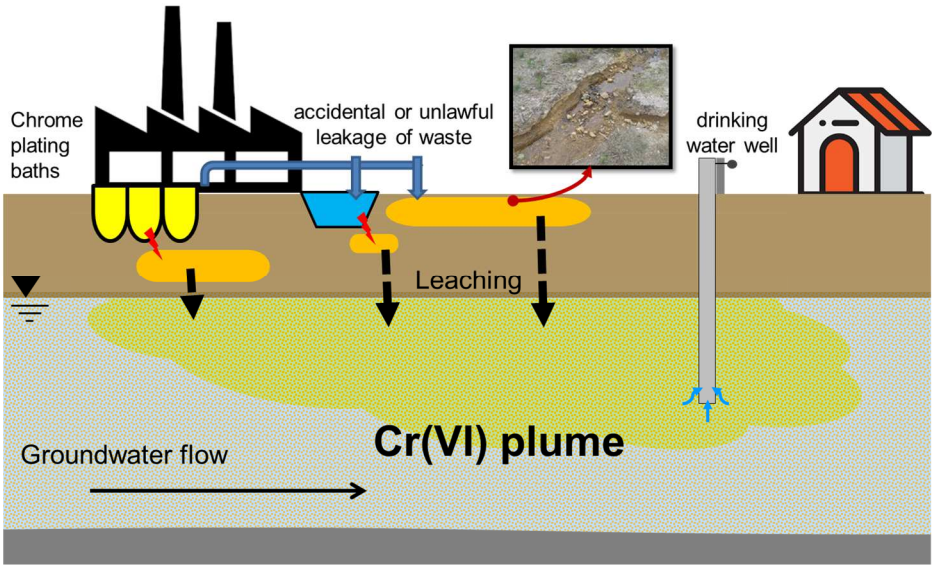


Figure 1.4: Cr(VI) disposal scheme; leaching and plume in groundwater.

In uncontaminated groundwater, chromium concentration is lower than 0.001 ppm (Hashim et al., 2011). Chromium levels in soil vary according to area and the degree of contamination from anthropogenic chromium sources. Soil tests have shown chromium concentrations ranging from 1 to 1000 mg/kg (WHO, 2000). In the USA, the study of chromium plume has become topical; Hinkley groundwater contamination, famous because of the Erin Brockovich class-action lawsuit, had expanded to 9.7 km long and 6.4 km wide. Given the extensive territorial expansion, new plumes were discovered recently, during the installation of a groundwater monitoring well (Vesselinov et al., 2013). Plume characterization is a challenging but not unique problem because multiple models and contamination scenarios are consistent with the site data and conceptual knowledge. Sharma et al., (2012) verified an association between health adversities among inhabitants and the presence of Cr (VI) in groundwater in Kanpur areas, India. Even in China the increased sensitivity to groundwater places more emphasis on chromium plumes (He and Wu, 2019); groundwater availability is one of the key factors determining local living conditions, especially in the arid and semiarid areas, such as the Loess Plateau in northwest China. In Italy, many of the 39 sites of national concern (SIN) contain high concentrations of total and Cr(VI) ("Tito" (Potenza), "Cogoleto" (Genova), "Livorno", etc.). In Lombardy, about 25% of the sites ranked in the Regional Plan of Contaminated Sites (SER-APHIM) are affected by Cr(VI) in groundwater and/or soil (Regione Lombardia, 2016; Azzellino et al., 2019).

1.4 Environmental regulation

As a consequence of mobility in water, groundwater resources are especially vulnerable to Cr(VI) contamination, with levels often shown to exceed the

internationally acceptable exposure limit of 50 $\mu g_{Cr(VI)}$/l (Guertin et al., 2005; Xafenias et al., 2013). Cr(VI) gained world-wide attention in 2000, thanks to the Julia Roberts' film, Erin Brockovich, which increased public awareness of contamination in groundwater.

The limits imposed to drinking water can be more stringent. Standards are generally set for total chromium, as it can convert between oxidation states both outside and inside the human body. In order to ensure that the greatest potential risk is addressed, EPA's regulation assumes that a measurement of total chromium is 100 percent Cr(VI) (USEPA, 2000). There is a provisional guideline value of 50 µg/l for total chromium (WHO 2004). The USA and Europe set 100 µg/l (USEPA 2017b; European Commission, 2018) as the upper limit for chromium; the European Commission proposes reducing the value to 25 µg/l within 10 years after the Directive enters into force. In 2014, the California State Water Resources Control Board (SWRCB, 2014) established 10 µg/L and 50 µg/L as the drinking water standard for hexavalent and total chromium, respectively. In Italy, Ministerial Decree 14th November 2016 introduced 10 µg/l as the limit for hexavalent chromium potable water.

The limits on the quality of groundwater are also of interest in the context of national regulations; however they are not often defined. Italian law limits for groundwater (CSC – Contamination Threshold Concentrations), defined in Annex 5 to the Part IV-Title V of the Legislative Decree 3rd April 2006 n°152 (D.Lgs. 152/2006), are equal to 50 µg/l for total chromium and 5 µg/l for hexavalent chromium (Regione Lombardia 2001; ARPA, 2012). CSCs represent "contamination levels of natural matrixes above which characterization and Risk Assessment is required" (article 270, paragraph 1 of D.Lgs 152/2006).

The soil predicted no-effect concentration (PNEC) of Cr (III) ranges between 3.3 $mg/kg_{w/w}$ for acidic conditions and 62 $mg/kg_{w/w}$ for other conditions (ECHA, 2014). In comparison, the PNEC value related to soil for Cr (VI) ranges between 0.006-0.15 $mg/kg_{w/w}$. In the absence of a dedicated soil regulation at EU level, there is no harmonization of permitted thresholds in soil at EU level. The guideline value for remediation action is 200 mg/kg for agricultural land and on industrial areas is 300 mg/kg soil (INSURE, 2017). In any case, if the concentration exceeds 100 mg/kg, and if it is above the natural background concentration, soil contamination and remediation requirements must be assessed (Dhal et al., 2013). Italian CSCs limits for total chromium in soils are equal to 150 mg/kg (as dry weight) for residential and park areas and 800 mg/kg for industrial and commercial sites. Italian law also establishes limits for hexavalent chromium: 2 and 15 mg/kg for residential/park and industrial/commercial sites respectively (European Commission, 2016).

2

CR(VI) CONTAMINATED SITES: REMEDIATION TECHNOLOGIES

2.1 Introduction to innovative technologies

P&T has been used to remediate chromium-contaminated plumes; this method requires long-term application to meet Cr(VI) remediation goals and may not be effective at remediating source-zones (USEPA 2007; European Commission 2017). Contamination in the vadose zone has typically been addressed via soil excavation and disposal. In the last decade, innovative and promising in situ clean up technologies for Cr(VI) have been tested, mostly at lab- and pilot-scale (Chrysochoou and Ting, 2011; Theologou et al., 2013). The technologies of interest are based on biological or chemical mechanisms, taking into account the sustainability approach (USEPA, 2000). Reagents like zero-valent iron, ferrous sulphate and sodium dithionite are commonly used in chemical treatment thanks to their interaction and reduction capability. Organic substrates are frequently injected in the subsoil to create a negative redox zone in biological processes. Chemical and biological treatments are strictly joined and there are commercial products that blend both actions. Given that the major mechanism for Cr(VI) remediation within an aqueous system is reduction, the aim is the stabilization of chromium reducing hexavalent chromium to the trivalent form (Dermatas et al., 2013). The amount of total chromium doesn't change, but groundwater is cleaned due to the precipitation of trivalent chromium (USEPA 1999). Mechanisms such as precipitation and sorption which can also be used for chromium immobilization and remediation are described (Loyaux-Lawniczak er al. 2001).

2.2 Chemical processes

2.2.1 Fundamentals

In general, applicable reagents for the chemical reduction of Cr (VI) have an iron or sulphur based composition, and can act either directly or indirectly (Beretta and Pellegrini, 2006; Ludwig et al. 2007) (Table 2.1).

Class	**Reagent**	**Ref.**
Reduced iron reagents	Iron(II) sulfate ($FeSO_4$)	Cheng et al. (2009)
	Iron(II) chloride ($FeCl_2$)	Seaman et al. (1999)
	Zero-valent iron (Fe(0))	Dutta et al. (2009)
Reduced sulfur reagents	Sulfur dioxide (SO_2)	Ahn et al. (2003)
	Hydrogen sulfide (H_2S)	Thornton and Amonette (1999)
	Sodium dithionite ($Na_2S_2O_4$)	Fruchter (2002a)
	Sodium metabisulfite ($Na_2S_2O_5$)	Bianco Prevot et al. (2018)
	Calcium polysulphurs (CaS_5, CaS_5)	Jacobs et al. (2001)
	Sodium bisulfite ($NaHSO_3$)	Jacobs et al. (2001)
	Calcium bisulfite ($Ca(HSO_3)_2$)	Jacobs et al. (2001)
Reagents with iron and sulfur	Iron sulphide (FeS)	Mullet et al. (2004)
	Pyrite (FeS_2)	Lin and Huang (2008)

Table 2.1**.** Chemical reagents for hexavalent chromium reduction.

Amongst the iron-based species that act directly on the reduction of Cr (VI), the most commonly used is nanoscaled zero-valent iron particles (nZVI) (Mueller et al., 2011).

$$3Fe^0+2HCrO4^-+14H^+ \rightarrow 3Fe^{2+}+2Cr^{3+}+8H_2O \quad (3)$$

Zerovalent iron is a strong reducing chemical injected as nZVI due to the greater reactivity compared to the bigger sized prowder. Acid conditions facilitate Cr (VI) reduction with Fe (0) (Němeček et al., 2014; Yoon et al. 2018). The reduction process with Fe(0) develops with a second order kinetics that may be simplified to a pseudo-first order kinetics when great amount of reducing agent is available. Natural bacteria living in groundwater are not affected by possible negative effects due to the introduction of Fe(0) within the aquifer.

Fe(0) is introduced in the environment as specific reactive or it is released by solid phase of soil due to changes in the redox condition of the system. In the pH range 5-10, the Fe(II) produced by oxidation of Fe(0) reacts with the chromate ion (CrO_4^{2-}) or with the hydrogen-chromate ion ($HCrO_4^-$) to create Fe(III)-Cr(III) hydroxide with a molar ratio Fe(III)/Cr(III) equal to 3 to 1 (Ludwig et al, 2007). Chemical reactions that involve chromate are the following (Khan and Plus, 2003):

$$3Fe^{2+}+CrO_4^{2-}+4(OH)^-+4H_2O \rightarrow Cr(OH)_3+3Fe(OH)_3 \quad (4)$$

$$3Fe^{2+}+CrO_4^{2-}+4(OH)^-+4H_2O \rightarrow 4Fe_{0,75}Cr_{0,25}(OH)_3 \quad (5)$$

There are many studies on ferrous sulphate ($FeSO_4$) in literature. Di Palma et al. (2013) compare the capabilities of $FeSO_4$ with the ones of Fe(0) in laboratory tests. Both reagents promote reduction of hexavalent chromium, but reaction time are faster with Fe(0) although it is required a greater amount of reagent for the same removal rate of pollutant.

The calcium polysulphurs (CaS_4, CaS_5) are also in common use (Storch et al. 2002); Chrysochoou et al. (2010) have shown that, when polysulphurs are used, the reducing conditions remain in the soil for a long time; neutral or basic pH values have provided greater reducing capacities. The reduction reaction of hexavalent chromium is the following:

$$10H^+ + 2CrO_4^{2-} + 3CaS_5 \rightarrow 2Cr(OH)_3 + 15S + 2Ca^{2+} + 2H_2O \quad (6)$$

Reagent may be oxidized to thiosulphate ($S_2O_3^{2-}$) if oxygen is present; the overall quality of the treatment may be compromised by the production and release of sulphate in groundwater. In Chrysochoou et al. (2012) a comparative evaluation of hexavalent chromium treatment in contaminated soil by calcium polysulfide and green-tea nanoscale zero-valent iron was also performed.
Sodium dithionite ($Na_2S_2O_4$) acts mainly indirectly, converting Fe (III) to Fe (II) (Beukes et al. 1999; Khan and Plus 2003; Xie and Cwiertny 2010). This ion plays an active role in the reduction of the pollutant, according to reactions (1) and (2). The process is favored in acidic conditions.

$$S_2O_4^{2-} \rightarrow 2SO_2^- \quad (7)$$

$$SO_2^- + Fe(III) + H_2O \rightarrow Fe(II) + SO_3^{2-} + 2H^+ \quad (8)$$

Sodium dithionite also directly contribute to the reduction of Cr(VI): its breakdown generates sulphites and thiosulphates that react in the medium and long term. The general reduction reaction of Cr(VI) due to hydrogen sulphite ion (HSO_3^-) is the following:

$$6H^+ + 2HCrO_4^- + 3HSO_3^- \rightarrow 2Cr^{3+} + 2SO_4^{2-} + S_2O_6^{2-} + 6H_2O \quad (9)$$

The velocity of reduction of Cr(VI) due to dithionite and its decomposition products increases with high pH values (Ludwig et al., 2007). The more iron ions are available, the more powerful is the action of dithionite; for this reason, the use of dithionite is often combined with the injection of ferrous substances. Ludwig et al. (2007) have studied the combined use of sodium dithionite and ferrous sulphate; care must be taken in the amount of ferrous sulphate injected because its overdose may result in production and accumulation of sulphate in groundwater.

The in situ reduction of Cr (VI) in an unsaturated zone by means of gaseous injections (Thornton et al. 2007) is an approach which has been little developed so far and principally concerns the use of hydrogen sulphide (H_2S) diluted in air. The efficacy is limited to acid or more neutral environments; for pH > 7.5, a significant collapse in the efficiency of the process may occur (Kim et al. 2001). The technology is especially suited to permeable soil, where the circulation of the gaseous reagent is enhanced. To promote the reduction of Cr (VI) to Cr (III) in unsaturated soil, there

needs to be adequate moisture content in the soil or in the gas current injected (Hua et al. 2003). Sulphurate hydrogen generates reduction of hexavalent chromium; reaction products are chromium hydroxides (insoluble) and sulphates:

$$8CrO_4^{2-} + 3H_2S + 10H^+ + 4H_2O \rightarrow 8Cr(OH)_3 + 3SO_4^{2-} \quad (10)$$

$$2CrO_4^{2-} + 3H_2S + 4H^+ \rightarrow 2Cr(OH)_3 + 3S + 2H_2O \quad (11)$$

High amount of iron in soil may provoke secondary unwanted reactions. Reaction kinetics are slow, in particular if iron is present; they may lead to production of sulphate and the subsequent increase of their concentration in groundwater. There is the possibility to treat the source of contamination in unsaturated soil, but the characteristics of contamination in vadose zone must be carefully studied to properly design the treatment (Oliver et al., 2003). This technique may reach the area that needs decontamination more efficiently than liquid solutions, but must be considered possible problems related to the management of gases (storage and distribution) and to the requirement of treatment for possible extracted gases.

2.2.2 Field applications

The characteristics of the aquifer and of the contamination must be studied in detail to careful plan the intervention. For example, a high permeability of the aquifer involves a high dosage of the reagent, problems for its dilution and inefficacy of the chemical action. Moreover, also the cost of the reagent must be taken into account among the disadvantages; for example, zerovalent iron tends to exhaust quickly. Attention should be paid to induced negative changes in the aquifer, including pH and redox potentials. On the other hand, an advantage is represented by the possibility to carry out quickly remediations; zero-valent iron, for example, acts very quickly.
It may not be necessary to insert large quantities of ferrous based reagents in soils where iron is already present in the matrix, which would cause inappropriate overdoses. The presence of iron in the aquifer is essential if dithionite is used; therefore, it is appropriate to proceed with simultaneous insertion of ferrous compounds if the soil lacks iron.

Němeček et al. (2014) carried out a pilot test in the Kortan site, Czech Republic. The site presents a diffuse contamination by Cr(VI) related to the presence of an industrial plant for the production of trivalent chromium salts destined for the tanning industry; it was active until the early years ‘90. The concentration of Cr(VI) in groundwater is about 3 mg/l; the groundwater is situated at an average depth of 5 m from the ground level; the water speed varies in the range 0.2-2.0 m/d. About 120 kg of nano-particles Fe(0) were injected in 60 m^3 of water in the pilot test, reaching a concentration of 2 g/l. The reducing solution was injected over the entire thickness of the aquifer by 3 wells; 5 other wells were then used to monitor the test. There was a significant increase of pH of the groundwater, with values about 8 in the injection wells. The redox potential of groundwater has gone from values of 500 mV to -400 mV in the injection wells. The concentration of Cr(VI) decreased significantly in the first few days (up to 0.05 mg/l) in the monitoring wells, but the values returned

comparable to the initial ones about 8 months later the beginnig of the test, due to the progressive consumption of Fe (0).
Laboratory test results of Singh et al. (2011) show that the almost total removal of Cr(VI) in a short time (120 minutes) may be achieved handing out a reducing solution with concentration of nano-particles Fe (0) equal to 10 g/l. The concentration of Cr(VI) decreased by 99% after 40 days compared to the initial value in the in situ test with injection of a reducing solution at 5 g/l.
Khan and Plus (2003) state that in situ treatment with sodium dithionite is a viable option for the full-scale project at the U.S. Coast Guard Air Support Center (North Carolina). Pilot studies showed reductions of hexavalent chromium below 10 µg/l injecting 17 kg of $Na_2S_2O_4$. The area of influence of the injection had a radius equal to of 1 m. The reducing zone created reached redox potentials equal to -700 mV and the absence of oxygen was observed for 48 weeks; moreover, an increase of Fe (II) concentrations had occurred. The iron naturally presents in the silicate minerals of the coastal sediments contributed to the reduction of hexavalent chromium in the long term.
Ludwig et al. (2007) studied the combined use of ferrous sulfate and sodium dithionite to create an in situ reducing zone for the treatment of Cr(VI) released from a disused ferrochrome production plant in South Carolina (USA). 4500 liters of reducing solution were injected in the pilot test; concentrations of ferrous sulphate and sodium dithionite were equal to 0.20 M and pH was 3.5. Treatment solution was injected in the area with the highest concentration of Cr(VI) using two wells at a depth between 3.0 and 4.5 meters from the ground level; saturated area was located at 1.5 meters below ground surface. The average concentration of Cr(VI) detected in the injection well and in the monitoring wells decreased from 6 mg/l to less than 0.01 mg/l after 421 days from reagents injection. Data showed a substantial reduction of concentration of Fe(II) probably due to the progressive depletion of ferric hydroxide in the aquifer transformed into Fe(II) for interaction with sodium dithionite.
In Italy, in Spinetta Marengo (AL) a pilot scale application was carried out injecting albite, a formulation containing 79-92% of sodium dithionite and 2-25% sodium carbonate (Eupolis Lombardia 2015). The unsaturated area (gravelly sand) was treated in this case. The injections in n. 4 points were carried out by direct push technique between -2 and -4 m below ground surface; a total volume of 1000 l of solution was injected at the flow rate of 70 l/min and 10 bar of pressure. A radius of influence of 1.1 m was estimated from the injection point using tomographic monitoring; the volume of treated soil was approximately 6-9 m^3. The concentration of hexavalent chromium in the area affected by injections decreased by an order of magnitude (from about 1600 mg/kg to 200 mg/kg). Soil samples collected after 1, 4 and 6 months from the injection showed the stability of trivalent chromium hydroxides. An increase in sulfides concentration (up to 60 mg / kg) was observed. Treatment costs were estimated at 53 euros/m^3, excluding tomographic monitoring and chemical sampling. Injection with direct push technique was more efficient than traditional injection using wells considering the radius of influence, although influence radius of this treatment is restricted. This treatment is innovative for the possibility for reclamation of the

vadose area; however, costs become significant for applications deeper than few meters from the ground level.
Calcium polysulfide was used in a pilot test in a metal plating plant (Storch et al., 2002). Reagent was introduced into the aquifer by direct injection; hexavalent chromium, chlorinated hydrocarbons and nitrates were present in the groundwater. Reducing conditions were created in the aquifer and a radius of influence of about 9 m from the injection point was pointed out by monitoring. A rapid decrease of Cr(VI) concentrations was observed. Furthermore, there were no changes in the concentrations of iron, manganese and arsenic dissolved in groundwater in the first 30 days following the treatment.
CaS_4 was used in a contaminated site in Tezze sul Brenta (VI), Italy (Eupolis Lombardia 2015). Both the unsaturated area of the soil and the saturated one were contaminated. The aquifer was highly permeable (10^{-2}-10^{-3} m/s) and for this reason injection through well was ineffective due to the run-off of the chemical reagent. A cement "reactive cell" (9 m^2) was built to reduce the permeability in the treatment area (about two orders of magnitude). Chemical solution (2400 kg CaS_4, mixed with 2000 l of water) was injected through 21 valve tubes arranged in the cell. The monitoring within the cell showed that the redox potential was strongly negative (-300 mV); concentration of Cr(VI) decreased from 4000 µg/l to <1 µg/l. The risk of reagent run-off can therefore be avoided by dimensioning an adequate perimeter and bottom confinement system (not watertight), which allows to create a calm volume and to obtain adequate contact times. However, the building of "reactive cells" involves higher costs due to excavation and disposal of contaminated land.
Technology based on gases is not widespread due to the complexity in managing the intervention. Thornton et al. (2007) estimated a treatment time equal to 48 days using an air mixture containing 200 ppm H_2S and a flow rate of 9 m^3/min. Pilot tests were carried out in New Mexico (White Sands Missile Range) injecting hydrogen sulphide diluted with air in a contaminated unsaturated soil. Injection was carried out for 76 days in a well, also using extraction wells. 70% of the hexavalent chromium has been reduced and immobilized; its concentration in soil decreased from 8.1 to 1.1 mg/kg.
An Italian experience (Eupolis Lombardia 2015) concerns the use of a mixture with 4% hydrogen in a current of nitrogen at a working galvanic plant in the municipality of Rho (MI). This technique was carried out for a preliminary treatment of the aquifer and of the unsaturated layer. Concentrations in the deep layers have been reduced while it was necessary to excavate and dispose the source of contamination due to the higher concentrations of chromium present in the layer directly in contact with the chromium plating tanks.

2.3 Biological processes

2.3.1 Fundamentals

Somenahally et al. (2013) state that the bio-reduction of hexavalent chromium is an important treatment strategy: many microbial communities have been identified over years that can adapt to soils with this type of contamination. This work does not

study in depth considerations regarding the many studies carried out on "resistant and reducing chromium" pure cultures of microorganisms (bacteria and fungi). The examples of microorganisms that act in the presence of sufficient substrate are many, the metabolic processes used for the reduction of chromium, are both direct and indirect, with actions in anaerobic, but also aerobic conditions (Ontañon et al., 2014). Natural biological attenuation can therefore contribute to the processes of decontamination of a polluted site. These phenomena are essential only if the natural reductants are present in the aquifer, the concentrations do not exceed the reduction capability of the aquifer and the removal kinetics are compatible with the chromium transportation time in the aquifer from the source area to the point of compliance (Franzetti et al., 2017). This aspect is often the most critical one and it carries out to more stringent interventions for in situ chromium reduction. A detailed knowledge of the hydraulic and chemical-biological conditions of the aquifer is required to understand the contributions of adsorption, dilution and dispersion to the natural attenuation of an aquifer contaminated by hexavalent chromium (Jeyasingh and Philip, 2005). The performance of biological in situ Cr(VI) treatments, relying on either induced or direct mechanisms, is influenced by the abundance of suitable electron acceptors and donors, as well as carbon sources and nutrients for the microorganisms, often requiring external supplements for a balance (Pous et al., 2018). The control of supply rates can be a crucial step to avoid unwanted side reactions and the accumulation of undesired substances (e.g. excess substrates or nutrients, fermentation products) with deterioration of soil and water quality (Vanbroekhoven et al., 2007). Studies, that aim to support the development of microbial communities without the dosing of substrates, have been developed recently; bioelectrochemical systems (BES) (Chapter 3) and, more generally, the application of currents in the subsoil through electrodes are an example. The influence of electromagnetism on organisms dates back to the end of the nineteenth century, however the researces that investigates the field effects on microorganisms is very limited (Beretta et al., 2019). Exposure to electromagnetic fields tends to enhance rather than reduce cell activity, with possible applicative consequences in the field of biotechnology, including biological techniques for depollution. Applying electrostatic field or a field generated by direct current correlated phenomena could result; an overview is proposed in Figure 2.1.

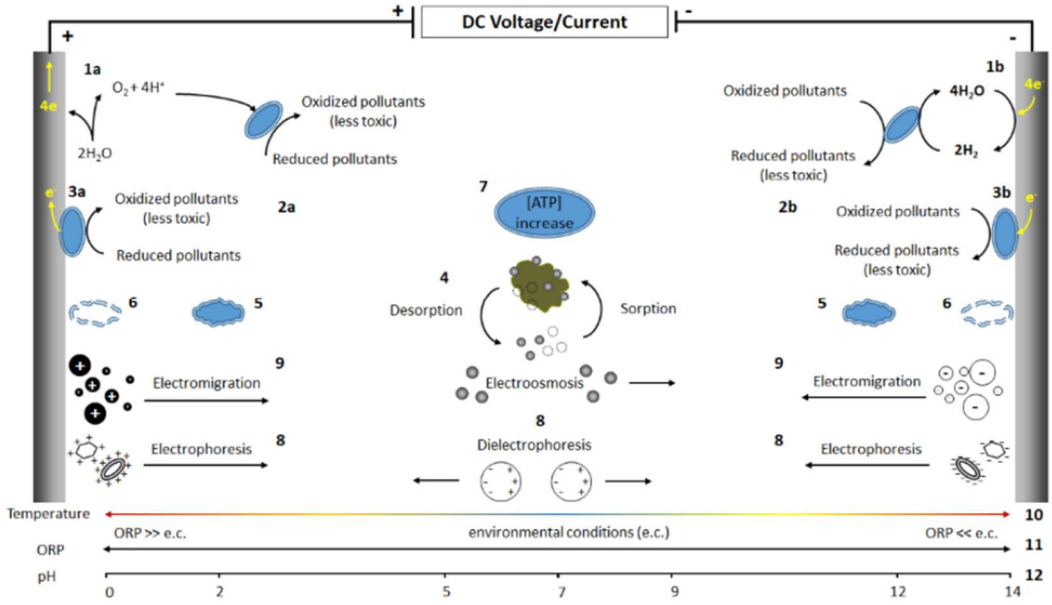


Figure 2.1: Scheme of the various phenomena resulting from the application of an electrostatic field or a field generated by direct current: 1) hydrolysis resulting in O_2 production (a) and H_2 production (b); 2) partial oxidation (a)/reduction (b) of pollutants; 3) solid electrodes as electron acceptor (a)/donor (b); 4) increase in pollutant bioavailability; 5) modification in the physiology and morphology of the cell; 6) loss of membrane integrity, with release of cytoplasmic material and cell death; 7) increase in intracellular ATP concentration; 8) increase in the transport of organic molecules, nutrients, and bacterial cells due to electroosmosis, electrophoresis and dielectrophoresis; 9) transport of dissolved ions due to electromigration; 10) increase in temperature near the electrodes; 11) divergence of the redox potential from the environmental conditions; 12) pH variation close to the electrodes (Beretta et al., 2019).

For some common microorganisms, suitably insulated and exposed to fields, increases in the cell activity were noted, with effects on the biomass growth rate and the metabolic kinetics.

The most surveyed fields are certainly the electrostatic; She et al. (2006), in a sysyem with a current of 10 mA, stimulated dehydrogenase activity thanks to the simultaneous production of O_2 and H_2 at the electrodes. Zhang et al. (2013) affirm that in bioelectrochemical tests the electrochemical assistance provided the electrons and accelerated the electron transfer rate in the microbial reduction of 2,4-dichlorophenoxyacetic. Zhang et al. (2014) observed an increase in the mineralisation efficiency of 2-fluoroaniline by an aerobic culture exposed to a direct current of 10-15 mA, as a result of the increased activity of the catechol dioxygenase and the selection of microorganisms with specific degradative abilities. In some experiments with electrostatic fields of 100-200 V/m or applied/induced currents below 20 mA, typically bioelectrochemical and bioelectrokinetic treatments, excluded important field effects

on the basis of biotransformation kinetics, evolution of the CO_2, microbial charges or enzymatic activity (Jackman et al., 1999; Wick et al., 2004; Harbottle et al., 2009).
Regarding magnetostatic fields, the most important applications in the environmental field involve wastewater treatment in activated sludge systems. In addition to a potential improvement in solid-liquid separation during the sedimentation step, increases in the degradation kinetics of the organic substance or increased biomass were related to exposure to magnetic fields.
The limited experiences concerning electromagnetic fields involve wastewater treatment and in particular the control of fouling. In Zhou et al. (2017), a pulsed electromagnetic field (square wave with frequency 100 Hz and intensity 5 μT) applied to a BES caused changes in the microbial community at the anode: a relatively greater abundance of *Geobacter spp.* was found (4-8%) than in the control. In comparison with electrostatic and magnetostatic fields, the experiments with electromagnetic fields were limited to an exposure duration of a few days at most, and it is not clear if there were microorganism adaptation.

2.3.1.1 Induced biological reduction

With reference to biological processes in a saturated zone, the administration of carbonaceous substances aimed at supporting an indirect bacterial action can be assessed (Ibbini et al. 2010; Somenahally et al. 2010; Bianco Prevot et al. 2018). Induced bio-reduction is based on the injection of organic carbon sources, whose rapid microbial degradation by indigenous heterotrophic microorganisms prompts anaerobic conditions and produce reductants, such as S^{2-}, Fe(II) and fermentation metabolites, able to mediate Cr(VI) reduction and precipitation (Brodie et al., 2011; Deng and Stone, 1996; Somenahally et al., 2013). Microorganisms use different electron acceptors after oxygen depending on availability, in sequence: nitrates, manganese and iron oxides, sulphates and, lastly, carbon dioxide. The new environmental conditions make it possible to reduce hexavalent chromium to the trivalent form; these conditions remain until the injected carbonaceous substrate is exhausted. The quality of the treated soil is generally higher than that treated with chemicals (Liao et al. 2014). Redox conditions of the aquifer are generally less reducing than those occuring using chemical compounds; several injection campaigns may be required to maintain the availability of carbonaceous substrates over time. The efficiency of Cr (VI) reduction through indirect biological processes tends to diminish as the concentration of the contaminant increases, because of the rise in toxicity (Viti et al. 2006). This type of process is therefore not advised for environments with high concentrations of Cr (VI) and where there is a lack of iron. Somenahally et al., (2013) carried out tests where assessed the impact on microbial communities increasing concentrations of chromium. In conclusion, they affirm that the injection of substrates promotes the reduction of chromium thanks to the development of bacterial activity that does not undergo negative impacts up to concentrations of hexavalent chromium equal to 3.0 mg/l.

Multiple substrates are injectable (Suthersan et al., 2002) and they may require different application methods depending on their characteristics. Carbonaceous substrates (molasses, fructose, lactate and whey syrup) can be cheap products deriving

from waste from production and food activities. Molasses is injected in a 10% (or less) aqueous solution and it is quickly transported with groundwater. It is rapidly degraded, resulting in the rapid creation of anaerobic conditions. Different qualities of molasses are available and they vary according to their sulfur content. The injection can also be done with a direct technique; however it may be more economical to make "ad hoc" wells due to the need for repeated injections over time. Fructose syrup contains less sulfur and can be used as an alternative to molasses. Sodium lactate ($C_3H_5NaO_3$) dissociates in groundwater in lactate ion $C_3H5O_3^-$ and sodium Na^+ ion. Lactate ion ferments to acetate, for example, producing hydrogen:

$$C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + CO_2 + H_2O + 2H_2 \quad (12)$$

Acetate may be used directly as an electron donor for the reduction process or, alternatively, it ferments releasing further hydrogen. Whey is a more complex product than lactate, which is effective in the aquifer for longer time due to a slower fermentation. Fresh whey is very cheap, but it is more difficult to find and be treated than whey powder. It is advisable to use a filtered product to reduce its nitrogen load. Ascorbic acid, or vitamin C ($C_6H_8O_6$), like other organic acids, certainly represents a promising alternative, as it does not exhibit any toxic features. At pH ≤ 7, it reduces Cr (VI) efficiently, transforming itself into dehydroascorbic acid (Xu et al. 2004). Bianco Prevot et al. (2018) have, however, encountered high levels of Cr (VI) reduction in the environment with a pH up to 9.

Chemical processes occasionally complement biological technologies, as, for example, in Němeček et al. (2016), where there is the combined use of zerovalent iron and iron lactate. Many registered trademark reagents on the market incorporate the advantages of the two approaches. These products can therefore have a long effect thanks to their contents of slow-release products; however their use may be less sustainable due to their higher costs and to the greater presence of by-products.

2.3.1.2 Direct mechanisms

Selected microorganisms are capable of direct Cr(VI) bio-reduction (Elangovan et al., 2010; Yang et al., 2009) and biosorption (Liu et al., 2012), both anaerobically and aerobically (Thatoi et al., 2014; Viti et al., 2014). Nevertheless, the presence of suitable microorganisms in conjunction with favourable environmental conditions are essential. The injection of selected bacterial suspensions to reduce the Cr (VI) directly (chromium-reducing microorganisms) appears difficult to apply both in saturated and unsaturated zones. In fact, the in situ development and maintenance of these microorganisms is difficult (Hohener and Ponsin, 2014; Němeček et al. 2014; Thatoi et al. 2014; Qu et al. 2018). As further proof, there is a lack of literature recording encouraging experiences.
The known Cr(VI) reducing bacteria belong to different taxa including *Escherichia coli, Pseudomonas putida, Desulfovibrio sp., Bacillus sp., Shewanella sp., Arthrobacter sp., Streptomyces sp. MC1* and *Microbacterium sp. CR-07* (Focardi et al., 2013). Many chromate resistant bacteria possess chromate reductase enzymes, which are either localized in the membrane fraction (Cheung and Gu, 2007; Wang et

al., 1990) or in the cytosolic fraction (Bae et al., 2005). Membrane bound reductase enzymes are predominant in anaerobic Cr(VI) bacteria, whereas cytosolic chromate reductases are soluble proteins which are ubiquitous in aerobic chromate reducing bacteria. Although the anaerobic Cr(VI) respiration has never been rigorously shown, the membrane bound reductases of anaerobic bacteria are supposed to be related to the reduction of Cr(VI) as the terminal electron acceptor (Panneerselvam et al., 2013).

In the presence of oxygen, the reduction of Cr(VI) is commonly associated with soluble chromate reductases and requires NAD(P)H as electron donor (Horitsu et al. 1987, Thatoi et al. 2014). The mechanisms associated with Cr(VI) reduction can involve a direct, one step or two steps electron transfer. The *Escherichia coli* YieF Cr(VI) reductase transfers three electrons to Cr(VI) in one step to produce Cr(III), and one to molecular oxygen generating reactive oxygen species (ROS) (Ackerley et al., 2004). The Cr(VI) reductase ChrR from *Pseudomonas putida* involves a one/two steps mechanism in which one/two electrons are donated from NAD(P)H to generate the intermediate Cr(V)/Cr(IV) that is further reduced to Cr(III) by one/two additional electrons (Cheung and Gu, 2007; Thatoi et al., 2014). Under aerobic conditions, most Cr(VI)-resistant microorganisms tolerate up to 1500 mg Cr(VI)/l (Camargo et al., 2004), however, the rate of chromium reduction is directly related to the concentration of the contaminant and physical parameters, such as pH and temperature (Tahri Joutey et al., 2014). One of the first studies with Cr(VI)-reducing bacteria, achieved almost 100% of chromate reduction in 2.0 mg/L Cr(VI) solution within 90 h by *P. putida* PRS2000 and *P. fluorescens* LB303 (Ishibashi et al., 1990). Similar results were obtained by the soil-isolated strains *Bacillus* sp. E29 and *Arthrobacter crystallopoietes* strain ES32 that achieved the reduction of 82% and 90% of Cr(VI) in less than 6 h and 12 h, respectively (Camargo et al. 2004). Much higher Cr(VI) concentrations were removed by *Serratia proteamaculans*. Within 48 h, 100 mg Cr(VI)/l was reduced (corresponding to 100% of dichromate added) under aerobic conditions (Tahri Joutey et al., 2014). In the same study, the authors demonstrated that *S. proteamaculans* was also able to reduce chromate anaerobically, but the process was more efficient in the presence of oxygen.

Chromium-resistant microorganisms have also been found in marine environments. A Cr(VI)-resistant bacterium isolated from seawater and identified as *Exiguobacterium indicum* achieved nearly 92%, 50% and 46% reduction for 100, 500 and 1000 mg Cr(VI)/l respectively, after 192 h of incubation (Mohapatra et al., 2017).

Under anaerobic conditions Cr(VI) can serve as final electron acceptor in a process that usually involves membrane bound reductases (Thatoi et al., 2014), but also soluble enzymes (e.g. soluble cytochrome c_3 from *Desulfovibrio vulgaris*) have been observed to reduce Cr(VI) (Lovley and Phillips, 1994). Considering glucose as electron donor, the overall reaction is provided in equation (Barrera-Díaz et al., 2012):

$$C_6H_{12}O_6 + 8CrO_4^{2-}{}_{(aq)} + 14H_2O \rightarrow 8Cr(OH)_{3(s)} + 10OH^-{}_{(aq)} + 6HCO^-{}_{(aq)} \qquad (13)$$

Cr(VI) reduction in anaerobic conditions was reported in several microorganisms. Both *P. dechromaticans* and in *Enterobacter cloacae* are able to use Cr(VI) as terminal electron acceptor (Thatoi et al., 2014; Wang et al., 1989). Gene expression of *Shewanella oneidensis* MR-1 during Cr(VI) reduction was studied (Bencheikh-

Latmani, 2005). Under Cr(VI) reducing conditions, 83 genes were upregulated. Among the others, genes involved in the reduction of Fe(III) and Mn(IV) were also upregulated. Further studies with mutant strains confirmed the involvement of *mtrA*, *mtrB*, *mtrC*, and *omcA* in the reduction of Cr(VI) (Belchik et al., 2011; Bencheikh-Latmani, 2005). In anaerobic environments, iron(II) and sulphide can also play a role in Cr(VI) reduction (Němeček et al., 2016; Thatoi et al., 2014). Iron-reducing bacteria (IRB) reduce Fe(III) to Fe(II), and biologically produced Fe(II) can be re-oxidized reducing Cr(VI) to Cr(III) (Wielinga et al., 2001; Barrera-Díaz et al., 2012). Sulphate is an electron acceptor widely used by several bacterial groups for the degradation of the organic matter in anaerobic environments (Jørgensen, 1982). In sulphate rich environments Cr(VI) can react with sulphide produced by sulphate-reducing bacteria (SRB) to produce Cr(III) that precipitates (Barrera-Díaz et al., 2012). *Clostridium chromiireducens* sp. a Cr(VI)-resistant, Gram-positive, spore-forming, obligate anaerobe, was identified for its ability to reduce Cr(VI) at a contaminated site (Inglett et al., 2011). Many previous reports confirmed also autotrophic reduction of chromium, mostly using hydrogen as electron donor (Battaglia-Brunet et al., 2002; Chung et al., 2006). A Gram-negative bacterium, capable of reducing hexavalent chromium, was isolated from a contaminated site; 16S rRNA analysis revealed that it was belonging to *Pseudomonas* genus, with high similarity to *P. synxantha* (Mclean and Beveridge, 2001; McLean et al., 2000). Marsh and McInerney (2001) demonstrated reduction of Cr(VI) with hydrogen and carbon dioxide/$NaHCO_3$ as electron donor and carbon source, respectively, carried out by an anaerobic mixed culture developed from aquifer sediment.
In addition to laboratory studies focused on the elucidation of possible mechanisms used by bacteria for Cr(VI) reduction, the ability to reduce Cr(VI) in soil-aquifer systems has been also reported but needs to be further investigated.

2.3.1.3 Phytoremediation

For shallower unsaturated soil, phytoremediation treatment should be mentioned (Nayak et al. 2018). Plants extract the nutrients necessary for growth through the root system, including metal ions; also hexavalent chromium can be absorbed although it is not useful for the metabolism of the plant. The process is certainly slow, but recent studies have shown how it can be accelerated, for example, by boosting the growth of the plants (Ranieri and Petros 2014). To define a phytoremediation treatment, it is crucial to evaluate whether the physical chemical features of the soil and the meteorological/climatic conditions of the site are compatible with the plant species to be used. In the case of phytoextraction, it is necessary also to take into account the periodic discharge of biomass, which contains chromium mainly in trivalent form (Chai et al. 2009; Lotfy et al., 2014; Raptis et al., 2018). The technology is not advised for environments with high concentrations of Cr (VI). This technology can be useful in cases of slight contamination included in the first centimeters of the subsoil, or as a finishing for an already reclaimed soil.

2.3.2 Field applications

The pH of the aquifer must be compatible with biological action; furthermore, adequate time is required for the development of microbial activity, substrate fermentation and the generation of reducing conditions.

A full-scale treatment with whey was conducted in Emeryville, California (Rynk, 2004). Initial contamination was very high (up to 630 mg/l) with a diffuse plume. 22 injection wells were realized, both in the source of contamination and along the plume. During one year of injections, each of 1000 l of serum (diluted with water), a total of 57 m^3 of product was introduced. One year after treatment, concentrations decreased up to 0.02 mg/l.

In a site near Bergamo, the phreatic aquifer (a mix of conglomerate, gravel and sand, with hydraulic gradient of 0.2-0.3%) was affected by Cr(VI) with values up to 20 mg/l, in a plume 260 m wide and 2.6 km long. Despite the installation of a P&T, the dissolved plume remained quite stable. In 2011 a patented product, based on food-grade organic nutrients for bacteria biostimulation, was injected at 4 points located about 40 m downgradient of the pollution source area. After 40 days, Cr(VI) in the monitoring wells, located 50 m and 300 m downgradient of the source area, decreased to values <5 µg/l. These results allowed turning off the P&T, even if dissolved iron, manganese and arsenic were found in the injection area as side-effect.

Registered trademark reagents MRC® and 3D-Me® were injected into two contaminated sites in the Lombardy Region (Eupolis Lombardia 2015). Site n. 1 is a chromium plating facility (5000 m^2) hexavalent chromium was found in soil up to 2000 mg/kg and in the shallow groundwater 100 mg/l on average (concentration peaks up to 690 mg/l). Soil at the site is silty gravel and sand, with local lenses of sandy silt with gravel. The water table is at 12 m b.g.s. (± 5 m) and the aquitard is at 28 m b.g.s. In 2008, a hydraulic barrier was installed as the emergency action. After 3 years operation, a stable Cr(VI) concentration in groundwater was achieved, though it was very high (20 mg/l). In order to speed up site remediation, in 2012 injections of a mixture of MRC® and 3D-Me® were carried out. The injection system consisted of 10 HDPE injection clusters screened at different depths (14-19 m b.g.s. and 20-25 m b.g.s.). A total volume of 28000 l of reagent was injected. Post-treatment monitoring showed a very rapid decrease in redox potential (down to about -400 mV); total organic carbon increased from about zero up to 1800 mg/l, suggesting the presence of organic substrates in groundwater. Oxygen and nitrates dropped down, while iron and manganese rose respectively up to 260 mg/l and 113 mg/l. Cr(VI) concentrations in groundwater decreased by 59 % to 99%, depending on the specific monitoring well, reaching residual concentrations of about 100 µg/l. Site n. 2 is a metal processing factory; Cr(VI) was found in groundwater (200-600 µg/l) and in soil (up to 5000 mg/kg). The lithology of the area is very heterogeneous, with presence of boulders.

Since 2007 a hydraulic barrier has been operating at the site to prevent Cr(VI) migration in groundwater. In 2012, 1300 kg of 3D-Me® and 1300 kg of MRC® were injected through 8 vertical wells in the saturated soil zone, over an area of about 1000 m^2. Injections in the capillary fringe were also combined (800 kg of 3D-Me® and 800 kg of MRC® at 16 points over an area of about 250 m^2), in order to protect the aquifer

from vadose zone leaching. Cr(VI) concentrations decreases in all monitoring wells, resulting in groundwater concentrations at the point of compliance of about 150 µg/l, above the target value.
EHC-M® was used for full-scale remediation at a former chromium plating factory in Veneto (Eupolis Lombardia 2015). In the semi-confined silty sand aquifer, a 30 m long Cr(VI) plume, with values up to 200 mg/l, was observed. Initially, a P&T was installed but, as no appreciable reduction in chromium concentration was observed in more than 3 years operation, stabilization via injection of EHC-M® was carried out. In the pollution source area and immediately downgradient, direct push injections (90 kg EHC-M®) at 46 points were carried out in the unsaturated soil and groundwater. The distance between the points of injection was about 1 m. The injection pressure was up to 15 atm. A rapid decrease in redox potential (down to values of -300 mV in a couple of weeks) and Cr(VI) (down to values <5 µg/l in few months) were observed. Later on, redox potential stabilized at values around zero. Dissolved iron, manganese and sulphates also increased in groundwater.

From these cases emerge the criticality to guarantee the homogeneous distribution of the injected reagents into the subsoil. Ground fluctuations can lead to contaminant peaks in the water, if pollution in the unsaturated area is not treated. Treatments in the unsaturated soil followed by specific treatments in groundwater can complete the recovery process.

2.4 Chemical-physical processes

2.4.1 Fundamentals

Electrokinetics is a remediation technique for both the saturated and the unsaturated soil zone, based on the application of a low constant electric field between two or more electrodes (positive/anode and negative/cathode) (Zhang et al. 2010a; Wei at al. 2016; Yan et al. 2018). The field causes two important transport mechanisms, almost independent from soil intrinsic permeability: (a) Electromigration (transport of ionic species in bulk solution, according to the electric field direction); (b) Electroosmosis (bulk pore fluid migration, including neutral or charged dissolved species, from the positive to negative electrode). Electrodes are applied in the ground generating an electric field of 50-150 Volts to achieve the electrokinetic separation of chromium ions present in water (Wieczorek et al. 2005). Also an anion ($Cr_2O_7^{2-}$, CrO_4^{2-}, $HCrO_4^-$) movement is produced applying an electric field in the unsaturated soil. The electrodes with ceramic coatings are submerged in a process fluid that keeps stable the pH. Despite this, negative ions attracted to the anode create an acid front, lowering of the pH of the soil; the opposite situation happens at the cathode. This behavior is due to the H^+ and OH^- ions generated by electrolysis which migrate across the soil to the opposite electrodes. The cathodic flow must be pumped out, whereas chromium can accumulate at the anode as a precipitate. The moisture content must be enough to allow ion migration, but saturation must be not reach to optimize the process. Therefore it remains potentially one of the few in situ systems suitable for the

removal of Cr(VI) from the unsaturated area of the soil. It allows also a contextual removal of other metal ions. It can be used as the first step in a system based on several treatment technologies for the complete remediation of the unsaturated and saturated area of a contaminated site. Fine particle size and low permeability improve the results of the process. Among the difficulties must be cited the treatment of the extracted liquids besides the installation and maintenance of the system and the electrodes. It can be difficult to create homogeneous treatment zones and areas can be created with low electrical conductivity. Treatment aberrations may occur if there are particular heterogeneity in the subsoil that reduce the migration efficiency. The process is not efficient if there are different metal ions at different concentrations. The application of electrodes is also constantly evolving; alternatively, or in addition, to the electrokinetics processes, hexavalent chromium reduction can be carried out electrochemically (Chapter 4).

Soil flushing is used to treat unsaturated soil contaminated by leachable pollutants through suitable chemical agents (Tang et al. 2011; Yan et al., 2012a). In the case of Cr (VI), given its high solubility in water, the use of these latter may not be necessary (Yaqiao and Lei 2016). Aqueous solutions containing surfactants, chelating agents (eg EDTA) or acid solutions (eg HCl, H_2SO_4, HNO_3 o CH_3COOH) can be used if results are unsatisfactory or to reduce treatment durations. Soil flushing for Cr (VI), in general, has satisfactory results in alkaline, permeable, and homogeneous soils whereas rocky formations or layers of less permeable soil help to create preferential flows that leave the untreated zone. After extracting Cr(VI) from the soil, the solution containing the pollutant continues to filter into the unsaturated area until it reaches the level of the groundwater, where it is then removed through an extraction system (eg P&T). The extracting solution must be put in contact as closely as possible with the solid matrix; possible techniques are: surface flooding or irrigation, construction of filter beds or vertical/horizontal injection wells and trench infiltration systems. The equipment required is simple to install and operate. The use of a soil flushing system improves the performance of a possible P&T system installed for the treatment of contaminated groundwater thanks to the increase of the hydraulic gradient and to the greater solubilization and mobility of Cr(VI). The treatment of hexavalent chromium is mainly based to the reduction of the compound to its trivalent form rather than extracting it and treating it in the extracted water; for this reason full-scale interventions are not widespread. The characteristics of the aquifer and/or of the contamination in the unsaturated area must also be studied in detail for an accurate design of the intervention. Unexpected side and vertical diffusion of the contamination could occur, with consequent possible leakage of water contaminated by Cr (VI) from the extraction system if the initial modelling of the system has not been accurate. Among the site-specific soil conditions, the most favourable situations are good hydraulic conductivity, low content of iron oxide, clay and organic carbon, low cation exchange capacity and high pH (USEPA, 2000). The efficiency of the process decreases significantly with poorly porous or poorly homogeneous soils due to the possible creation of preferential flows that inhibit the treatment of large portions of polluted soil.

2.4.2 Field applications

The technologies discussed can be used for the removal of Cr(VI) from the unsaturated area of the soil, directly treating the source of contamination.

Electrokinetics full-scale applications have been satisfactory, although significant limitations in the process were observed when Cr (VI) concentration was low compared to non-target ion concentrations (CLAIRE, 2009). The equipment had to be optimised to reduce costs (Voccinate et al. 2016). Among the most recent laboratory-scale studies, Zhang et al. (2010a) propose to insert a second electric field (vertical) besides the horizontal one generated by the introduction of two electrodes perpendicular to the ground level. The vertical dispersion of ions is also taken into account generating a 2D electric field. The metal ions move along the resultant of the attraction forces in the presence of the two electric fields.

There are no complete applications of soil flushing in literature due to the usually reduction of chromium to the trivalent form. Trivalent chromium remains adherent to the solid matrix and therefore it is not necessary to pump groundwater.

2.5 Full scale implementation

With reference to the mentioned in situ remediation technologies for Cr (VI), they can be implemented in full scale using a range of approaches, depending on the technology, the zone to be treated, and the geological/hydrogeological features of the site. Reactive zones (RZs) and permeable reactive barriers (PRB) are discussed. Barriers are less widespread for the treatment of hexavalent chromium; however, they have prospects for future development.

RZs involve the generation of a zone with suitable physical-chemical features in the portion of ground/aquifer to be treated by injecting appropriate reagents, without soil excavation. They are the most widely used, in view of their versatility and the possibility of reaching considerable depths (Fruchter 2002b). The injections can take place upstream from the source of the contamination, next to it, and/or downstream (USEPA 2002). Reagents must be placed in the subsoil with an adequate pressure to obtain their correct distribution in the area to be treated. To intercept a plume in a saturated zone, lines of injection points can be used, perpendicular to the direction of the flow (Jeyasingh 2011). Within 10 m from g.l. and with lithology that is not excessively coarse (therefore, excluding gravel and pebbles), the reagents can be administered using “direct push” type systems, which require significant injection pressure to facilitate the distribution (OHIO EPA 2005). The zone of influence tends to diminish significantly as the viscosity of the fluid to be administered increases. The injection wells in a saturated zone can also reach very considerable depths, provided they use adequate pressure (USEPA 1989). It is advisable to distribute the injection points along the vertical. It is also necessary to carry out pilot tests to evaluate the distribution of the chemicals in the subsoil (ASTM 2013). Complexity due to the correct location of the injection points are compensated for the lack of excavation of

contaminated soil. In addition to the plume geometry, some more hydrogeological parameters must be considered in the evaluation phase of the applicability of these techniques to groundwater (NICOLE 2012):

- thickness of the unsaturated area (to decide the injection depth);
- lithostratigraphic sequence (to identify the fenestration of wells);
- horizontal and vertical hydraulic conductivity (for the distribution of reagents in the aquifer);
- groundwater velocity (to decide the injection flow rate and to evaluate residence and dilution times).

The hydrogeochemical parameters of interest are: dissolved oxygen, anions (nitrates, nitrites, sulfates, sulfides), total and dissolved iron, manganese, CO_2, CH_4, alkalinity, pH and TOC.

High groundwater velocities can wash out the reducing reagent if aqueous solutions are injected (in particular those aimed at promoting chemical reduction mechanisms). Instead, poor and not homogeneous distribution in groundwater may occur using viscous products to promote biological processes.

In summary, some common advantages of in situ RZs within the saturated zone are:

- the capability to treat directly the source of contamination;
- lower installations compared to P&T;
- reduced problems to carry out the treatment if the site presents problems of accessibility, presence of buildings or infrastructures.

The main disadvantages are instead:

- injections can reach limited depths, also due to the increasing costs with depth;
- design difficulties, for example for the quantification of the necessary reagents.

In very heterogeneous soil, the creation of RZs can result in treatments of the contamination that are not homogeneous, with zones of finer lithology barely involved in the process (Jeyasingh et al. 2011). The PRBs, which can only be used in saturated zones, consist of the substitution of the aquifer material with allocthonous material, through which the groundwater has to pass for the decontamination (USEPA 1988; ITRC 2011). This enables the achievement of a homogeneous treatment zone, regardless of the heterogeneity of the aquifer under examination. The PRBs are technically and economically sustainable if the depth of the installation does not exceed 25 m from g.l. (USEPA 1989; Sethi et al. 2011; Obiri-Nyarko et al. 2014; Faisal et al. 2018). Aquifers with high hydraulic conductivity are difficult to treat with this type of installation, because the reactive layer must have permeability of at least an order of magnitude greater than the aquifer to intercept effectively the contaminated plume. To increase the permeability of the barrier, it is necessary to increase its thickness so that the contaminant has an adequate hydraulic residence time in the RZs (Scherer et al. 2002; USEPA 2012). The use of reactive chemicals in the PRBs must consider possible problems of progressive fouling of the barrier. This is the case with the use of iron-based reagents, with the precipitation of the chemical species of Cr (III) and Fe (III) (Fuller et al. 2013; Liu et al. 2015). PRBs are well suited to the implementation of biological processes, in which case they are called “Biobarriers” (NAVFAC 2004; Careghini et al. 2013). PRBs for the treatment of Cr(VI) are based

on chemical-physical or biological mechanisms; the main involved mechanisms are those of reduction (chemically or biologically induced) and subsequent precipitation. Zero-valent iron is one of the most used filling material (Marrazzo, 2009). The construction of barriers with solid organic-based substrates (mulch, compost, sawdust, wheat straw) is also widespread. Finally, a suitable support material for the inoculation of dedicated microorganisms must be envisaged in relation to the application in PRBs of chromium resistant/reducers microbial species (Hohener and Ponsin, 2014). The applicability of these studies is actually mainly aimed at the treatment of water in plants; treatments in the subsoil with these biomasses are not yet developed.
In summary, the application of a PRB might be precluded by these hydrogeological factors: high groundwater velocities, presence of preferential routes, high aquifer permeability, high rate of heterogeneity in the aquifer and excessive depth of the aquifer. Regarding the hydrogeochemical factors, the presence of high concentrations of some compounds in groundwater may generate an excessive demand of reactive material due to the consumption by parasitic reactions (eg. sulphates, nitrates, carbonates, dissolved oxygen, silicates for PRB based on Fe (0), dissolved oxygen, nitrates for PRB based on biodegradation).
Therefore, it is necessary to take into account the type of reactive material, the quantity needed and its longevity to correctly plan the treatment. It might also be required a pre-treatment of the water upstream to the PRB. The disposal of the contaminated soil resulting from the excavation operations and any exhausted reactive materials must be taken into account in the cost/benefit analysis.
In summary, some advantages are:

- passive technique that can guarantee years of operation (depending on the reactive material) with minimal management operations, mainly related to monitoring;
- classified as "green technology" by USEPA (in particular the configuration based on the use of biodegradable carbon substrates) (USEPA 2017a).

As for the disadvantages, instead:

- construction of the barrier can be expensive in areas with buildings or many underground services;
- for Fe(0) based PRBs, the precipitation of chromium salts can cause the progressive coating of Fe(0), reducing the efficiency of the reactive material;
- the permeability of the reactive zone can be reduced due to the creation of biomass in case of biodegradation processes.

The number of contaminated sites treated using PRB with nano-particles Fe(0) is increasing in the USA, while its spread is still limited to few cases in Europe. Wilkin et al. (2014) reports the performance of a continuous PRB (length: 46 m; depth: 7.3 m; width: 0.6 m) based on Fe (0) installed in 1996 and still active in Elizabeth City (North Carolina, USA). The PRB was designed to treat waters containing Cr(VI) at concentrations >10 mg/l and some chlorinated solvents (TCE, cis-DCE, VC). During years, monitoring did not show significant changes in the pH in groundwater upstream (about 6), downstream (8) and within the reactive zone (10). Instead, a gradual decrease in the system's capability to maintain reducing conditions has been observed over years. Concentrations of Cr(VI) measured in piezometers within and downstream

the PRB were always between <0.1 and 3 μg/l, with an average removal efficiency of 99.9% over 15 years of activity. Jeyasingh et al. (2011) tested the applicability of biological PRBs for the treatment of groundwater contaminated by Cr(VI) at 50 mg/l; they carried out a pilot test to simulate the saturated area under dynamic conditions (Darcian water speed: $5{,}4 \cdot 10^{-8}$ m/s). The pilot (3 m long, 1 m wide and 0.5 m deep) was built in laboratory inserting a support layer (sand) for the biomass of 0.1 m wide at 1 m from the water inlet into the system. Laboratory selected biomass and molasses were added in the reactive zone; molasses was also periodically injected as a carbonaceous substrate. During test, Cr(VI) concentrations below limit of quantification were observed in all the piezometers placed at 50 cm downstream of the reactive zone.

2.6 Conclusion

In situ strategies with the objective of reducing Cr(VI) coupled with the oxidation of chemicals are also applied in Fe(0) permeable reactive barriers or by injecting nano-Fe(0), $Na_2S_2O_4$ or hydrogen sulphide to develop reactive zones in the aquifer (Chirwa and Molokwane, 2011; Tseng and Bielefeldt, 2002; Turick et al., 1998). The applicability and effectiveness of these approaches are limited by high costs, poor chemical distributions and undesired side reactions in the subsurface (Pous et al., 2018). Recent studies have shown also the possibility of using microorganisms to promote Cr(VI) reduction and immobilization in contaminated groundwater and/or soil.
In addition the fact that Cr(VI), as any metal, is not removed with in situ remediation, but only changes its oxidation state, requires to carefully evaluate the long-term stability of reduced products and possible re-oxidation mechanisms (Butler et al., 2015; Varadharajan et al., 2017).

The experience resumed pointed out that Cr(VI) groundwater pollution cannot be solved unless the unsaturated zone is treated as well. Soil heterogeneity is a critical issue and mixtures usually have to be injected at different depths. Localized iron, manganese, arsenic and sulphate side-contamination can result in groundwater after injections. An interesting topic that emerges from these studies is the possibility to provide a complete remediation treatment for a contaminated site. Some technologies are specific for groundwater contamination, but are less common the case studies that have also included treatment in the contaminated unsaturated area. For this reason, it is useful to evaluate the described technologies in relation to the peculiarities of the site and the possibility of adopting differentiated treatments. Even at the economical level it might be cheaper to plan interventions using more technologies rather than carry out only a treatment whose final results would slowly arrive.
In conclusion, the majority of in situ innovative technologies of hexavalent chromium aim at reducing the compound (Table 2.2). The trivalent form deposits on the solid matrix due to the precipitation of the hydroxides and it is considered less toxic, stable and not susceptible to reoxidation under typical ambient conditions, as presented in the literature by many authors.

	Process	Treatable Matrix	Application scale
Chemical processes	Reduction of Cr(VI) to Cr(III)	Groundwater/unsaturated soil	Full scale
Induced Bioremediation	Reduction of Cr(VI) to Cr(III)	Groundwater	Full scale
Bioremediation (direct action)	Reduction of Cr(VI) to Cr(III)	Groundwater	Laboratory scale
Soil flushing	Extraction of Cr(VI) (contaminated groundwater)	Unsaturated soil	Pilot scale
Electrokinetic separation	Extraction of Cr(VI) (contaminated groundwater)	Groundwater/unsaturated soil	Laboratory scale
Phytoremediation	Extraction of Cr(VI) (contaminated plants)	Unsaturated soil (surface layer)	Pilot scale

Table 2.2**.** Main concepts for the considered technologies.

No technology is able to completely remove chromium in a contaminated site; it is essential to evaluate the benefits of the removals in order to reach the removal targets. Furthermore, a technology can be adapted to the characteristics of a particular site. As a consequence, it is necessary to understand how to combine the characteristics of one or more technologies to achieve the objectives to fulfil the remediation of a site considering both environmental and economic sustainability.

3

BIOELECTROCHEMICAL SYSTEMS AND THEIR FIELDS OF APPLICATION

3.1 Fundamentals

Microbial bioelectrochemical systems (BESs), a new emerging technology that combines biological processes with electrochemical systems, interconverts electrical and chemical energy, enabling electricity generation, hydrogen production, chemical synthesis, wastewater treatment, desalination, and remediation (Xie et al., 2015). In BESs, electron transfer is continuously promoted/controlled in terms of current or voltage application between the bioelectrodes (Figure 3.1), close to which electrochemically active microorganisms are located with the role of catalyst (Bajracharya, 2016). BESs provides both oxidation (anode) and reduction (cathode) reactions that integrate microbial electro-chemical removal mechanisms (Wang et al., 2015). In principle, solid-state electrodes are attractive electron acceptors or donors because they can provide a low-cost, low-maintenance, continuous sink/source for electrons (Logan, 2009; Zhang et al., 2010b; Wang and Ren, 2013; Lu et al., 2014).

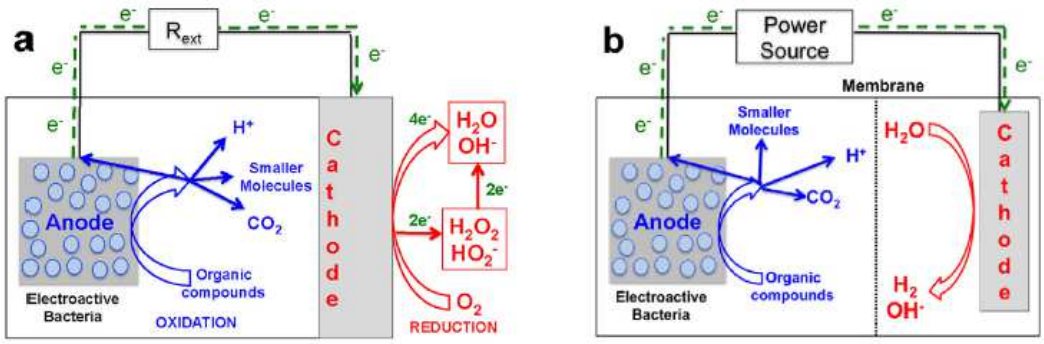


Figure 3.1: Microbial Fuel Cells (a) and Microbial Electrolysis Cells (b) graphical scheme (Santoro et al., 2017).

The nature of the reactions at the electrodes is decisive for the type and the outcome of a BES. In Microbial Fuel Cells (MFCs) (Figure 3.1a), oxidation of electron donor at the anode is coupled with a reduction of species with comparable or higher redox potential at the cathode. The net potential of the MFC, as the sum of anodic and cathodic potentials, is positive, therefore spontaneous electron flow from the anode to the cathode occurs (Rabaey et al., 2009; ElMekawy et al., 2013). Electrical energy is gained as a result of thermodynamically favourable reactions; available chemical energy in biodegradable organic matter is converted into electricity by the oxidative capability of anaerobic microorganisms (Gude et al., 2013; Logan et al., 2006; Santoro et al., 2017).
The reaction can be evaluated in terms of Gibbs free energy, expressed in units of Joule [J]. It provides a measure of the maximum work that can be derived from the reaction and it is calculated through:

$$\Delta G_r = \Delta G_0 + RTln(\pi) \quad (14)$$

where:

- ΔGr_0 [J] is the Gibbs free energy in standard condition (temperature equal to 298,15 K, pressure equal to 1 bar and concentration of all species equal to 1 M);
- R is the universal gas constant (8,31447 J mol^{-1} K^{-1});
- T [K] is the absolute temperature;
- Π [/] is the reaction quotient calculated as the activities of the products divided by those of the reagents.

The expression that links energy to the electromotive force of the cell, E_{emf} [V], defined as the potential difference between the cathode (E_{cat}) and the anode (E_{an}), is:

$$W = E_{emf} Q = -\Delta G \quad (15)$$

where:

- Q = *n**F it is the charge transferred in the reaction, expressed in Coulomb [C] and determined by the number of electrons exchanged;
- *n* is the number of electrons per mole and per reaction;
- F is the constant of Faraday (9,64853 × 10^4 C/mol).

Combining these two equations, it is possible to calculate E_{emf}:

$$E_{emf} = - \Delta G/nF \quad (16)$$

If all the reactions are evaluated under the standard conditions, $\pi = 1$:

$$E_{emf}^{0} = - \Delta G_0/nF \quad (17)$$

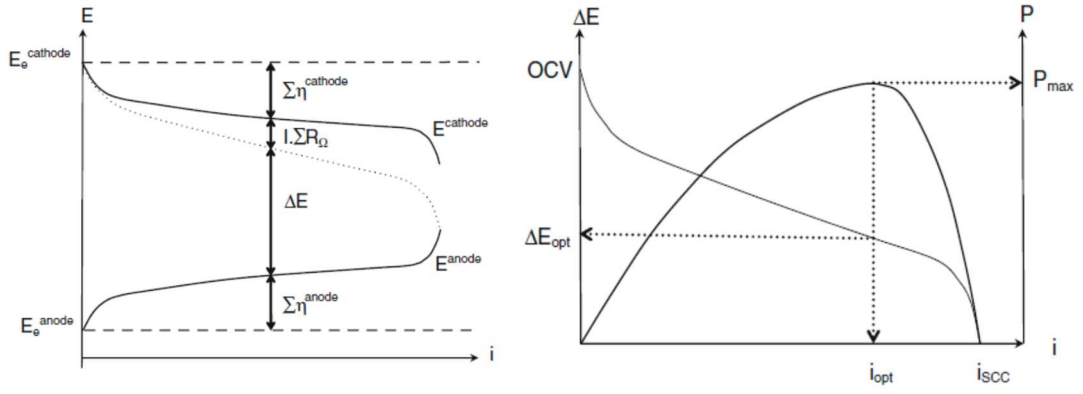
where:

- E_{emf}^{0} [V] is the standard electromotive force of the cell.

Generalizing, the equation of Nernst is derived:

$$E_{emf} = E_{emf}^{0} - RT/nF \cdot ln(\pi) \quad (18)$$

The advantage of this last equation is to directly provide a positive value of the electromotive force for any favored reaction. The calculation of this value provides the maximum limit of the cell voltage; the cell voltage in a BES is determined by the Open Circuit Voltage (OCV) lowered with losses that occur as current is produced. The OCV is the difference between the cathodic and anodic equilibrium potential (E_{cat} and E_{an}). The losses that occur in BESs are the over potentials of the anode and the cathode (Figure 3.2). Plotting the cell voltage and electrode potentials in function of the current density produced, which is called a polarization curve, is one of the most revealing methods to express the cell performance.



a b

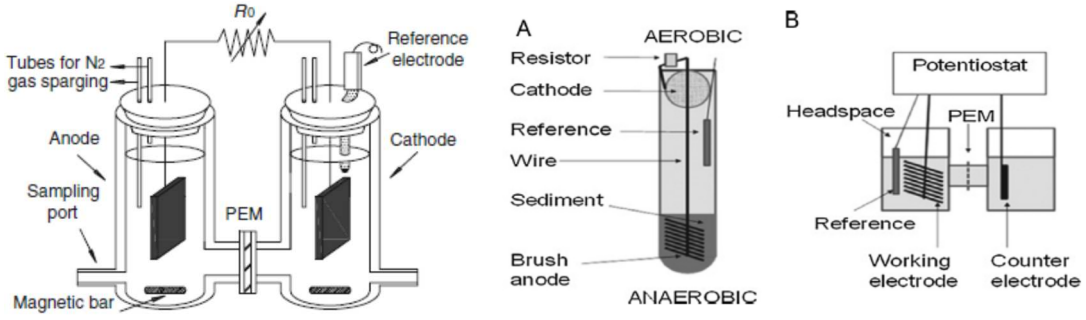
Figure 3.2. Schematic overview of MFC anodic and cathodic overpotentials (Σηanode and Σηcathode) in function of the current density (a); the cell voltage (ΔE) and the power density (P) in function of the current density (b) (Clauwaert et al., 2008).

Conversely, with electrochemical devices (Figure 3.1b) where small amounts of electrical power are made available to microbes who act as biocatalysts, energy is supplied to allow or enhance thermodynamically unfavourable processes (Rabaey and Rozendal, 2010). Thanks to external power input to force electron flow, the oxidation of an electron donor at the anode can be coupled to reduction of lower redox potential species at the cathode.

3.1.1 Materials and Systems Monitoring

Reactors consist of glassware, ion exchange membranes, and electrodes mainly made of carbon materials (Figure 3.3). Electrodes are immersed in electrolytes, which contain both the reagents and the products of the bio-electrochemical reactions, and are connected via an external circuit.
Membranes provide a separation structure to isolate different bulk liquids, to optimise the operating conditions without affecting the microbial community, to prevent undesired substrate transport, and to facilitate the transfer of ionic species from one chamber to another for charge balance, increasing, however, the internal resistance of the system (Kokabian and Gude, 2015). These membranes are classified in cationic, anionic and bipolar, based on migration species. PEM (Proton Exchange Membrane) scores high for selectivity but low for stability; CEM (Cation Exchange Membrane) has a greater resistance and is less selective but generally shows greater stability (ElMekawy et al., 2013). Applying membranes lies in the development of a pH gradient between anode and cathode which involves an additional energy loss; another disadvantage with membranes could be the cost (Kadier et al., 2016). To limit pH unbalance, bipolar exchange membranes (BPMs) could be assembled by a cathodic and an anodic layer forming a wet bonding interface, where water splits into protons and hydroxyl ions (Kozaderova, 2018; Chacon-Carrera, 2019).

In electrochemical experiments, electrode surface may catalyze chemical reactions; the size affects the magnitude of the currents passed which can affect signal to noise. However, electrodes are not the only limiting factor for electrochemical experiments, the potentiostat also has a limited range of operation. Both the electrodes and the connections can present differences in terms of material, shape and size. The desirable features of bioelectrodes are high conductivity, stability, and biocompatibility; design developed from nonporous to modern porous prototype that are particle-based, fiber-based, or monolithic (Xie et al., 2015). The most promising strategies use porous structures conducive to microbial colonization and surface materials that promote Extracellular Electron Transfer (EET).



a b

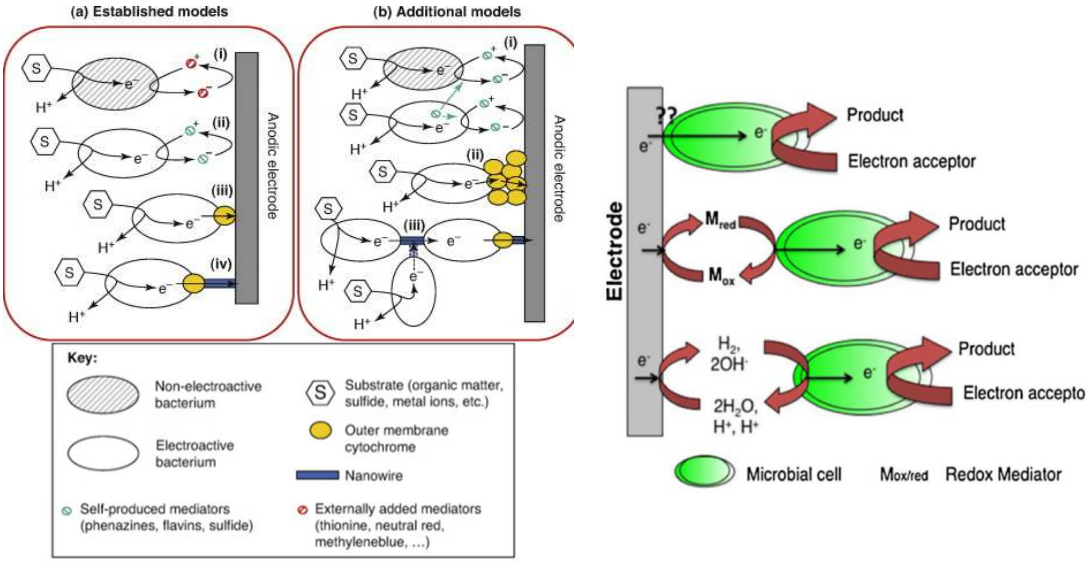
Figure 3.3. Schematic of a Double chamber Microbial Fuel Cell (2C-MFC) (a) (Wang et al., 2008); two-step method used to first enrich (A) a diverse, electrically active sediment biofilm on MFCs brush anode that was then transplanted to form (B) the working electrode of a dual-chamber (b) (Kadier et al., 2016).

To effectively apply BESs, both biological and electrochemical losses need to be further minimized (Clauwaert et al., 2008). Furthermore, both the ohmic cell resistance and the pH gradients need to be minimized. The losses can be mainly classified as activation overpotentials, concentration polarization and ohmic losses (ElMekawy et al., 2013). The direct flow of electrons from the bacteria to the electrode is hindered mainly by the transfer resistances which are known as overpotentials. Concentration polarization occurs when compounds are being oxidized faster at the anode than they can be transported to the surface, and this could be due to the large oxidative force of the anode. Ohmic losses are due to electrical resistances of the electrodes, membrane and electrolyte. It has been observed that cathode resistance could be significantly reduced by increasing the size of the cathode compared to the anode (Fan et al., 2008).

Further work is needed to understand important biological and engineering issues that underpin BES technology; only a few pilot studies have been run in real-world conditions and more pilot studies and scaled-up demonstration projects are needed to prove the reliability of the systems by bacteria (European Commision, 2013). The costs of reactor materials have to be decreased, and the volumetric biocatalyst activity in the systems has to be increased substantially.

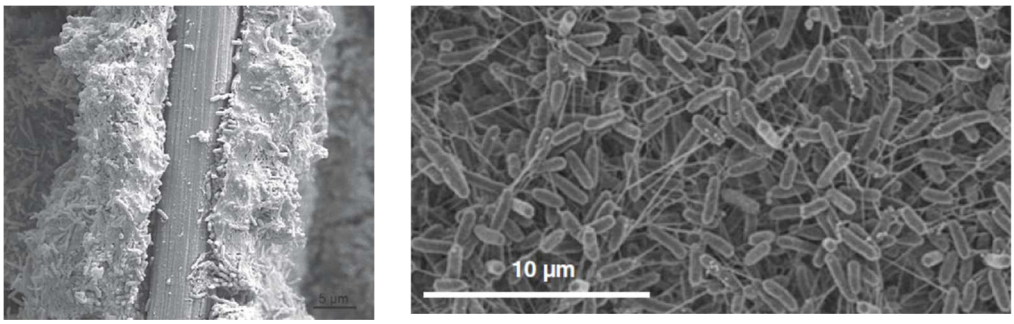
3.1.2 Role of microorganisms

Certain microorganisms have demonstrated the ability to use the electrodes as inexhaustible electrons acceptors/donors (Figure 3.4, 3.5), via a process typically referred to as EET, and to catalyze redox reactions in order to produce a flow of electrons (Logan et al., 2006; Rabaey et al., 2009; Shi et al., 2016). Microorganisms, capable of maximizing their energy gain, when there is a shortage of soluble electron acceptors, can take advantage of solid electron acceptors. Therefore, the EET concerns the mechanism of transport of electrons inside and outside the microbial cells. EET and even biochemistry of so-called ‘electroactive’ bacteria (EAB) have gained deeper insights and shown that electroactives are more abundant and important than considered, so far (Aulenta and Harnisch, 2017). Electrodes serve as electron acceptors or donors for EAB, and the rate of electron flow between electrodes and EAB is related to their metabolic activities.



a) b)

Figure 3.4. Models representations of electron transfer from microorganisms to the electrodes in BES (a): it occurs via exogenous or self-secreted (by microbes) redox mediators and oxidation of reduced primary metabolites (i, ii) and via cell-membrane-bound cytochromes and/or electrically conductive pili (nanowires) (iii, iv). Mechanisms from electrodes to microorganisms: it occurs by direct route, mediated by electron shuttles, and indirectly by oxidation of hydrogen by microorganisms (Patil et al., 2014).



a) b)

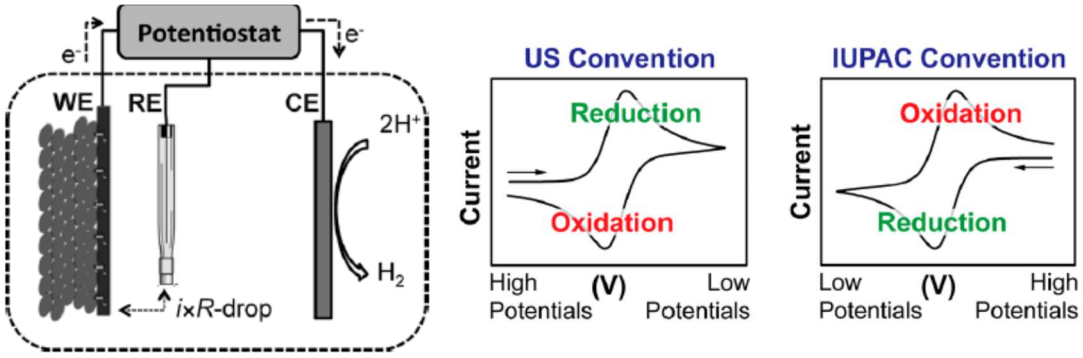
Figure 3.5. Scanning electron microscopic images of a mixed culture bioelectrocatalytic anodic biofilm derived from wastewater on a carbon fibre electrode at magnifications of 5 µm (Rif.) (a); Nanowires electrically conductive produced by Shewanella oneidensis strain MR-1 (b). The nanowires are considered as the extension of outer membrane and perform EET by electron hopping.

Microorganisms that have EET capability can be used for biotechnological applications, including bioremediation, biomining and the production of biofuels and nanomaterials (Shi et al., 2016):

- specific microorganisms use metal-containing minerals as electron sinks for heterotrophy-based respiration and electron and/or energy sources for autotrophic growth;
- the microbial cell envelope is an electrical and physical barrier that can be overcome; some microorganisms can extend their redox-active surface by forming microbial nanowires, which transfer electrons to distal minerals;
- minerals that contain metal ions can also function as electrical conductors and batteries to facilitate electron exchange among different groups of microorganisms.

Researchers have therefore attempted to develop methodologies for electrochemically modulating microbial metabolisms using electron-shuttling compounds (redox mediators) that mediate electron transfer between electrodes and intracellular redox molecules (Kumar et al., 2017a).
EAB alter their gene-expression patterns and metabolic pathways in response to changes in intracellular redox states, for example in response to shifts in electrode potentials (Hirose et al., 2019). This finding suggests that an electrode can be used for the direct control of gene expression in EAB and opens up a novel biotechnology platform, termed “electrogenetics”.
The research on EAB developed also with cyclic voltammetry (CV) (Virdis, 2018). CV experiments on electroactive microbial biofilms needed for a three-electrode set-up (M3Cs): a working electrode (WE), a reference electrode (RE), and a counter electrode (CE) (Figure 3.6a). Using this set-up, a current–potential polarization curve can be recorded using a potentiostat for controlling the voltage between the WE and the RE and for measuring the current flow between the WE and CE (Zoski, 2007). During CV, a potential is applied to the WE; a CV scan starts with a certain scan rate, thus, the flowing current is recorded. The derived spectrum, which shows the current as a function of potential, is called a cyclic voltammetric curve (Harnisch and Freguia, 2012) (Figure 3.6b). CV is a powerful technique for the study of the EET of electroactive microbial biofilms (Harnisch and Freguia, 2012):

- formal potentials of redox species;
- reversibility of redox species and its dependency on the scan rate;
- studying the influence of the mass transfer;
- elucidating which redox couples are responsible for the development of turnover currents, i.e. the identification of the bioelectrocatalytically active sites;
- distinguishing between adsorbed and diffusive natures of the mediators.



a b

Figure 3.6. A potentiostatic three-electrode experiment on an anodic biofilm that is housed in a single-chamber electrochemical cell (a) (Harnisch and Freguia, 2012); the counter reaction at the CE is the reductive hydrogen evolution from protons. Convention commonly used to report CV data (b) (Elgrishi et al., 2018).

When WE potential is changed, two different types of current can flow: capacitive currents and faradaic currents. Whereas faradaic currents are related to an oxidation or reduction reaction, capacitive currents are not. For low biofilm coverage or biofilms with low electrocatalytic activities, the capacitive current may always mask the faradic current, even at low scan rates, and thus allow no CV analysis. Low scan rates allow the resolution of close redox peaks, which become indistinguishable as the scan rate is increased. At a slow scan rate, four peaks systems can be revealed.
No theoretical support has been available so far to explain some of the behaviour observed through transient CV; theoretical models that exploit transient electroanalysis of microbial systems could be developed (Rousseau et al., 2014).

Data gained by a purely electrochemical study needs to be combined with data from, e.g., molecular biology and metabolomics, in order to gain a better understanding of the mechanisms of cell–electrode interactions at the molecular level (Fricke et al., 2008).

3.1.3 Applications

Not only MFC and Microbial Electrolysis Cells (MEC) describe BESs (Figure 3.7); fields of application are very different. However, there are a number of biotechnological barriers that first have to be overcome before this application would be feasible at the commercial level. Costs have to be competitive with other water treatment and chemical production processes before BES can be adopted on a commercial scale (European Commission 2013).

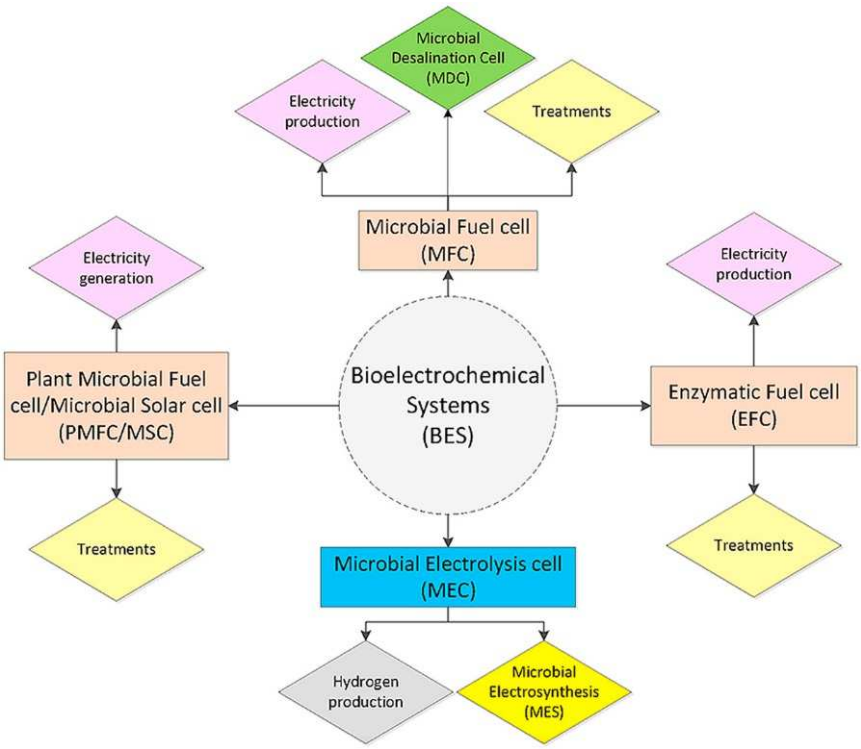


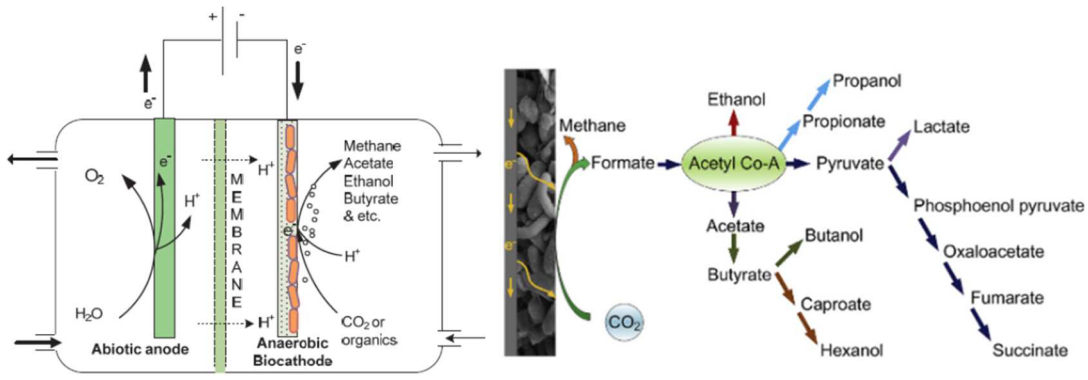
Figure 3.7. BESs applications (Bajracharya, 2016)

Research has broadened the utility of BESs to processes such as resource recovery and chemical synthesis. The production of energy-rich chemicals such as hydrogen, H_2O_2, caustic soda, ethanol, methane or even organic molecules is achieved with lower energy required compared to typical electrolytic reactions (Escapa et al., 2016; Kadier et al., 2016).

The possibility of sequestration of atmospheric CO_2 exploiting a bioelectrochemical reduction at the cathode, is an application of environmental interest. Electro-Synthesis (ES) is the use of inorganic (es. CO_2) (Figure 3.8); bioelectrochemical reduction of CO_2, electricity-driven, is a specific application of autotrophic bioproduction (Rabaey and Rozendal, 2010). Microbial ability enables reduction reactions to be catalyzed by accepting the electron (energy source) from the cathode with the production of organics; there is no need for the external supply of hydrogen as the microbe can obtain the protons directly from electrolyte. Electro-autotrophic bacteria, such as *Clostridium ljungdahlii*, utilize electrical currents as an electron source from the cathode to reduce CO_2 to extracellular, multicarbon, exquisite products through autotrophic conversion. Jabeen and Rarooq (2016) tested *Clostridium ljungdahlii* as electro-autotrophic bacteria to reduce CO_2 obtaining organic acids and alcohols. A pre-enrichment method (to accelerate bio-fuel production) is suggested: anaerobic enrichment on fructose, inoculation of cathode at –400 mV, and carbon dioxide provided as sole carbon source to switch from heterotrophic to autotrophic growth. *Clostridium ljungdahlii* and *Clostridium*

aceticum are reported to be used in a biocathode (-400 mV); acetate, 2-oxobutyrate and formate were produced (Nevin et al., 2011 in Tremblay and Zhang, 2015).

Anaerobic metabolisms of homoacetogenic bacteria are known for the metabolic conversion of CO_2 to acetate and other multi-carbon compounds. An oxidation reaction at the anode produces protons and electrons for the cathodic reduction and an electric power source (by applying external electrical energy) drives the electrons from anode to cathode through an external circuit. Several lithoautotrophs are reported for the metabolic reduction of CO_2. When homoacetogenic bacteria catalyze the CO_2 reduction reaction, the major product of CO_2 reduction is mainly acetate (Bajracharya, 2016). However, the products can extend to alcohols and other carboxylates. In another case, if methanogens are present then methane (CH_4) is ultimately produced. The BES of volatile fatty acids and alcohols directly from CO_2 is a sustainable alternative for non-renewable, petroleum-based polymer production.



a b

Figure 3.8. Principle of microbial electrosynthesis at the cathode (Bajracharya, 2016) (a); possible metabolic pathways for microbial electrochemical CO_2 reduction that leads to the generation of a variety of high value organic compounds beyond acetic acid and CH_4 (Jiang et al., 2019).

The conversion of CO_2 to produce organic compounds as methane (for fuel) or bioplastics (e.g. poly-ß-hydroxybutyrate) implies reduction of greenhouse gas emissions (European Commision, 2013). The electrosynthesis at bio-cathode is a novel approach for the bio-production by microbial fixation of CO_2.

Fermentation is an anabolic metabolic process in which glucose is degraded into other organic compounds, producing energy. It consists in the transformation of a glucose molecule into two pyruvic acid molecules that are subsequently reduced to lactic acid with a low energy yield. Lactate fermentation is involved in the preparation of numerous foods including, for example, yogurt, kefir, capers and sauerkraut. In Electro-fermentation (EF), microbial fermentative metabolism is electrochemically controlled with electrodes (Moscoviz et al., 2016) that can act as either electron sinks or sources that allow unbalanced fermentation. In EF starting from organic compounds (e.g. acetate, butyrate) commonly found in industrial wastewaters to produce ethanol

biofuel). The EF process is the subject of recent studies; in Xue et al. (2018) the cathode poised at -0.1 V vs SHE stimulated ~~the~~ lactic acid productivity that resulted 4.73 times higher compared to that in the open circuit control. Both mixed culture and pure were evaluated; *Corynebacterium glutamicum* showed higher lactate yield from glucose in a bioelectrochemical reactor with the cathode poised at -0.4 V (vs. SHE) (Sasaki et al., 2014). Xafenias and Mapelli, (2014) investigated the use of electric potential to bioelectrochemically ferment glycerol, a cheap by-product of biodiesel production, into valuable 1,3-propanediol (1,3-PDO). The 1,3-PDO production rates were increased up to 6 times in EF, compared to non-EF, and high concentrations up to 42 g 1,3-PDO/l were achieved in fed-batch mode. These actions demonstrated that low cathodic potential is beneficial for lactate production; nevertheless, more studies on applied potential and mediation mechanisms wpuld be beneficial.

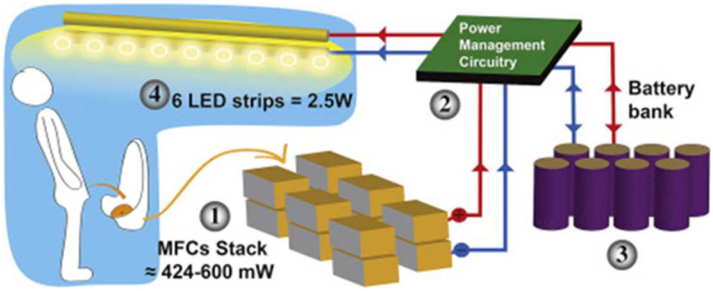
BESs have been extensively studied for wastewater valorisation and treatment (Figure 3.9a-c); the volume of wastewater from all sources will increase and the cost of wastewater treatment will also rise (Zou and He, 2018). Organic waste in wastewater contains more internal energy than the amount of energy required to treat the wastewater. If that energy could be released, little or no extra energy would be needed during the treatment process (Li et al., 2015). However, MFC maximum power outputs achieved thus far are in the order of 100 W/m^3, which is rather low in terms of investment costs compared to anaerobic digesters that produce electrical power up to 1000 W/m^3 (Pham et al. 2006). The largest energy savings may come from reducing costs for aeration; in addition, as MECs operate under anaerobic conditions, the production of sludge could be significantly reduced compared with the activated sludge process.

Power generation of low power devices (Figure 3.9d-e) and desalination (Figure 3.9f) have been intensively developed, especially in recent years. MFCs are also being explored as sustainable power supplies for robots (‘gastro-bots’) using biomass to generate electricity in artificial stomachs (Ieropoulos et al., 2008). Sediment MFCs are typically sufficient to power small devices such as radio sensors or meteorological buoys in remote areas (Figure 3.9g); the anode is embedded in the (anoxic) sediment, while the cathode is placed above seawater, where oxygen is available. MFCs are being used to power remote biosensors (Figure 3.9h-i), such as tools for monitoring water quality, as example to monitor BOD in water (Di Lorenzo et al., 2014). Sensors can be put in position and the collected data transmitted wirelessly. Batteries that have traditionally been used to power the sensors and data transmissions can be replaced with self-sustaining MFCs (Shantaram et al., 2005). There is a growing interest in the development of even smaller BESs, in which the effective chamber volumes are reduced to the (sub) microliter regime (Figure 3.9j-k). They are still limited by relatively low volumetric power density and coulombic efficiency, probably due to their high internal resistance (ElMekawy et al., 2013; Zhang et al., 2017).

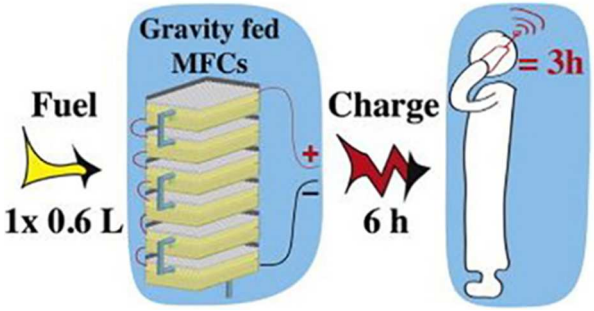
a

b

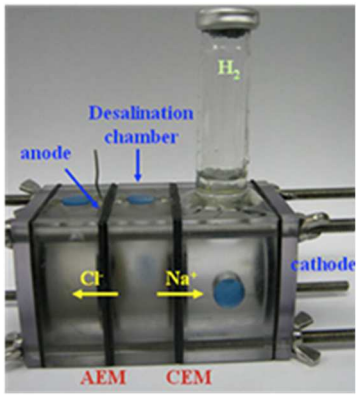
c


Power Management Circuitry
4 6 LED strips = 2.5W
2
Battery bank
1 MFCs Stack ≈ 424-600 mW
3

d

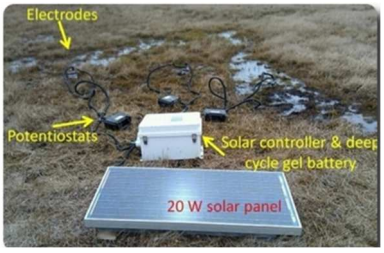

Gravity fed MFCs
Fuel
1x 0.6 L
Charge
6 h
= 3h
+
–

E

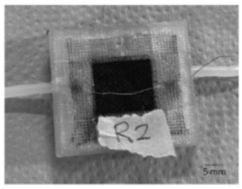

H2
Desalination chamber
anode
cathode
Cl-
Na+
AEM
CEM

f

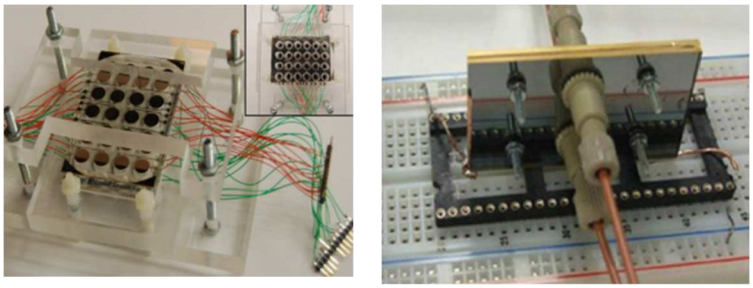
g


Electrodes
Potentiostats
Solar controller & deep cycle gel battery
20 W solar panel

h


R2

i



j k

Figure 3.9. Floating MFC, pilot plant in Milan (Cristiani et al., 2015) (a); MFC/MEC pilot plant feeded with winery effuents, USA (Rif.) (b); MFC Pilot plant streams from brewery (Australia) (Rif.) (c); power generation of low power devices (d-e); desalination (f); benthic unattended generator (g); sensor to monitor microbial respiration in drained thaw lake basins (Alaska) (Rif.) (h-i); microfluidic MFC development (ElMekawy et al., 2013).

BES application for agriculture has not been well explored. BES may be applied to treat the waste/wastewater from agricultural production, minimizing contaminants, producing bioenergy, and recovering useful nutrients and can also be used to supply irrigation water via desalinating brackish water (Li et al., 2015). The energy generated in BES can be used as a power source for wireless sensors monitoring the key parameters for agricultural activities.

BESs for stimulation of bioremediation reactions (Logan, 2008), reclaiming and recovering metals, including hexavalent chromium (Chapter 4), is a new and promising approach in water treatment (Nancharaiah et al., 2015).

3.2 BESs for remediation

Solid-state electrodes that could be used as terminal electron acceptors/donor have raised the possibility that they could also be employed to accelerate the microbial catalyzed contaminants remediation in soils and groundwater (Lu et al., 2014). Microbial electro-remediation represents an opportunity to develop a robust and sustainable technology in a context with different contaminants that are already present in groundwater bodies and soils (Rosenbaum et al., 2011; Pous et al. 2017). BES technology proved its flexibility, as it has been adapted for ex situ or in situ treatment applications depending on the target pollutant (Figure 3.10, 3.11) (Harnisch et al., 2011). In BESs, limited energy and chemicals are required with respect to other bioremediation technologies (Wang and Ren, 2014). The electrical signal generated in BESs also provides great opportunities for real-time monitoring of target contaminant concentration (Zhou et al., 2017; Wu et al., 2018b; Zhao et al., 2018) and in-situ microbial activity (Williams et al., 2010). Generally, positive effects on microorganism activity are reported with fields that are electrostatic or generated by direct currents until around 100 V/m (or induced currents of about 10 mA); some

positive laboratory experiences with BESs have been reported (Beretta et al., 2019). Electric field, in fact, induces changes in physicochemical and hydrologic properties of soil, with effects on distribution and chemical speciation of contaminants and natural soil constituents (Wang et al. 2016, 2015; Wu et al 2018a).

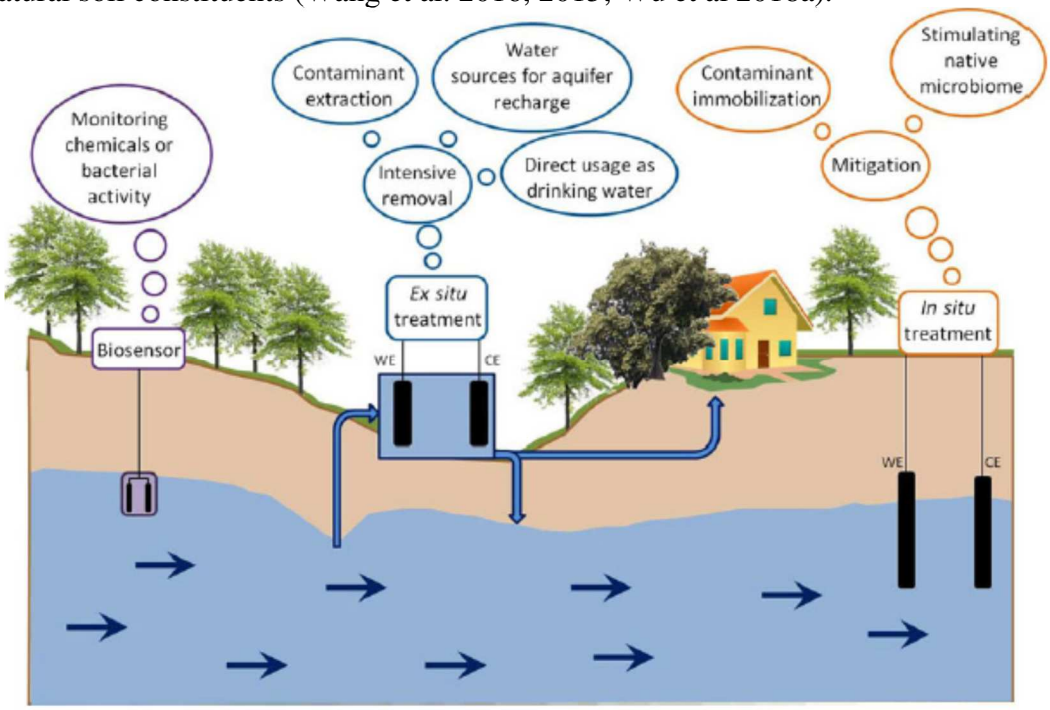


Figure 3.10. Framework of opportunities for microbial electrochemical technologies in groundwater (Pous et al., 2018).

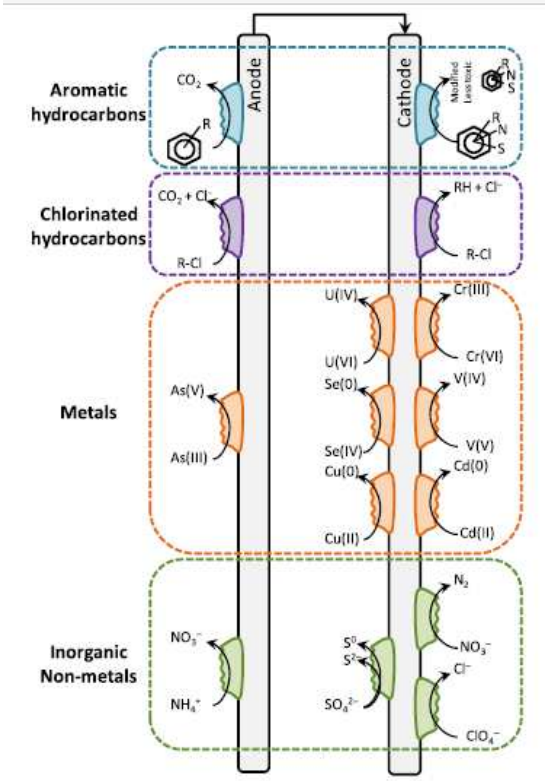


Figure 3.11. Summary of electrochemical reactions for the different pollutants treated in groundwater (Pous et al., 2018).

Through biologically-mediated oxidation (at the anode) and reduction (at the cathode), BESs potentially provide a flexible platform for the treatment of many pollutants frequently found at contaminated sites and even co-contaminations (Lovley, 2011; Wang et al. 2015). Solid electrodes can serve, in fact, either as electron sink, for the oxidation of petroleum hydrocarbons (Daghio et al., 2017; Palma et al., 2018; Zhang et al., 2010b) or As(III) (Nguyen et al., 2016b), or as electron donor, for reduction of chlorinated hydrocarbons (Lai et al., 2017; Leitão et al., 2017), nitrates (Cecconet et al., 2018) or oxidized metals, including Cr(VI) (Gregory and Lovley, 2005).

The discovery that microorganisms (e.g. *Shewanella, Geobacter*) are capable of forming highly conductive networks of filaments that transfer electrons along their length with organic metallic-like conductivity, provides an explanation for the ability of species to grow in subsurface environments and effectively remediate groundwater contaminated with hydrocarbon fuels or similar contaminants (Lovely, 2011).

An integrated anaerobic/aerobic process to completely degrade chlorinated compounds has also been proposed (FCEE, 2004; Harnisch et al., 2011); higher chlorinated compounds, such as PCE, are anaerobically transformed to lower chlorinated compounds, such as cis-DCE, which are subsequently oxidized, through metabolic or co-metabolic processes, under aerobic condition. The in situ enhancement of dechlorination is often obtained by injection of different types of fermentable substrates such as glucose, formate, yeast extract, methanol, lactate, propionate, butyrate, benzoate and ethanol, which are fermented, to H_2 by

autochthonous microorganisms (Aulenta et al., 2011). Aulenta et al. (2007; 2009) described the use of a polarized carbon electrode (i.e., –450mV vs. SHE) in combination with a redox mediator (i.e., methyl viologen) as electron donor for the microbial dechlorination process. The potential of the cathode is set at a value that does not allow H_2 production from water electrolysis, thus minimizing the occurrence of competitive reactions, such as methanogenesis. *Geobacter* is capable of partially dechlorinating PCE to cis-DCE using a polarized (-300 mV vs. SHE) graphite cathode as the sole electron donor, in the absence of any externally added redox mediator (Strycharz et al., 2008; Di Battista et al., 2012). The inability of this microorganism to dechlorinate beyond cis-DCE represents a major limitation in its practical application. Recently, the main attention has been given to the oxidative dechlorination of a less chlorinated solvent. One possible explanation for the poor degradation efficiencies is the presence at the anode of microbiologically hostile conditions (Lai et al 2015). In order to optimize the anodic compartment, abiotic batch experiments were performed with various anode materials (i.e., graphite rods and titanium mesh anode coated with mixed metal oxides (MMO)) and setups (i.e., electrodes embedded within a bed of silica beads or graphite granule). The MMO anode displayed higher efficiency (>90%) for oxygen generation compared to the graphite electrodes (Lai et al., 2017).

Petroleum hydrocarbons (i.e., PAH, BTEX, phenols, mineral oil, CHC) are detected in over 50% of European contaminated sites. Bioremediation is typically regarded as a sustainable technique (Ghosal et al., 2016; Varjani and Upasani, 2017; Nzila, 2018; Daghio et al., 2018); strategies involve O_2 delivery into the subsurface. However, relatively high energy input associated with areation (e.g., blowers), O_2 consumption by side-reactions (Fe^{2+}, S^{2-} oxidation) and O_2 diffusion away from the reaction zone limited the advantages of the technique. With BES, completely anaerobic, microorganisms catalyze oxidation by using solid-state electrodes as terminal electron acceptors (Espinoza Tofalos et al., 2018). The feasibility of an electrode-based bioremediation approach involving crude oil, gasoline, or aromatic hydrocarbons as target contaminants has been recently presented in a number of lab-scale studies (Rakoczy et al., 2013; Wang et al., 2012; Zhang et al., 2010b; Morris et al., 2009; Palma et al., 2016). Microbial communities enriched during hydrocarbon oxidation in BESs have been fully characterized in a few cases only. Among them, microbial communities dominated by microorganisms belonging to the *phylum Chloroflexi* and to the genus *Nitrospira* were described during PAH degradation in soil (Yan et al., 2012b), while Lu et al. (2014) highlighted that *β-Proteobacteria and γ-Proteobacteria* were the most abundant classes after total petroleum hydrocarbon soil remediation with BES. Interestingly, Rakoczy et al. (2013) noticed that the families *Desulfobulbaceae* and *Desulfobacteraceae* were enriched on the anode during benzene degradation in sulfide rich groundwater. This observation suggested that microorganisms involved in the sulfur cycle can play a key role in bioelectrochemical processes.

The ability to produce chemicals from CO_2 could be of particular interest for wastewater treatment. However such a system could also be studied for an aquifer; in fact, cathodic biocatalysts produce various types of carbon source that could be quickly used by autochthonous microorganisms in aquifers. The reaction of carbonates,

commonly found in aquifers, could provide substrates without having to inject the by-products for the development of biological activity, as described in the previous chapters.

3.2.1 Feasible interference in groundwater

Another challenge for bioelectrochemical treatment of contaminated groundwater is the presence, in addition to the contaminants, of a mixture of different naturally occurring inorganic (calcium, magnesium, carbonate, nitrates, and sulphates) and organic chemicals (e.g. humic acids) (Squillace et al., 2002). Magnesium and calcium can produce precipitates with the consequent passivation of the cathode (Santini et al., 2017). Identifying a correlation between the decreasing performances of the device and the increasing quantity of scale deposition, they noticed carbonate scale deposition penetrating the cathode cross section over time.

Bioelectrochemically assisted reduction has been recently reported for nitrate (Duca and Koper, 2012; Pous et al., 2013); electrocatalytic removal is a promising alternative to bacterial denitrification to N_2, which can be returned to the atmosphere. BESs for nitrate reduction, have been extensively applied, especially for nitrogen removal from civil or industrial wastewater. Its presence in groundwater originates from a number of nonpoint sources, including geological origins, septic tanks, improper use of animal manures, cultivation, precipitation and fertilizers. A pure culture of the metal-reducing microorganism *Geobacter metallireducens* was shown to reduce nitrate to nitrite with a graphite cathode set at –300mV vs. SHE, with the expected stoichiometry of electron consumption (Gregory et al., 2004). The complete reduction of nitrate to nitrogen gas has been observed with mixed cultures in the cathode chamber of an MFC (Clauwaert et al., 2007); sustained nitrate reduction rates were obtained only when the cathode potential was lower than 0 mV vs. SHE. Xie et al. (2014), in a -252 mV versus SHE poised biocathode, observed lower perchlorate reductions (E^0 ClO_4^-/Cl^- = 1.28 V) in the presence of nitrate (E^0 NO_3^-/N_2 = 1.25 V) and total suppression of the process at about 130 mgNO_3/L. This inhibition of perchlorate reduction in the presence of nitrate is not specific to bioelectrochemical reduction as it was also observed when using organic carbon or hydrogen as electron donors (Ricardo et al., 2012; Zhao et al., 2011). Most of the perchlorate-reducing bacteria are denitrifiers (Nozawa-Inoue et al., 2011) and have a substrate preference over nitrate that allows faster cell growth. Therefore, perchlorate reduction starts only after nitrate is depleted (Bardiya and Bae, 2011).

Sulphate reduction was observed when the cathodic potential was poised 0.26 V vs. SHE, with a minimum energy requirement of 0.7 V, while maximum removal occurred at 1.4 V applied. The reduction of sulphate led to sulphide production, which was entrapped in the ionic form thanks to the high biocathode pH (i.e. pH of 10) obtained during the process (Coma et al., 2013).

Lai et al. (2015) investigated the potential effects of nitrate (about 16 mg/L) and sulphate (215 mg/L) on bioelectrochemical reductive dechlorination of cis-dichloroethene in real contaminated groundwater. Tests with poised biocathode in the potential range -550/-750 mV vs SHE revealed that the cathode potential has a key role in selecting the target pollutant. Nitrate reduction always took place, but as the cathode potential ~~got~~ lowered, sulphate reduction and methanogenesis activities

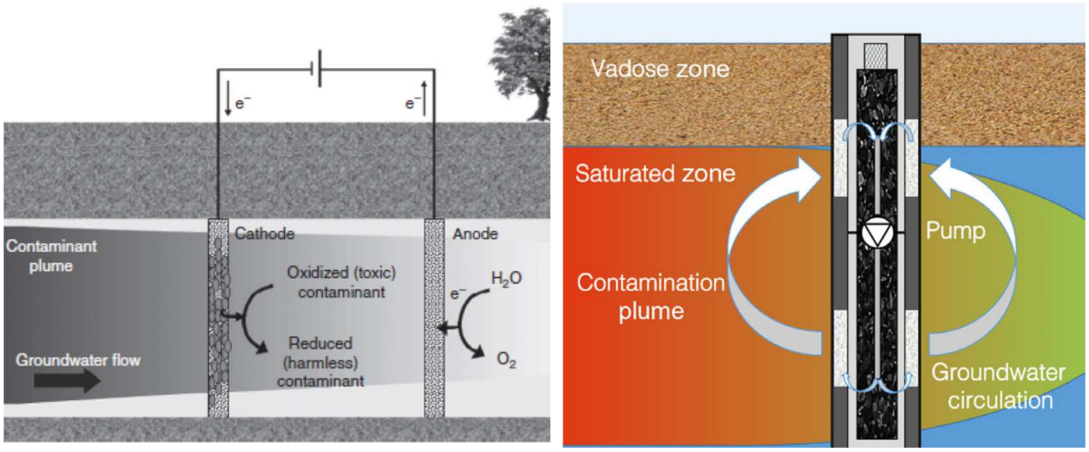
increased. Even if reductive dechlorination was not inhibited, the electricity consumption in the reactor was raised due to cross-reactions. Nguyen et al. (2016a), compared the denitrifying activity in a biocathode with or without sulphate (50 mgS-SO_4^{2-}/L), and observed inhibition of denitrification in the presence of sulphate, with both decreases in the nitrate removal rate and nitrite intermediate accumulation in the system.

So far, the study of co-contaminants with BESs is limited but, theoretically, as reduction potentials of nitrate and sulphate are similar to the reduction potential of several pollutants, they can be electron competitors in the remediation process and affect the microbial community at the biocathode (Bardiya and Bae, 2011; Daghio et al., 2017).

3.2.2 Lab tests and full scale option

In Huang et al., (2011a) a MFC was inserted into waterlogged soil to enhance the biodegradation of phenol and, simultaneously, electricity generation. The highest power density reached was 29.45 mW/m^2. Under closed-circuit conditions operated for 10 days, about 90% of the phenol was removed; the phenol degradation rate constant under closed-circuit conditions was approximately 23 times higher than under non-MFC conditions. The degradation of phenol was also positively correlated with the removal of soluble COD and particulate COD, indicating that the removal of organic pollutants and COD in waterlogged soils could be enhanced by a soil MFC. Domınguez-Garay et al. (2017) developed a so-called bioelectroventing strategy for achieving an effective clean-up of the atrazine-polluted soils able to restore the prepollution conditions.

In principle, BESs could be employed in a variety of remediation schemes either in situ (e.g., in a permeable biobarrier) or ex situ (e.g., aboveground bioreactor). For in-situ applications, the management of the specialized equipment required must be optimized to reduce costs and make technology competitive (Voccinate, 2016). Electrodes could be inserted directly into the aquifer medium or conversely installed into water-wells for full scale application (Figure 3.12). The Electrodes, made of conductive and noncorrosive materials, could be placed within the aquifer to form a permeable and reactive barrier which intercepts (and treats) the contamination plume. If needed, the potential generated by the reaction can be augmented using an external power supply. Palma et al. (2017) describe a BES configuration, the ‘bioelectric well’, which can be installed directly within groundwater wells.



a) b)

Figure 3.12. BES for in situ treatment of groundwater contaminated with oxidized contaminants (Harnisch et al., 2011) (a); cross-sectional view of an in situ groundwater bioremediation system based on the bioelectric well concept (b) (Palma et al. 2017a).

4

CR(VI) REDUCTION IN BIOELECTROCHEMICAL SYSTEMS

4.1 Introduction

This chapter reviews the research experiences with BESs for the treatment of chromium-contaminated water, focusing on the perspectives and opportunities for Cr(VI) bioelectrochemical remediation for groundwater and the open research issues.

Several experimental works investigated hexavalent chromium cathodic reduction in MFCs with bioanodes and either abiotic cathodes or bio-cathodes (Figure 4.1); most of the experiences have focused so far on wastewater treatment coupled with energy recovery in MFCs (Sophia and Saikant 2016). Only a single study evaluated Cr(VI) detoxification at the bioanode, via bacterial protection mechanisms (Yeon et al., 2011). Table 4.1 (Attachment A) summarizes the available research experiences of Cr(VI) reduction, focusing on the electrode materials and inoculum, cathode potential, pH, Cr(VI) concentrations and the observed removal rates and efficiencies.

Due to the high standard redox potential, comparable, or even higher, in specific conditions, with other commonly used electron acceptors in BESs, Cr(VI) was principally considered by several studies as a theoretically favourable electron acceptor, to be reduced at the cathode in typical MFC configuration for power production (Figure 4.2). This concept has been demonstrated for the first time in a dual-chamber MFC (2C-MFC) by Wang et al. (2008) who, using acetate as electron donor and Cr(VI) solution at pH 2 as acceptor, observed higher power densities than for O_2 and hexacyanoferrate (Wang et al., 2008). The half-cell Cr(VI) reduction potential and the stoichiometry of the reaction are however strongly dependent on chromium species, concentration and pH conditions. In water solution, dichromate $(Cr_2O_7)^{2-}$ form prevails for total chromium concentrations above approximately 1 g/L (Palmer and Puls, 1994; Palmer and Wittbrodt, 1991). At lower concentrations, as typically occurs in groundwater plumes or natural surface water, the dominant species

is $(HCrO_4)^-$ at pH between 1 and about 6-6.5, and $(CrO_4)^{2-}$ at neutral or alkaline conditions (Figure 4.3a). High positive standard reduction potential E^0 (vs SHE), of both $(Cr_2O_7)^{2-}$ and $(HCrO_4)^-$ indicates thermodynamically favourable reaction in acidic environments, conducive to high power density generation in BESs (Nancharaiah et al., 2015; Sophia and Sai, 2016; Wang et al., 2008). On the contrary, $(CrO_4)^{2-}$ lower potential implies that chromium reduction, in the neutral pH range of natural waters, may require external energy supply, depending on Cr(VI) concentration and especially where other species are present.

The predominant form of trivalent chromium at acidic pH (pH < 4) is dissolved Cr^{3+}; in the pH range 5-8, Cr(III) forms soluble $Cr(OH)_2^+$ and $Cr(OH)^{2+}$, coexisting with $Cr(OH)_3$ or Cr_2O_3 precipitates at pH above 6.5 (Molokwane and Nkhalambayausi-Chirwa, 2009) (Figure 4.3b).

In addition, not only are Cr(VI) and produced Cr(III) species involved in cathodic reduction processes but they also have effects on other key factors for overall efficiency and long-term operation of BESs, such as electrolyte conductivity, internal resistance of the system, electrode and, in the case of biocathodes, on the activity of microorganisms, impacting on (Lu et al., 2015).

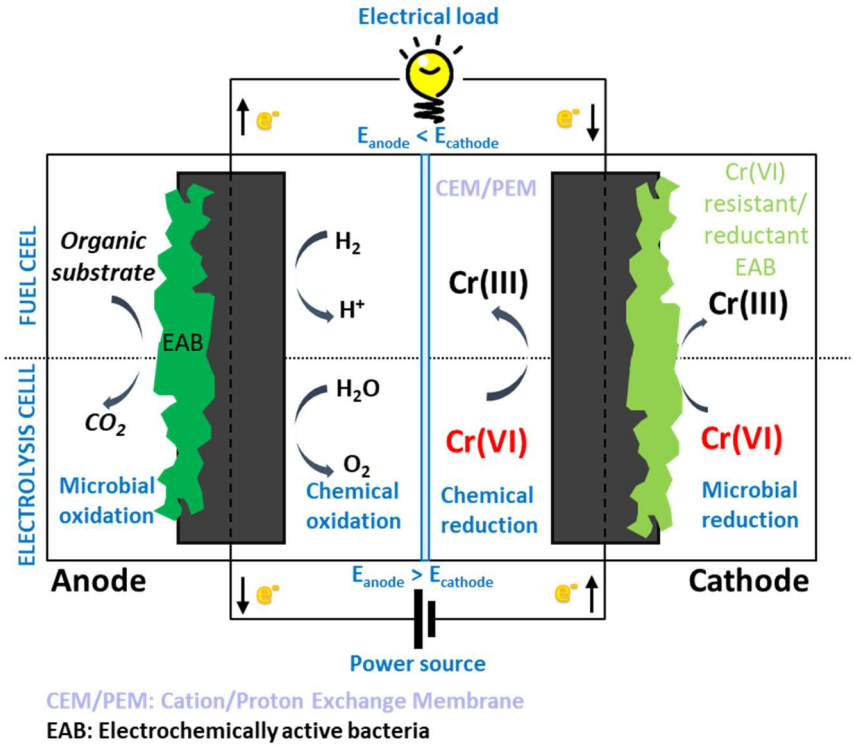
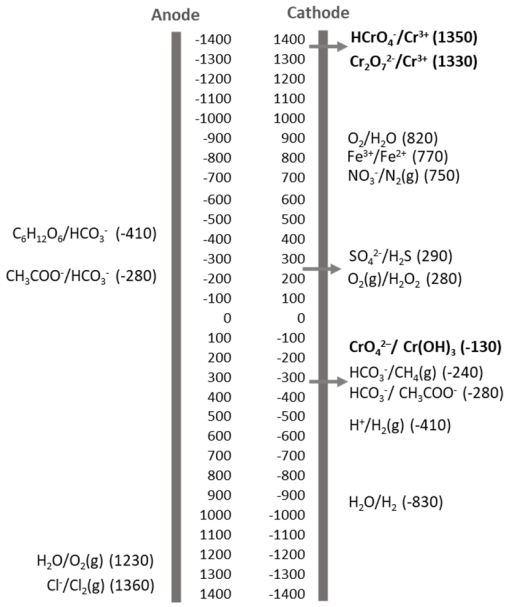


Figure 4.1. Schematic overview of BES system for Cr(VI) reduction as MFC with energy harvesting or MEC with external supply (Clauwaert et al., 2008, modified). In MFCs, oxidation of electron donor at the anode is coupled with a reduction of species with comparable or higher redox potential at the cathode. The net potential of the MFC, as the sum of anodic and cathodic potentials, is positive, so a spontaneous electron flow from the anode to the cathode occurs. Conversely, in MEC, thanks to

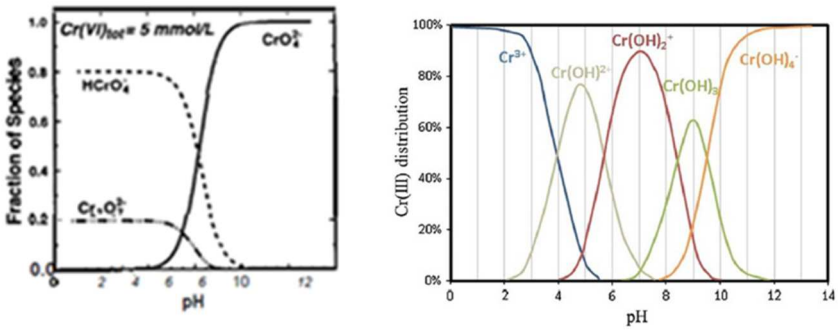
external power input to force electron flow, the oxidation of an electron donor at the anode can be coupled with a reduction of lower redox potential species at the cathode

$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$
$HCrO_4^- + 7H^+ + 3e^- \rightarrow Cr^{3+} + 4H_2O$
$HCrO_4^- + 4H^+ + 3e^- \rightarrow Cr(OH)^{2+} + 3H_2O$
$CrO_4^{2-} + 4H_2O + 3e^- \rightarrow Cr(OH)_{3(s,\ hydrated)} + 5OH^-$
$O_2 + 4H^+ + 4e^- \rightarrow H_2O$
$NO_3^- + 6H^+ + 5e^- \rightarrow 1/2\ N_2 + 3H_2O$
$Fe^{3+} + e^- \rightarrow Fe^{2+}$
$SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O$
$HCO_3^- + 9H^+ + 8e^- \rightarrow CH_4(g) + 3H_2O$
$2HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O$
$2H^+ + 2e^- \rightarrow H_{2(g)}$
$MnO_{2(s)} + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$



a) b)

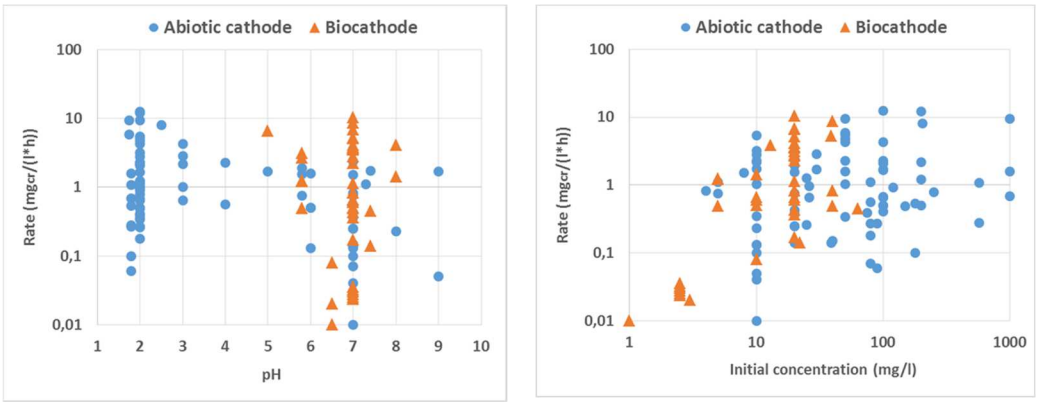
Figure 4.2. Reaction of selected species of interest for BESs application in Cr(VI) remediation (a); microbe-electrode redox tower for Cr(VI) reduction in BESs (b). Theoretical standard potentials (E_h^0) at 25 °C (mV vs SHE) of selected species of interest for BESs application in remediation, sourced from Wang et al. (2008), Clauwaert et al., (2009), Nancharaiah et al. (2015).



a) b)

Figure 4.3. Distribution of Cr(VI) (a) and Cr(III) (b) species as a function of pH (Barrera-Díaz et al., 2012; Xafenias et al., 2015).

All the reviewed experiences promoting hexavalent chromium reduction with abiotic cathodes are in the MFC configuration and aimed to exploit Cr(VI) as a cathodic oxidant to improve power production. Most studies focused, in fact, on operating conditions in which Cr(VI) exhibits high reduction potential and fast electrochemical reaction rates, i.e. at acidic pH and high Cr(VI) concentrations, indicatively above 5 mg/L (Li et al., 2008; Wang et al., 2008; Yu et al., 2018). Figure 4.4 shows the observed reduction rates in function of pH and initial concentration of Cr(VI), for BESs with abiotic or biotic cathodes, respectively.



a) b)

Figure 4.4. Cr(VI) removal rate [mgCrVI/L/h] as a function of pH (a) and initial concentration (b) in the reported research. Each dot relates to a test reported in reviewed scientific papers.

It is possible to notice that biocathodes, operating at neutral pH, reach comparable removal rates to those obtained with abiotic cathodes at acidic pH. Moreover, Cr(VI) concentration does not significantly affect the rate. Above 30-50 mg/l approximately, chromium concentrations become unfavourable to biocathodes activity. Common concentrations in groundwater Cr(VI) contaminated plumes often fall below these limits; therefore, BESs could be effective in addressing contaminated plumes. Biologically mediated Cr(VI) reduction at biocathodes may overcome the main open issues to the treatment of hexavalent chromium contaminated natural water i.e. i) the limited efficiency of electrochemical reduction at neutral pH, typical of natural waters and ii) the progressive deterioration of the reduction rates as Cr(III) deposits onto the cathode surface (Li et al., 2018). The general advantage of biocathodes in respect to abiotic cathodes relates to the improved sustainability of biocatalysts associated with the environmental compatibility of the biocatalysts operating conditions and lower costs of construction and operation (Lai et al., 2018). Moreover, the biofilm on the cathode may in some way protect and improve the electrode long term efficiency by preventing or delaying Cr(III) deposition (Huang et al., 2011a, 2011b).

4.2 Microbial communities in Cr(VI) reducing BES

Biologically mediated Cr(VI) reduction at biocathodes (Figure 4.5) may overcome the main open issues to the treatment of contaminated natural water i.e. i) the limited efficiency of electrochemical reduction at neutral pH, typical of natural waters and ii) the progressive deterioration of the reduction rates as Cr(III) deposits onto the cathode surface (Li et al., 2018). The general advantage of biocathodes in respect to abiotic cathodes as summarized by He and Angenent (2006) is related to improved sustainability of biocatalysts associated with the environmental compatibility of the biocatalysts operating conditions and lower costs of construction and operation. In case of Cr(VI), a biocathode, with respect to an abiotic system, may take advantage of multiple bacterial Cr(VI) reduction mechanisms, and the biofilm on the cathode may somehow protect and improve the electrode long term efficiency by preventing or delaying Cr(III) deposition (Huang et al., 2011a, 2011b).

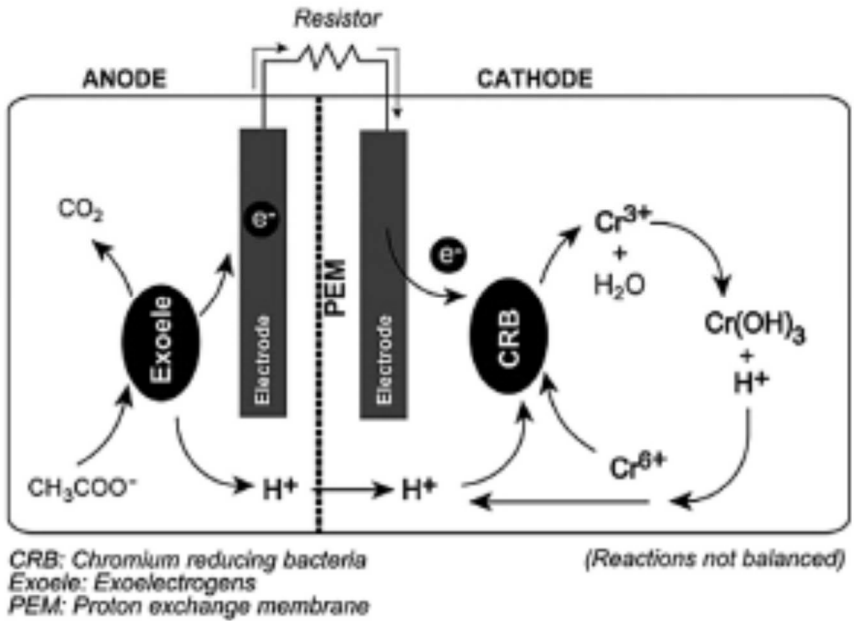


Figure 4.5. Schematic of Cr(VI) reduction in the biocathode of an MFC (Lu et al., 2015).

The previously described biological mechanisms (Chapter 2.3.1) can take place also in BES. Electroactive Cr-reducing microorganisms in the cathodic biofilm or the production of hydrogen at the cathode of a BES could also favour the litoautotrophic reduction of Cr(VI) by hydrogenotrophic bacteria (Battaglia-Brunet et al., 2002; Chung et al., 2006; Marsh and McInerney, 2001), or IRB or SRB involved in bioelectrochemical processes may operate indirect reduction of Cr(VI).

Several laboratory experiences of Cr(VI) reduction in anaerobic biocathodes have been reported. So far, no experience is reported in the literature of Cr(VI) reduction in aerobic biocathodes. Despite the fact that oxygen is unquestionably the preferred final electron acceptor for microorganisms, possible uses of electrons from the cathode by

Cr(VI)-reducing bacteria in the biocathode for Cr(VI) bioreduction, yet decreasing the coulombic efficiency of the system, may not be excluded at all. Moreover, Cr(VI)-reducing bacteria may be favoured over other species in an environment with specific toxicity, even though tolerance to high Cr(VI) concentrations, up to 1 g Cr(VI)/L or even above, have often been documented (Sagar et al., 2012).
The first test with Cr(VI) reducing biocathode was performed by Tandukar et al. (2009), who inoculated the cathodic compartment of a 2C-MFC (PEM membrane) with a mix of a denitrifying and methanogenic mixed culture, dosing bicarbonate as the sole carbon source. Anaerobic mixed culture fed with acetate served as anode inoculum. With graphite plate electrodes and an external 1000 Ω resistor, the authors reported power densities of 7.0 mW/m^2 and 55.5 mW/m^2, depending on initial Cr(VI) concentration (22 and 63 mg/L, respectively). Maximum specific Cr(VI) reduction rate, about 0.46 $mgCrVI/g_{VSS}/h$, was registered at 63 mgCr(VI)/L initial concentration. Analysis of the Cr(VI) reduction community by 16S rRNA gene sequences showed a predominance of phylotypes related to *Trichococcus pasteurii* and *P. aeruginosa*. Even considering the small amount of substrate that can leak from the anode even using a CEM (Jung et al., 2007) and organic carbon released in cell lysis, most Cr(VI) reduction was obtained with autotrophic conditions. In a batch-fed dual chamber MFC, with the cathode inoculated with a mixed microbial consortium from a Cr(VI) contaminated site and 39.2 mg Cr(VI)/L, Huang et al. (2010) observed a specific reduction rate of about 2.4 $mgCrVI/g_{VSS}/h$, and 3.9 W/m^2 maximum power production at a current density of 11.1 mA/m^2. Anaerobic pure cultures were also tested (Hsu, 2011; Hsu et al., 2012; X. Wu et al., 2018c; Xafenias et al., 2013a). Hsu et al. (2012) compared Cr(VI) reduction by six *Shewanella* strains at the cathode of MFCs in repeated cycles, observing initially the use of the electrode as the sole electron source in all tested strains. The variability in Cr(VI) reduction was supposed to be associated with different mechanisms of chromium reduction, not even identified, for each *Shewanella* strain, and other factors such as biofilm attachment to the electrode. Repeated Cr(VI) injections showed a general decrease in the MFCs performances and high residual Cr(VI) concentrations, which were explained with microorganisms' finite tolerance limit to Cr(VI) exposure and gradual fouling of the system by biological or reduced chromium species, limiting the active surface area of the cathode. Xafenias et al. (2013a) inoculated the cathode of an MFC and a MEC with *S. oneidensis* MR-1 fed with lactate. The combined use of the electrode and lactate as electron donors allowed bioelectrochemical and non-bioelectrochemical Cr(VI) reduction at the same time, even though the contribution of the two different mechanisms to the overall process was not recognized. In Wu at al. (2018) *Bacillus sp.* showed efficient Cr(VI)-reducing ability in both heterotrophic and autotrophic environments. The Cr(VI) removal rate reached 2.56 mg/L h, which was 1.75 times higher than that of the MFC with the sterile control cathode.

4.3 Effects of pH, materials and other operational parameters

As in abiotic cathode, the performance in Cr(VI) reduction in biocathodes is affected by pH and Cr(VI) initial concentration, although in different ways and for different reasons. Extreme pH values (indicatively pH< 5 or >8) and/or high chromium concentrations, typically 10-100 mg/L, can inhibit microbial activity. Tandukar et al. (2009), for instance, reported that initial Cr(VI) concentrations above 80 mg/L inhibited the reduction rates in a denitrifying community, and Li et al. (2014) observed 10 mgCr(VI)/L to irreversibly lose microbial activity in a single chamber MFC inoculated with municipal wastewater. Below toxic levels, increased initial Cr(VI) concentration, in accordance with thermodynamics, was associated with improved specific chromium reduction rate and MFC's power production (Huang et al., 2010; Li et al., 2014).
The pH, with its effects on the surface properties of the cells, including cell surface hydrophobicity, net surface electrostatic charge as well as biofilm structure may also heavily affect complex biological and electrochemical reactions at biocathode. In the case of Cr(VI), variation in pH may also affect the enzymes activity, as well as producing Cr(III) precipitation or bioadsorption (Malaviya and Singh, 2014). In Huang et al. (2011b), 50 mg/L initial Cr(VI) concentration inhibited the catalytic activity of electrochemical bacteria in the biocathode, whereas, at 20 mg/L Cr(VI) concentration, increased (+27.3%) and decreased (-21%) chromium reduction efficiencies were respectively reported in acidic (pH = 5) and alkaline catholyte (pH=8), with respect to neutral pH. A 0.22 cell net potential increase, from 0.54 V at pH 8.0 to 0.76 V at pH 5.0, beyond the theoretical value of 0.177 V derived by Nernst's law, was associated with a pH decrease in the cathodic compartment, actually indicating a positive response of microorganisms' activity to pH decline (Huang et al., 2011b). Similar effects have also been reported previously for denitrifying biocathodes (Clauwaert et al., 2009). Obviously, pH also affected the Cr(III) precipitation, with 9.3 mg/L dissolved Cr(III) at the end of the test at pH 5.0, in comparison to 0.3 mg/L at pH 8.0.

Huang et al. (2010) also identified, together with Cr(VI) concentration, high conductivity of the electrolyte, i.e. improved ion transport between the biofilm and bulk phase, as a key factor for efficient Cr(VI) reduction and power production. Increased conductivity of the solution, from 1.5 mS/cm to 10.6 mS/cm, raised the specific Cr(VI) reduction rate by about 25%, from 2.4 mg/(L g_{VSS}/h) to about 3.0 mg/(L g_{VSS}/h).
As to electrode materials, most experiences tested graphite or carbon-based electrodes. Huang et al. (2010) modified graphite electrodes by covering the cathode with graphite granules to increase the electrode specific surface, in order to promote bacterial attachment and electrical connection between bacteria and the electrode surface. Huang et al. (2011a) tested the performance of both graphite fiber/felt and granular graphite cathodes. In a tubular 2CMFC, with cathode to anode surface ratio (C/A) of 3, at pH 7 and temperature 22 °C, graphite fiber biocathode showed a higher specific Cr(VI) reduction rate and power generation with respect to the same kind of reactor

with either graphite felt and granular graphite biocathodes. Specific Cr(VI) reduction rates on the graphite fiber cathodes, 12.4–20.6 mg/g_{VSS}/h, were about 10-100 folds higher than the values reported for biocatalyzed carbon plate or graphite granule cathode in H-type MFCs with about the same Cr(VI) concentrations (Huang et al., 2010; Tandukar et al., 2009a). These results underline the coordinated role of cathode surface area and reactor architecture on the biocathode performance. In Wu et al. (2016b) NaX zeolite-modified graphite felts were used as electrodes (anode and cathode) in 2CMFCs; NaX zeolite was proved to enhance the hydrophilicity of the graphite felt facilitating bacterial adhesion and electrochemical reaction, by decreasing mass transport resistances. Two different fabrication methods for the NaX zeolite-modified graphite felts were tested, the first one without any pre-treatment of the felt and the second one with HNO_3 pre-treatment. Both methods, especially the latter, allowed excellent performance, with significant improvement in both electricity generation and Cr(VI) reduction rates, in comparison to graphite felts MFC. HNO_3 pre-process remarkably enhanced NaX loading mass on the graphite felt, by decreasing the organic residues on the graphite surface. NaX zeolite-modified graphite felts MFC, at an initial Cr(VI) concentration of 20 mg/L, resulted in maximum voltage above 410 mV, 29 mW/m^2 power density, and complete removal of Cr(VI) in 3 hours, with a rate 8.2 times faster than simple graphite felts MFC. Nanostructured graphene was also tested recently with positive results, (T. Song et al., 2016). The maximum power density in an MFC with graphene biocathode was 5.7 times higher than the one produced with graphite felt biocathode. In fact, electricity production increased from 28.6 to 164 mW/m^2 and improved efficiency in Cr(VI) reduction was also obtained, with 100% reduction in a 40 mgCr(VI)/L solution within 48 h, in comparison to only 58% reduction with graphite felt.

The most widely tested configuration with biocathodes is the 2CMFC, although a significant experience with single chamber reactor exists (Li et al., 2014). Organic substrate removal at the anode and cathodic chromium reduction were reflected in the open circuit potential of the system and Cr(III) deposition on the cathode, as revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy. Cr(VI) conversion efficiencies in the range 89-99% depending on initial Cr(VI) concentrations were observed, even the open circuit control demonstrated that other mechanisms than electron reduction on cathodes, including bioadsorption or bioreduction by not electroactive bacteria, contributed to the dissolved Cr(VI) declining in time in the single chamber MFC.

To increase microbial concentration and prevent cathode premature passivation due to Cr(III) precipitates during the system set-up, Wu et al. (2015) proposed an ex-situ acclimatization method for Cr(VI)-reducing biocathodes. The electrode was initially enriched with exoelectrogenic biofilm as an MFC anode, and the system was subsequently established using the anode as biocathode. This method allowed mature biofilm to be obtained in a shorter period of acclimatization (<19 days in the authors' experience) compared to traditional in situ methods, with Cr(VI) removal reaching 79% in 24 h, about four times higher than the one observed in the MFC with in situ acclimated cathode. The improved performance was attributed not only to the avoidance of premature Cr(III) precipitates on the electrode, during biofilm

acclimatization, but also to enhanced bacterial growth rates in heterotrophic anodic environment, leading to high microbial density and bacterial coverage of the electrode, possibly limiting the effects of Cr(VI) toxicity to the microorganisms, at the anode/cathode inversion.

Tests with poised cathode, in potentiostatically controlled MEC experiments, demonstrated that an optimal potential range typically exists for enhancing Cr(VI) reduction performances in biocathodes (Liang et al., 2009; Xafenias et al., 2015b). Theoretically, from Nernst's law, in MFC with chromium reducing cathode and acetate-oxidizing bioanode, the open circuit voltage at pH 7.0 and 25 °C is about 0.68 V, resulting in about 0.4 V theoretical cathode potential (Huang et al., 2010). Lower set cathode potentials would promote Cr(VI) reduction process. Huang et al. (2011a) compared the behaviour of a potentiostatically controlled BES (with cathode operated at 200, -150, -300 and -450 mV vs SHE) to an MFC operating with 200 Ω external load. Cathode at -150/-300 mV set potential promoted fast start-up time (19 days compared to 26 days in the uncontrolled MFC or 28 days in +200 mV set cathode system) and Cr(VI) reduction, with almost complete removal of 20 mg/L in 24 h, with respect to 43-70% with the other systems). Furthermore, +200 mV and -450 mV poised cathode limited bacterial growth, whereas -150 and -300 mV had beneficial effects. In all the tests, the reduction of Cr(VI) was attributed to microorganisms directly accepting electrons from the electrode surface and transferring them to Cr(VI) as, even in the test at the most negative potential, no production of hydrogen gas was observed. Optimal set potential can provide an appropriate selective pressure for adaptation of the microbial community in the system, leading to enhancements of microbial electrochemical interaction with the cathode. The difference between Cr(VI) reduction potential and the cathode set potential represents the maximum energy to be gained by the cathodic microorganisms; thus, the lower the set cathode potential is, the more energy microorganisms will potentially to be obtained. However, if the cathode potential is set too low and goes beyond the self-regulation capability of microbial consortia, the energy gain by the cathodic microorganisms is lost. Probably -150 and -300 mV set potentials allowed the biomass to gain more energy than 200 mV set potential. Although theoretically most favourable, -450 mV may have exceeded the self-regulation capability of the microbial consortia, with no positive effect on power generation and Cr(VI) reduction (Huang et al., 2011a). Xafenias et al. (2013a) demonstrated the positive impact of riboflavin, a naturally produced mediator, in potentiostatically-controlled *Shewanella oneidensis* MR-1 biocathodes for Cr(VI) reduction. Different configurations, with lactate supplied as electron donor in inoculated and abiotic systems and with or without riboflavin addiction, were tested. At 20 mg/L initial Cr(VI) concentration, in a –300 mV poised biocathode fed with lactate, up to 45% Cr(VI) reduction was observed in 4 hours in comparison to 5% Cr(VI) reduction in a biotic system with no lactate and 15% reduction in abiotic systems with lactate. In 2CMFC with *S. oneidensis* MR-1 fed with lactate in both anodic and cathodic compartments, Cr(VI) reduction at the cathode (10 mgCr(VI)/L initial concentration) was coupled with 32.5 mA/m^2 maximum current density production (Xafenias et al., 2013a).

Most of the reviewed studies evaluated bioelectrochemical Cr(VI) removal in batch mode, even recently few experiences with continuous-flow reactors have been published. Chen et al. (2016) used a 3 L cylindrical single chamber reactor, filled with sulphur granules, with graphite felt cathode, carbon rod anode, and anaerobic sludge as inoculum. The reactor was continuously fed with 100 mg/L Cr(VI) synthetic wastewater with no organic C source (16 hours hydraulic retention time) and run in galvanostatic mode (current 10 – 60 mA). Cr(VI) reduction in the effluent ranging from between 43% and 97%, directly proportional to the externally supplied current. SO_4^{2-} in the effluent, as well as the increased removal rate with increasing current, highlighted that both sulphur and hydrogen autotrophic bacteria were responsible for Cr(VI) reduction by using the S granules in the reactor and H_2 produced by the cathode as electron donors. A similar system, single chamber cylindrical reactor operated in galvanostatic (200 mA) continuous flow mode (20 h HRT) was adopted by Wang et al. (Wang et al., 2018a) for the removal of Cr(VI) and nitrates from synthetic wastewater. In Habibul et al. (2016), the Cr(VI) removal efficiency in a plant aided microbial reached 99% under various conditions. Efficiencies increased with the increasing initial Cr(VI) concentration (9.5 and 19 mg/l). The mass balance and XPS analysis results demonstrate that most Cr(III) precipitated in the form of the $Cr(OH)_3$ or was adsorbed onto the electrodes. Experiments with no external organic carbon supply show that plants can provide, through root exudates, carbon sources for microbes, and bioelectrochemical reduction was the main mechanism for Cr(VI) removal.

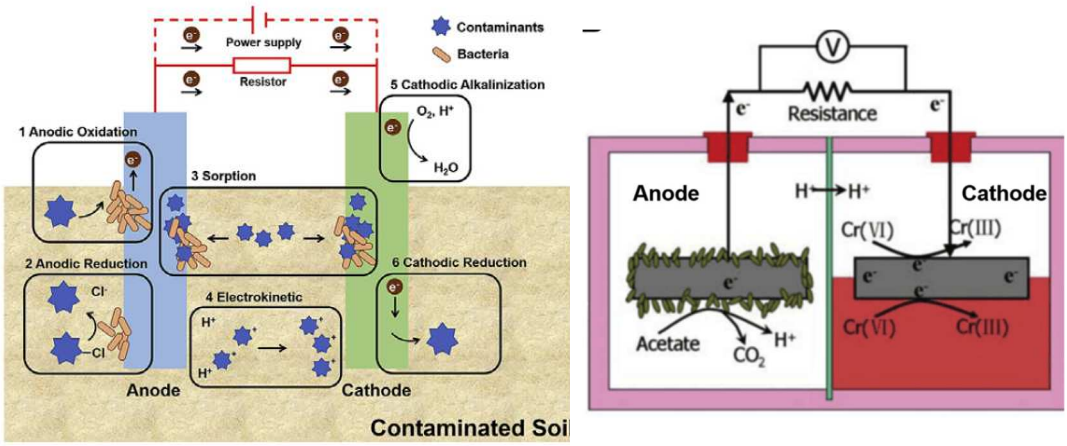
4.4 Applications for remediation

The reviewed laboratory research, even all those targeting treatment of Cr(VI) contaminated wastewater/industrial effluents, offer the first proof of concept also for the chance of bioelectrochemical Cr(VI) remediation. Experiences with conventional bioreduction processes, under both aerobic and anaerobic conditions, by either pure cultures or mixed consortia, report Cr(VI) bioreduction rates in a 0.1-13.5 mg/l/h range (Chai et al., 2009; Kavita and Keharia, 2012; Molokwane et al., 2008; Sharma and Adholeya, 2012; Somenahally et al., 2013), which are fully comparable to the values, 0.1-6.6 mg/l/h, observed in Cr(VI) reducing biocathodes. So, with comparable results, bioelectrochemical Cr(VI) remediation over the conventional bioremediation presents the following advantages: i) the chance of excluding any external chemical supply to support the microorganisms' actions, ii) ease of monitoring and control of the process, and iii) the potential recovery of reduced chromium deposited on the electrode.

It should be highlighted that all the reviewed studies deal with batch laboratory tests under conditions quite different from natural Cr(VI) contaminated water/groundwater. It would thus be useful to perform evaluations under dynamic water flow conditions as in contaminated aquifers, maybe with real groundwater, as reported by Gregory and Lovley (Gregory and Lovley, 2005) for uranium contaminated aquifers. To groundwater remediation, it is important to take into consideration specific properties that potentially affect BES operation (Pous et al.,

2018). Although Cr(VI) contamination is likely increase the specific conductivity, typical groundwater low values (indicatively well below 2 mS/cm) can have a negative impact on BESs by implying higher ohmic and transport losses (Bruce E. Logan et al., 2006). Moreover, pH shifts due to electrochemical Cr(VI) reduction in low buffering capacity systems may directly harm the electroactive bacteria and their removal performance (Clauwaert et al., 2008). Another challenge for bioelectrochemical treatment of contaminated groundwater, as described in Chapter 3.2.1, is the presence, in addition to the contaminants, of a mixture of different naturally occurring inorganic (calcium, magnesium, carbonate, nitrates and sulphates, metals) and organic chemicals (e.g. humic acids) (Squillace et al., 2002). Magnesium and calcium can produce precipitates with the consequent passivation of the cathode (Santini et al., 2017). The bioelectrochemical reduction has been recently reported for nitrate (Duca and Koper, 2012; Chen et al., 2016; Wang et al., 2018a) and sulphate (Coma et al., 2013). So far, the study of co-contaminants with BESs is limited but, theoretically, as reduction potentials of nitrate and sulphate are similar to the reduction potential of several pollutants, they can be electron competitors in the remediation process and affect the microbial community at the biocathode (Bardiya and Bae, 2011). Wang et al. (Wang et al., 2018a) evaluated the simultaneous autotrophic denitrification and the reduction of Cr(VI) under different pH conditions (6, 7 and 8). The highest removal efficiencies, 97% and 73% for nitrates and Cr(VI) respectively, were obtained at pH 7. The stable combined reduction was mainly ascribed to *Pseudomonas, Halomonas* and *Thauera* species.
A continuous-flow BES was proposed for the contextual removal of p-fluoronitrobenzene (p-FNB), nitrates and hexavalent chromium from synthetic wastewater (Chen et al., 2016). With respect to single pollutant removal, removal efficiencies were lower, even when Cr(VI) and nitrates reduction increased by increased p-FNB inlet concentrations, whose biodegradation provided organic carbon to the biomass.

Few bioeletrochemical tests for Cr(VI) contaminated soil remediation have been so far reported. Soil type and external resistance significantly affected the current and Cr(VI) removal efficiency in soil MFCs. MFCs with red clay soil and fluvo-aquic soil, operated at external resistances of 100 and 1000 Ω for 16 days, were tested by Wang et al. (Wang et al., 2016). The red soil generated a higher current in MFCs, but showed a lower Cr(VI) removal efficiency than fluvo-aquic soil, implying that the red soil may contain more electron acceptors that competed with Cr(VI) reduction reaction. The percentage of Cr(VI) removal in 16 days operation was relevant, about 60-90% in the MFCs with respect to 32-46% in the open circuit controls. MFC-based technology has the potential to remediate Cr(VI)-contaminated soil (Figure 4.6); the efficiency varied between soil types and can be improved with high current.



a) b)

Figure 4.6. Different bioremediation mechanisms with soil (Wu et al., 2018c) (a); 2C-MFC with only cathode in soil (Wang et al., 2016a).

Experiences were also conducted for phytoremediation with MFC that integrates plants, microbes, and electrochemical elements together to create renewable energy (Nitisoravut et al., 2017; Guan et al., 2019). Plants can utilize atmospheric CO_2 via photosynthesis under sunlight and secrete root exudates. Meanwhile, microbes in soils can use organic matters in the rhizosphere through metabolism to generate electrons. Root exudates can serve as the electron donor for microbes to promote the removal of Cr(VI).

Although the knowledge that low voltage electric fields can induce the migration of metals in soil (Wu et al., 2016a; Zhou et al., 2005), a limited number of studies attempted bioelectrochemical remediation of metal contaminated soil or sediments. Despite the first positive results, the potential of BESs for remediation of Cr(VI) contaminated groundwater and soil deserves further exploration.

5

CRITERIA FOR IN SITU TECHNOLOGIES SCREENING

5.1 Introduction

Decision Guides are not available in the literature for the selection of the most suitable reclamation technology according to site-specific conditions; some concepts can be found in technical reports prepared by US agencies (USEPA, 2008; USEPA 2011; SRNL 2011; USDE 2011) that define scenarios for sites contaminated by inorganic pollutants. Soils and groundwater in oxidizing conditions is the most interesting scenario due to the high potential risk of spreading of contamination: chromium remains in the hexavalent form in this scenario if not properly treated. Some criteria are presented in the preliminary phase of assessing possible technologies applicable according to the site-specific characteristics of sites. The actual efficacy of the technologies identified should nevertheless be verified in laboratory trials and pilot tests.

Innovative technologies described in Chapter 2, which have reached full-scale application, are presented, subdivided according to the typology of mechanism used and potentially treatable zone (Table 5.1). Some only apply in saturated or unsaturated zones; both unsaturated and saturated zones should also be further separated to take account of the fact that the full involvement of the contaminated matrix in the treatment is generally tied to the depth of the contamination from ground level (g.l.).

Technology	Unsaturated 0–1 m	Unsaturated 1–10 m	Unsaturated > 10 m	Saturated < 10 m	Saturated 10–25 m	Saturated > 25 m
Chemical process with solutions or slurry	-	-	-	x	x	x
Chemical process with gaseous reagent	-	x	x	-	-	-
Indirect biological process	-	-	-	x	x	x
Biological process-Phytoremediation	x	-	-	-	-	-
Chemical-physical process-Electrokinetics	x	x	-	x	-	-
Chemical-physical process-Flushing	x	x	-	-	-	-

Table 5.1**.** Potential applicability (x: yes, -: no) of innovative technologies depending on the zone and maximum depth of soil to be treated.

5.2 Scenarios and selection criteria

The scenarios refer to saturated or unsaturated permeable soil, in aerobic or, at most, anoxic conditions. In reducing environments, typically soil with low permeability and rich in organic substance, the redox conditions encourage the abundance of chemical species of Cr (III) rather than of Cr (VI). It is therefore rare to encounter significant contamination of Cr (VI) in these contexts (Christoph et al. 2012).

Table 5.2 shows the most influential factors on the choice of potentially applicable technologies: pH, concentration of Cr (VI), availability of iron in the soil, and homogeneity of the soil.

Factor	Scenario	Value
Soil pH	Acid	5 ÷ 7
	Alkaline	7 ÷ 9
Cr (VI) concentration	Low	< 10^2 mg kg^{-1} unsaturated soil; < 10 mg L^{-1} in aquifer
	High	> 10^2 mg kg^{-1} unsaturated soil; > 10 mg L^{-1} in aquifer
Fe concentration in soil	Low	< 1 g Fe kg^{-1}
	High	> 1 g Fe kg^{-1}
Soil homogeneity	Yes	Variation of hydraulic conductivity or intrinsic permeability within 2 orders of magnitude
	No	Variation of hydraulic conductivity or intrinsic permeability more than 2 orders of magnitude

Table 5.2**.** Factors which influence the choice of technology.

It is useful to subdivide soils according to their pH. The use of some reactive chemicals, for example, is advised for acid or neutral environments, in view of the significant loss of efficiency for basic pHs, or vice-versa. Soil flushing for chromium is not suitable in acid soils because of the lower mobility of its chemical species.

High concentrations of hexavalent chromium can limit the feasibility of some technologies. Regarding biological treatments, the capacity of the microorganisms to survive at high concentrations of Cr (VI) (up to a few grams per liter in water) could be mediated, not just by enzymes and/or very specific transport proteins, but also by sub-cellular structures, which interact with the metals themselves (Baldi and Barbieri 2008). Many microorganisms are able to grow and survive at high concentrations of Cr (VI), developing mechanisms of resistance and tolerance to the pollutant (Kamaludden et al. 2003; Viti et al. 2006; Ashraf et al. 2017). The use of selected inoculations, if able to remain in situ, would therefore not have limitations, even in contexts with a high level of contamination. Vice-versa, action in indirect biological treatments could be inhibited with dissolved contamination above 10 mg L^{-1} Cr (VI) or soil contamination above 10^2 mg kg^{-1} (Hassan et al. 2017).
The presence of Fe (II) ions allows redox reactions, with the reduction of Cr (VI). For the technologies that promote the development of reducing conditions in a reactive zone, the presence of iron in the solid matrix is a determining factor. In general, the matrix is considered to be at a high iron content if the concentration exceeds 0.1% in weight, or 1 g kg^{-1} (Dresel et al. 2011); below this threshold, it becomes necessary to exclude technologies that use the iron as an essential element of the action mechanism. The releases of Fe (II) from the solid matrix must be sufficient to balance the quantity of Cr (VI) to be reduced; according to reactions (1) and (2), the indicative ratio in solution is Cr (VI): Fe (II) = 1:3 by weight (Mukhopadhyay et al. 2007).
Almost all natural soils are highly variable in their properties. The heterogeneity of the soil is linked to the presence of different lithologies (Elkateb et al. 2003). The presence of heterogeneity limits the efficacy of the technologies, which envisage injection and dispersal of a reactive. As mentioned, the PRBs appear to overcome these problems. It is possible to quantify the homogeneity of the layer to be treated using parameters, such as the hydraulic conductivity or the intrinsic permeability of the layer to be treated (Uzielli et al. 2008).

Having taken the above into account, tables 5.3 and 5.4, that summarize suggestions on the exclusion of some technologies, are proposed.

pH [1]	Cr (VI) Concentration [2]	Fe Concentration in Soil [3]	Soil Homogeneity	Fe (0), $C_6H_8O_6$, H_2S	Sodium Dithionite	Calcium Polysulphurs	Indirect Biological Process	Phytoremediation	Electrokinetics	Soil Flushing
A	L	L	Yes	-	X	X	X	-	X	X
A	L	L	No	X [4]	X	X	X	-	X	X
A	L	H	Yes	-	-	X	-	-	X	X
A	L	H	No	X [4]	X[4]	X	X [4]		X	X
A	H	L	Yes	-	X	X	X	X	-	X
A	H	L	No	X [4]	X	X	X	X	-	X
A	H	H	Yes	-	-	X	X	X	-	X
A	H	H	No	X [4]	X [4]	X	X	X	-	X
B	L	L	Yes	X	X	-	X	-	X	-
B	L	L	No	X	X	X [4]	X	-	X	X
B	L	H	Yes	X	X	-	-	-	X	
B	L	H	No	X	X	X [4]	X [4]	-	X	X
B	H	L	Yes	X	X	-	X	X	-	-
B	H	L	No	X	X	X [4]	X	X	-	X
B	H	H	Yes	X	X	-	X	X	-	-
B	H	H	No	X	X	X [4]	X	X	-	X

[1] A = Acid, B = Alkaline; [2] H = High; L = Low; [3] H = High; L = Low; [4] Not recommended/Excluded only in case of injections.

Table 5.3**.** Low applicability/inapplicability (shown by an "X") of the innovative technologies examined. For each innovative technology mentioned, the conditions of the factors of Table 5.2, which advise against/exclude its application, are specified; the zone of applicability of the different technologies reported in Table 5.1 is implied.

Scenario	pH	Chromium Concentration	Fe concentration in soil	Soil Homogeneity	Proposed technologies							
					Soil Flushing	Phytoremediation	Electrokinetics	H_2S	Chemical Processes			Indirect Biological Process
									Fe(0), $C_6H_8O_6$	Dithionite	Calcium Polysulphurs	
U. (0-1 m)	<7	High					v					
		Low				v						
	>7	High		yes	v		v					
				no			v					
		Low		yes	v	v						
				no		v						
U. (1-10 m)	<7	High					v	v				
		Low						v				
	>7	High		yes	v		v					
				no			v					
		Low		yes	v							
				no								
U. (>10m)	<7							v				
	>7											
S. (<10 m)	<7	High	High	yes			v		v	v		v
				no			v		v *	v *		v *
			Low	yes			v		v			
				no			v		v *			
		Low	High	yes					v	v		v
				no					v *	v *		v *
			Low	yes					v			
				no					v *			
	>7	High	High	yes			v				v	v
				no			v				v *	v *
			Low	yes			v				v	
				no			v				v *	
		Low	High	yes							v	v
				no							v *	v *
			Low	yes							v	
				no							v *	
S. (10-25 m)	<7		High	yes					v	v		v
				no					v *	v *		v *
			Low	yes					v			
				no					v *			
	>7		High	yes							v	v
				no							v *	v *
			Low	yes							v	
				no							v *	
S. (>25 m)	<7		High	yes					v	v		v
				no					v *	v *		v *
			Low	yes					v			
				no					v *			
	>7		High	yes							v	v
				no							v *	v *
			Low	yes							v	
				no							v *	

Table 5.4. Summary sheet about the technologies, (v) possible choice under the conditions. (v*) Recommended technology with PRB, not through RZs.

5.3 Discussion and Conclusions

The innovative technologies considered aim at greater sustainability than traditional approaches persisting more for established practice than for real advantage. The intention is to provide support to operators and decision makers that wish to undertake the remediation of a site more directed towards a concept of sustainability (EU 2017).

Despite inapplicability under certain conditions, phytremediation, electrokinesis, and flushing can potentially be used. In relation to sustainability, time, and logistical limitations, phytoremediation is an interesting option from an economic and environmental standpoint, but cannot be used in sites with a high level of contamination, structures and/or land cover, and restricted remediation times.
Injections of reducing gases, electrokinesis, and flushing can potentially be used for unsaturated sub-surface soil; the latter two, however, are excluded for treating material more than 10 m from g.l. because the consequent technical-operational difficulties. Deep unsaturated ground (more than 10 m g.l.) therefore remains among the zones, with limited alternatives to treatment of the contamination by Cr (VI).
The contamination in a saturated zone can potentially be treated with all the innovative chemical and biological technologies mentioned and, in the case of depths within 10 m g.l., also with electrokinesis. In aquifers with low concentrations of Cr (VI), the indirect biological processes generally have lower costs (Chapter 2), even if remediation times are usually longer than the one of purely chemical processes.
For all zones, among the technologies that are the most innovative and without significant site-specific limitations, electrokinesis is promising (NICOLE 2018). Starting with this, there are also small-scale studies of remediation technologies underway, based on the application of low intensity electrical fields, for the reduction of chromium using electrochemical, biochemical, or bioelectrochemical processes (Chapter 3).

From the perspective of full-scale implementation, the administration of chemical agents can be carried out using injections (in wells and/or with the direct push technique) and/or in PRBs. In groundwater within 25 m g.l., PRBs offer greater advantages in heterogeneous soils. However, there are significant implications in terms of the cost and time taken to excavate as well as the disposal of the material resulting from the installation of the work.

The final choice of the best remediation option in a site depends, in any case, on additional sustainability factors, including the results of site-specific and laboratory tests. A lack of economic resources may lead in the direction of less onerous, but slower, technologies, just as the necessity of achieving quickly the remediation objectives for social purposes may lead to the exclusion of other technologies. Traditional treatment techniques should also not be excluded a priori from the assessment.

6

INDUCED BIOLOGICAL REDUCTION TESTS

6.1 Introduction

Experimental activity on biological technologies was focused on bio-induced reduction. This can be obtained in-situ with the injection of biodegradable organic substrates in the aquifer whose consumption by microorganisms depletes the different electron acceptors and causes reducing conditions conducive to Cr(VI) reduction to Cr(III) (Dhal et al., 2013). Bio-reduction of hexavalent chromium can result from both microbial direct action or indirect action due to the production of metabolites able to interact with the metal (Chai et al., 2009).

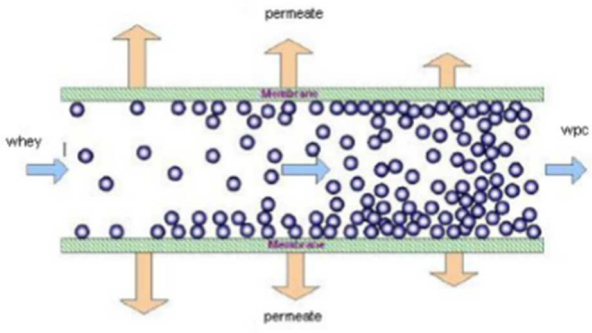
Specific tests were carried out to select valid alternative low-cost substrates to registered brand reagents that have been used for years. In this work two types of substrates, residues from the food industry i.e. permeate of cheese whey ultrafiltration and waste from brewing processes have been initially selected. Thanks to their density and viscosity, as well as biodegradability and non-toxicity, these substrates could be injected, directly or diluted with water, into the subsurface to promote biological activity.

Whey, which makes up 90% of the product in cheese making, is an important source of proteins and minerals but only recently has found use as a supplement for animal feed and/or the food industry, especially in the confectionery sector. It is mostly composed of water enriched in several soluble substances, such as lactose, proteins, mineral salts, soluble organic salts and non-protein nitrogen compounds. Vaccine whey can be classified according to the degree of acidity into three sub-categories (Figure 6.1a). For whey reuse often ultrafiltration process is required (Figure 6.1b) to separate proteins and fats from sugars. Ultrafiltration in fact results in i) ultrafiltration permeate, composed of water and lactose (80-99%), and ii) the so-called Whey Protein

Concentrate (WPC). The concentrate finds many applications in the industrial field, whereas the ultrafiltration permeate is essentially used in the confectionery sector.

Factor	Sweet whey	Acid whey	Casein whey
pH	6.1	4.6	4.4
	% on dry weigh		
Protein	12.1	5.3	8.2
Lactose	77.4	80.7	77.0
Ashes	9.7	14.0	14.8
Fat	0.8	-	-

a



b

Figure 6.1: Classification of whey as a function of pH and mean composition (a); Scheme of an ultrafiltration unit (b).

Beer production waste is characterized by a high content of organic substances, as well as water. Analyzing the typical process for beer production, it can be observed that, essentially, the waste stream is composed by waste from threshing material (18 kg/hl_{beer}) and purge flow from the fermenter (3 kg/hl_{beer}). The threshing residue is generated following the boiling and decanting of malt grains during the mashing phase. The unsoluble grain peel gets separated by gravity in wooden or metal vats. The residue from threshing is typically made up of cellulose, sugars, starch and proteins. In modern production processes fermentation takes place in the same tank (fermenter), followed by a maturation process at low temperatures with deposit on the bottom of the suspended yeasts. It is necessary to periodically remove part of the sediment, that constitutes the purge flow rich in vitamins, organic substances and the yeasts themselves.

Batch soil microcosms artificially contaminated with hexavalent chromium have been set-up in the laboratory for a preliminary evaluation of the possibility of promoting Cr(VI) reduction with the different substrates.

6.2 Materials and Methods

Several batch soil microcosms were prepared in 2.5 L Pyrex-bottles, equipped with two lateral necks for monitoring and sampling, using artificially Cr(VI) contaminated water, similar in composition to groundwater from the Milan area, two different soils (named "A" and "B"), from two different sites in Northern Italy, and samples of permeate from cheese whey ultrafiltration or waste from the brewing process, as organic substrates to promote indirect Cr(VI) bioreduction. Tables 6.1-6.3 summarize the results of initial characterization of water, soil and organic substrates, respectively.

Parameters	Value	Method
Dissolved oxygen (mg/l)	6.5±0.5	Standard Methods 4500-O (2012)
Nitrate (mg/l)	34±3	EPA 300.1 – Rev. 1 (1997)
Iron (mg/l)	0.10±0.01	EPA 6020B (2014)
Manganese (mg/l)	0.30±0.03	EPA 6020B (2014)
Sulphate (mg/l)	66±7	EPA 300.1 (1997)
Carbon dioxide (mg/l)	15	Saturation concentration at 20°C
Alkalinity (mg $CaCO_3$/l)	280±28	Standard Methods 2320 (1997)
Calcium (mg/l)	99±10	UNI EN ISO 17294 (2007)
Phosphate (mg P/l)	0.40±0.06	EPA 300.1 (1997)
pH (-)	7.2±0.2	EPA 150.1 (1982)

Table 6.1. Features of water used in the laboratory tests.

Parameter	Soil "A"	Soil "B"	Method
Particle size distribution	Slightly silty sand with gravel	Sand	ISO 11277 (2009)
Dry bulk density (kg/m^3)	1606±63	1478±22	ISO 11272 (1998)
Organic carbon (%)	0.59±0.03	0.27±0.02	UNI EN 15169 (2007)
pH (-)	8.52±0.01	8.5±0.1	Rayment and Higginson (1992)
Total heterotrophic bacteria (CFU/g d.w.)	10^4	10	Plate counts

Table 6.2. Soils used in the experiments.

Parameter	Ultrafiltration permeate of cheese whey ("Milanese company")	Waste from brewing process ("La Ribalta")	Method
COD (g/l)	60±12	122±24	MU 201 (2006)
Total Nitrogen (mg/l)	180	1810	ASTM D8083 (2016)
Total heterotrophic bacteria (CFU/100 ml)	10^6	10^3	Plate counts

Table 6.3. Organic substrates used in the experiments.

The addition of substrates must allow the complete consumption of electron acceptors from oxygen to sulphates (Table 6.4), even eccessive dosage should be avoided to limit the costs of remediation as well as undesired side effects of reducing conditions (e.g.eccessive methane production,…). The amount of substrates to be added in these tests for the available electron acceptors consumption was defined:

- for the permeate from ultrafiltration of cheese whey, based on the potential molecular hydrogen production, estimated according to the methods proposed by Pearson (2010), of lactose (assumed as the reference organic compound in permeate, making up about 35% on a weight basis). By applying a safety factor of 1.25, a volume of 5 ml of permeate per litre of aqueous phase was obtained.

The COD for the permeate was 60 mg COD/l. A theoretical COD of 1.12 g COD/$g_{lactose}$ was calculated, assunming lactose complete oxidation and a 53.2 $g_{lactose}$/L was estimated for the whey permeate

- As the substrate deriving from the brewing process contained yeasts and other organic components, including ethanol, but the actual composition was unknown, the volume of brewing residue to be added to the microcosms, 2.5 ml per litre of aqueous phase, was set to get a similar initial COD to the one of whey permeate tests (about 300 mg COD/l of aqueous phase).

Time →					
O_2	NO^{3-}	SO_4^{2-}	Mn(IV)	Fe(III)	CO_2
Aerobic	Anoxic		Anaerobic		Methanogenesis

Table 6.4. Ladder of electron acceptors consumption.

The details about the tests (soil, organic substrate, initial chromium concentration, solid to liquid ratio, temperature, Fe(II) addition, number of replicates, and sampling times) are summarized in Table 6.5.

Test	**Soil**	**C_0 (μg/l)**	**S**	**S/L (%)**	**T (°C)**	**Fe (II) addition (mg/l) (*)**	**Nr R**	**ST (d)**
1	A	10000	P	50	17±1	-	6	0, 8, 11, 18, 21, 28, 36
2	A	10000	B	50	17±1	-	6	0, 8, 11, 18, 21, 28, 36
3	A	5000	P	50	17±1	-	6	0, 8, 11, 18, 21, 28, 36
4	A	5000	B	50	17±1	-	6	0, 8, 11, 18, 21, 28, 36
5	B	10000	P	50	17±1	-	6	0, 11, 18, 21, 28, 33, 36
6	B	10000	B	50	17±1	-	6	0, 11, 18, 21, 28, 33, 36
7	B	5000	P	50	17±1	-	6	0, 11, 18, 21, 28, 33, 36
8	B	5000	B	50	17±1	-	6	0, 8, 18, 21, 28, 33, 36
9	A	-	P	25	25±2	-	3	0, 10, 30
10	A	-	B	25	25±2	-	3	0, 10, 30
11	B	-	P	25	25±2	-	4	0, 10, 30
12	B	-	B	25	25±2	-	4	0, 10, 30
13	B	10000	P	25	25±2	-	3	0, 7, 38
14	B	10000	B	25	25±2	-	3	0, 7, 38
15	B	5000	P	25	25±2	-	3	0, 7, 38
16	B	5000	B	25	25±2	-	3	0, 7, 38
17	B	10000	P	25	25±2	10	3	0, 7, 38
18	B	10000	B	25	25±2	10	3	0, 7, 38
19	B	5000	P	25	25±2	10	3	0, 7, 38
20	B	5000	B	25	25±2	10	3	0, 7, 38

Table 6.5. Details about the tests (C0: initial Cr(VI) concentration; S: Organic substrate - ultrafiltration permeate of cheese whey (P), and waste from brewing process (B); S/L: solid to liquid ratio; T: temperature; Nr R: number of replicates; ST: sampling times). After 7 d incubation, microcosms nr. 17 to nr. 19 were added of 10 mg/L of Fe(II) and incubated for further 31 d (*).

Tests 1-8 were incubated at 17±1 °C to simulate typical groundwater temperatures, Tests 9-20 were conducted at 25±2 °C, higher than typical groundwater temperatures; in such conditions Cr(III) precipitation is disadvantaged, but the biological processes are accelerated. Microcosms with 25% or 50% as solid to liquid ratio were performed; comparing the results of the tests the kinetics could be evaluated.
Iron can have a key role in the Cr(III) co-precipitation. Therefore, trying to explain the different results obtained with the two soils, in tests 9-12 (25±2 °C and a 25% solid to liquid ratio on weight basis) the release of Fe(II) by the two soils as a function of time was assessed.
To further highlight the Fe(II) role in Cr(VI) reduction, test 13-20 were performed for 38 d at 25 °C, 25% S/L, using only soil B.
In tests 17-20, $FeCl_2*4H_2O$ was added at a concentration equal to 10 mg Fe(II)/l, after 7 d of incubation. Such dose was set, according to stoichiometric calculation, in order to provide Fe(II) sufficient for almost complete Cr precipitation in the microcosms at 5 mg Cr(VI)/l initial concentration (Eq. 2).

During all the experiments (Figure 6.2), redox potential (ORP), dissolved oxygen (DO), temperature and pH were continuously monitored by means of a multiparametric laboratory analyzer (MARTINA - Multiple Analysis pRogrammable TItratioN Analyzer); a sampling time of 60 seconds was set in the tests.

In case of Cr(VI) above 10 µg/l, its quantification was performed by the spectrophotometric method (APHA 3500-Cr D; C-APAT–IRSA 2003) exploiting the reaction of hexavalent chromium with diphenylcarbazide and carrying out absorbance readings with mass spectrophotometer (Lange DR360000TM) at a wave length of 540 nm. Low Cr(VI) concentrations as well as total chromium analysis was performed by ICP-MS.

Figure 6.2: Microcosms Set UP and connection to MARTINA analyser.

6.3 Results and discussions

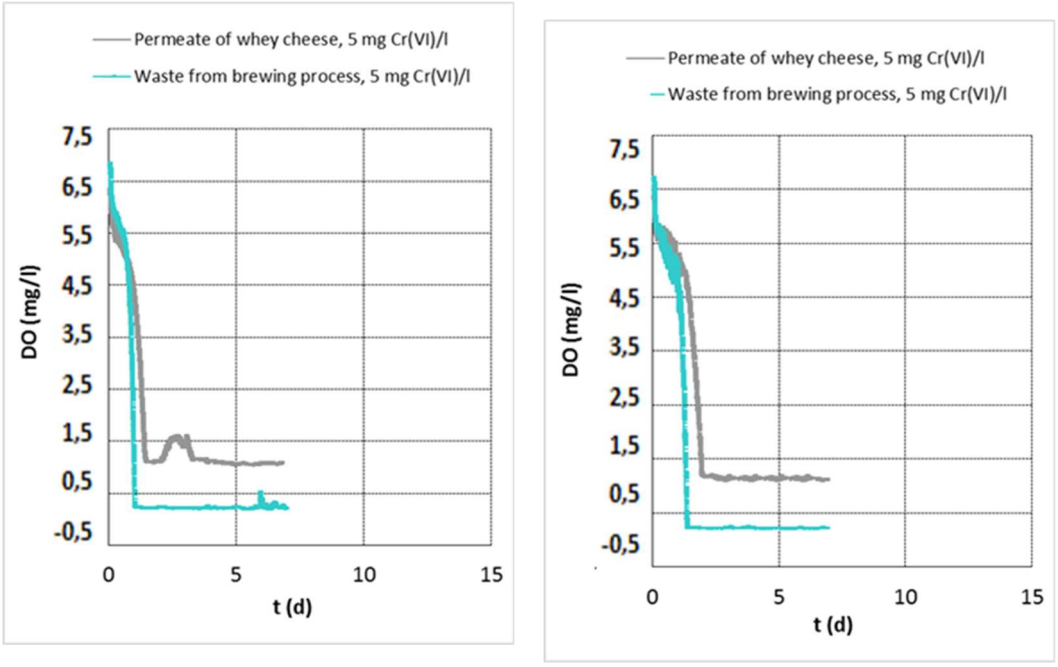
6.3.1 DO, ORP and pH behaviour

In all the microcosms the consumption of electron acceptors, in primis oxygen was quite rapid, with initial 5–6 mg/l dissolved oxygen (DO) concentrations reduced to values lower than 1 mg/l in 1-2 days (Table 6.6).

In the 50% S/L tests, oxygen and oxidation-reduction potential (ORP) decreased quicker in microcosms with soil A than in those with soil B, probably as a consequence of the higher initial total heterotrophic bacteria content of soil A, three orders of magnitude higher than B (Figure 6.3). With soil A and 5 mg/l initial Cr(VI) concentration ORP values of about -600 mV vs AgAgCl were obtained after 3-4 d incubation, with a very steep decrease after 3 d of treatment (Figure 6.4a). At 10 mg Cr(VI)/l initial concentration, ORP decreased more slowly, reaching in 7 days a slightly negative value (-50 mV vs AgAgCl) in microcosms with the brewery substrate, or still a positive value (130 mV vs AgAgCl) in those with whey permeate. However, ORP continued decreasing reaching -400 mV vs AgAgCl after 9 d in tests with brewery substrate and -300 mV vs AgAgCl in 11 d with whey permeate.
With soil B (Figure 6.4b), the fastest redox potential decrease was observed with brewery residue at 5 mg/l initial Cr(VI) concentration, where about -400 mV vs AgAgCl was registered in 5 days; 10 days were required to reach this ORP value in microcosms with the same substrate but 10 mg/l initial Cr(VI) concentration.
With whey permeate, after 10 days, values of – 600 mV and -200 mV vs AgAgCl were observed in microcosms at 5 mg Cr(VI)/l and 10 mg Cr(VI)/l initial concentrations, respectively.
In the 25% S/L tests and incubated at 25°C (tests 13-16), reducing conditions were registered within 5 days incubation, with ORP values all below -400 mV vs AgAgCl in 5-7 d.
Table 6.6 summarizes, for the different test,s the time required to reach dissolved oxygen below 1 mg/l and ORP lower than 0 V and 400 mV, as indicative of established reducing conditions. As already pointed out kinetics were faster in microcosms with soil A, higl S/L ratio and higher incubation temperature.

Test			DO	ORP (vs AgAgCl)	
			<1 mg/l	< 0 mV	< -400 mV
1	A	50	-	8	no
2	A	50	-	6.3	9.2
3	A	50	1.3	3.9	4
4	A	50	0.9	1.8	3.5
5	B	50	-	8.7	no
6	B	50	-	5.1	10.8
7	B	50	1.9	7.8	8.2
8	B	50	1.2	4	5.2
13	B	25	-	4	4.9
14	B	25	-	4.8	6.6
15	B	25	0.9	4.3	5.2
16	B	25	0.8	3.5	5.5

Table 6.6. Time (d) to achieve specific conditions in the microcosms. -: not measured; no: not reached by 11 d.



a b

Figure 6.3. Dissolved oxygen over time t, in 50% solid to liquid ratio on a weight basis microcosms (a) with soil A (test 3-4) and (b) soil B (test 7-8).

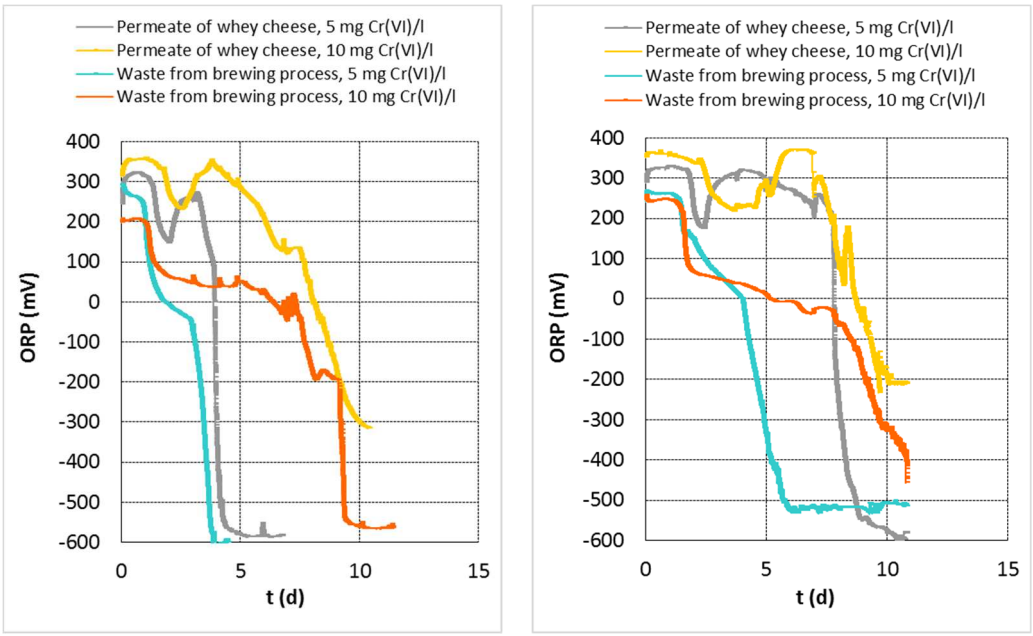


Figure 6.4. Redox potential (mV vs AgAgCl) over time t, in microcosms with 50% on weight basis solid to liquid ratio with a) soil A (test 1-4), and b) soil B (test 5-8);

In all the microcosms, initial pH value was in the range 7.0±0.5 and no significant variations were registered during the tests. In fact, after 36 d incubation, values in the range 6.7 ÷ 7.1 were measured with soil B and 6.7 ÷ 6.9 with soil A, resulting in not significant variations during the treatment.

6.3.2 Cr(VI) reduction

The effect of different soil on chromium reduction was observed in the 50% S/L at 17±1 °C for 36 d (tests1-8). In microcosms with soil A (Figure 6.5), a certain reduction in the dissolved Cr(VI) concentration was observed at the first monitoring, at 8 days incubation.

Following 36 d of incubation, residual values of about 1.3 µg/l were registered in all the microcosms, except in tests with 10 mg/l initial Cr(VI) concentration and whey permeate as substrate; in this latter, residual Cr(VI) concentration was about 2 mg/l.

With soil B, as shown in Figure 6.6, 11 d incubation were required to start observing a decline in Cr(VI) concentrations. The residual values after 36 days, ranged between 5 µg/l and 5500 µg/l. In particular, in the tests with 10 mg/l initial Cr(VI) concentration, the final concentrations were above 5.3 mg/l for both the substrates. Final values of 394 µg/l (whey permeate) and 5 µg/l (waste from brewing process) were obtained in the tests with 5 mg initial Cr(VI)/l.

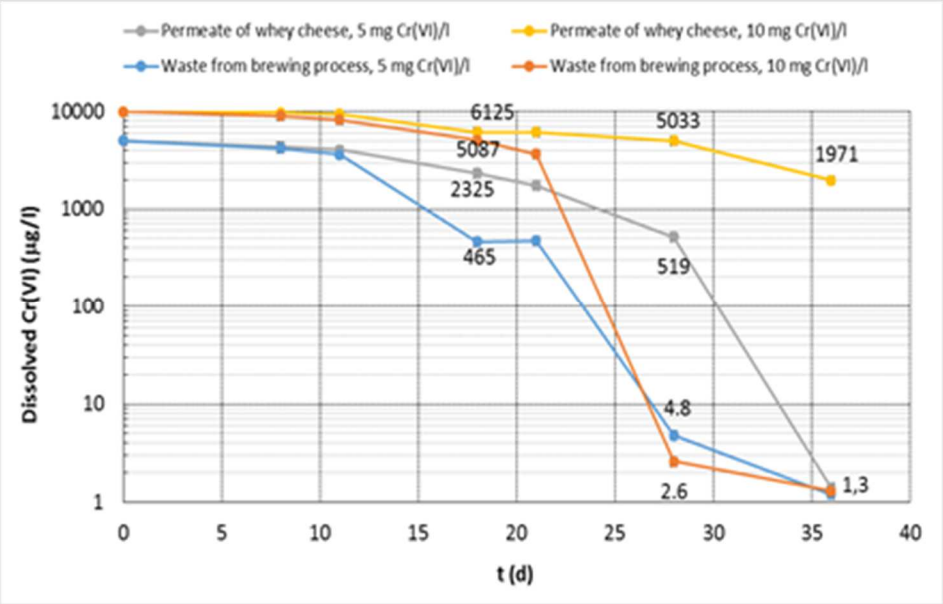


Figure 6.5: Dissolved Cr(VI) over time t, in 50% S/L soil A microcosms.

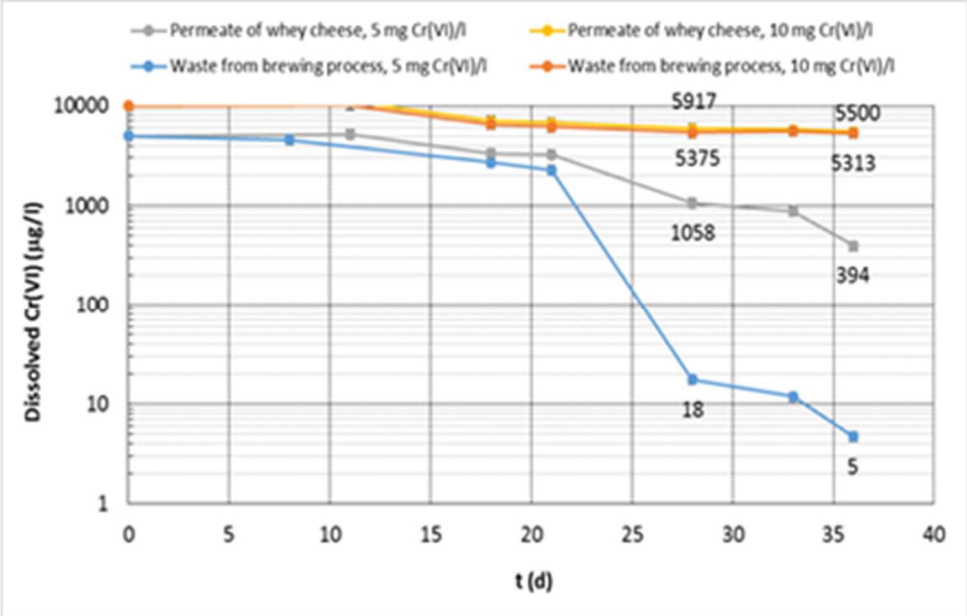


Figure 6.6: Dissolved Cr(VI) over time t, in 50% S/L soil B microcosms.

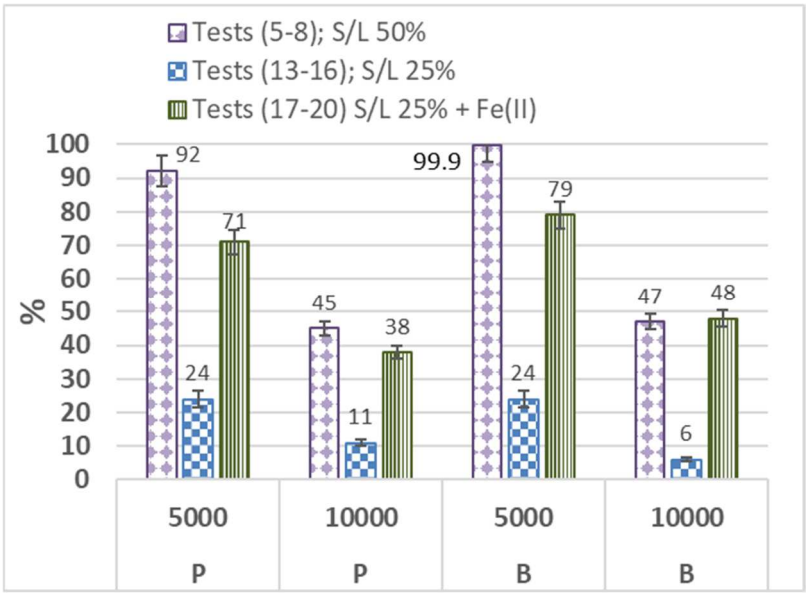
By comparing the microcosms at the same Cr(VI) initial concentration and organic substrate, the best performances were obtained with soil A. This result was explained considering the fundamental role iron plays in Cr(III) coprecipitation; tests revealed Fe(II) release by soil under reducing conditions occurred at a slower rate in soil B than in soil A; in fact, after 10 days, dissolved Fe(II) in soil B was <100 µg Fe(II)/l), whereas more than 1 mg Fe(II)/l was observed with soil A. A significant iron release (up to 850 µg Fe(II)/l) with soil B required more than 20 days.

In Figure 6.7 the dissolved Cr(VI) removal in the different soil B microcosms as a function of the initial concentration and S/L are compared. At the end of the tests (38 d), in microcosms at 50% S/L high efficiencies (>90% with both the substrates) were observed with 5 mg/L initial Cr(VI) concentration; at higher initial concentration, Cr(VI) removal was about 45% with both substrates.

At 25% S/L, Cr(VI) removal fell in the range 6%-24%, with the higher values in the tests at lower initial concentration. Fe(II) addition during incubation improved Cr(VI) removal by a factor about 3 in all the tests, excepting tests with brewery residue and 10 mg/L initial concentration where the effect of Fe(II) addition was more relevant.

Test	C_r(VI) removal (%)
1	80
2	100
3	100
4	100
5	45
6	47
7	92
8	100
13	11
14	6
15	24
16	24
17	38
18	48
19	71
20	79

a



b

Figure 6.7: Cr(VI) removal at the end of the tests. 5000 µg/l and 10000 µg/l initial Cr(VI) concentration; P: Permeate of cheese whey; B: waste from brewing process.

6.4 Conclusion

Lab scale batch tests, with two different soils (A and B) and solid/liquid ratios (25% and 50% on a weight basis), were carried out to test the feasibility of promoting Cr(VI) reduction by means of residues of the food industry, i.e. permeate from the ultrafiltration of cheese whey and waste from the brewing process. Initial Cr(VI) concentrations of 5000 or 10000 µg/L were considered as representative of typical values at the contaminated sites. In all the microcosms, dissolved oxygen decreased from about 6 mg/l to values <1 mg/l after 1-2 d incubation, and the redox potential from approximately +450 mV to -200 mV vs SHE by 11 days. The microcosms with

high initial concentration, 10 mg Cr(VI)/l, achieved stable reducing conditions (negative redox potential) during a longer period than those with low concentration. After about 40 days, the highest Cr(VI) abatements were obtained in soil A microcosms fed with beer distillation residues, as soil A was characterized by an about three orders of magnitude higher initial total heterotrophic bacteria content than soil B. Fe(II) availability was also a key factor in Cr(III) co-precipitation. In comparison to the theoretical conditions of the Pourbaix diagram, in real systems, according to our results stable redox potentials below -200 mV vs SHE are required to start appreciating Cr(VI) reduction.

In particular, the features of selected substrates are injectability, non-toxicity and non-recalcitrance. Interesting prospects in terms of reduced costs and overall environmental impacts; therefore, injection of this substrate into real aquifer could be potentially applicable. This technology could lead to the achievement of a concentration of hexavalent chromium below the threshold value established by current legislation in Italy (5 µgCr (VI)/L).

Further tests in the anaerobic field could be carried out for the design on a real scale and for a comparison with registered brand reagents, to understand their possible placement within the market of remediation technologies. However, the injection has peculiar limitations, such as the difficulty of appropriately defining dosages according to site-specific conditions (European Commission 2017). Too high doses can cause negative secondary effects in the aquifer and the accumulation of by-products; vice versa, insufficient contributions can cause a stall in the site remediation plan.

7

ELECTRO-SYNTHESIS AND ELECTRO-FERMENTATION TESTS

7.1 Introduction

Microbial production of chemicals in BES provides a highly attractive novel route for the generation of valuable products (such as H_2O_2, lactate, …) from electricity or even wastewater. The scientific advancement and system scale-ups of electrosynthesis (ES) and electrofermentation (EF) will depend on interdisciplinary collaborations (Jiang et al., 2019). This chapter resumed experiences aquired during a period at Chalmers University - Department of Biology and Biological Engineering, working on the project "Production of commodity chemicals and fuels in bioelectrochemical systems". This project is part of a bigger project recently funded by the Swedish Research Council Formas, aiming to develop a novel technology for producing chemicals and fuels from greenhouse CO_2 and "green" electricity. For the path of the PhD, aims were the acquirement of knowledge to managing these systems.

ES tests were conducted using a pure culture of *Clostridium chromiireducens* to evaluate its capability to grow up in an M3Cs system and use CO_2, the sole substrate added, producing organic byproducts. Moreover, a series of EF tests were also conducted to evaluate the potential of *Clostridium beijerinckii* to grow in a BES and to evaluate the substrate consumption rates as well as chemical production at varying biocathode conditions. Clostridium spp. were grown anaerobically in serum bottles under appropriate gas and temperature conditions, in a common growth medium prior to inoculation in Microbial 3-electrode Cells (M3C). The metabolic capabilities in BESs in an anaerobic environment are then evaluated. Clostridium is a genus of Gram-positive anaerobic, bacteria, which includes about 100 species, with common free-living bacteria and several significant human pathogens. Clostridium species inhabit soils and the intestinal tract of animals, including humans. The capacity to metabolize CO_2 for Clostridium genus was already studied in some process (but not in BESs) with hydrogen supply; as example, Ramachandriya et al. (2013) indicated that *Clostridium*

carboxidivorans produced 33% more ethanol and 66% less acetic acid compared to *Clostridium ragsdalei*, making *C. carboxidivorans* the better candidate for ethanol production. Clostridium genus could be detected in a natural system in different species; developments for the research could assess his ability to be chromium resistant; *Clostridium chromiireducens* was reported to help metal reduction in anaerobic soils (Inglett et al., 2011). Therefore, working with similar species has possible implications in what could be the action in a real matrix, such as soil or aquifer.

7.2 Materials and Methods

Minimal or Rich Medium (MM and RM) (Table 7.1) were considered as suitable growth media for Clostridium species. In the non-inoculated chambers a modified mineral medium with high conductivity seved as the electrolyte to maintain an electrochemical potential balance.

104b Medium – Modified (1 l distilled water)	**Minimal**	**Rich**	**No Growth**
	(g)	**(g)**	**(g)**
Triptone – refill from bucket	-	5	-
Peptone from Meat	-	5	-
Yeast	-	10	-
NaCl – sodium chloride – 7647-14-5	1	0.08	5.8
KH_2PO_4 – dihydrogen phosphate – 7778-77-0	5.25	0.04	5.25
K_2HPO_4 – di-potassium Hydrogen	10.7	0.04	10.7
NH_4Cl	1.5	-	-
$CaCl_2*2H_2O$	0.1	0.01	-
$MgSO_4*7H_2O$	0.2	0.02	-
$MnSO_4*H2O$	0.002	-	-
$NaMoO_4*H_2O$	0.001	-	-
$NaHCO_3$	-	0.4	-

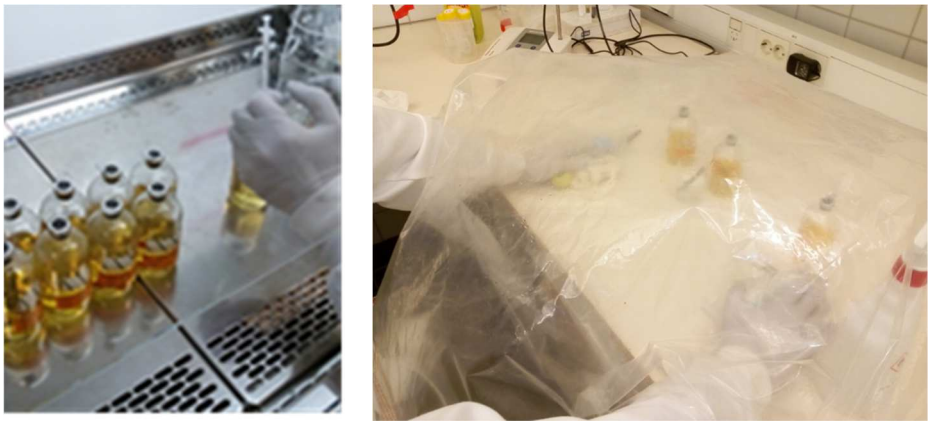
Table 7.1. Mineral media used during incubation and BES tests.

To the prepared solution 1 g Na-resazurin (solution 0.1% w/v) was added as a redox indicator, and medium was sparged with 100% N_2 gas for 30–45 min to assure anaerobic conditions (Figure 7.1a). Serum bottles were prepared crimping a certain volume (150-200 ml) of the solution in laminar fume hood. After autoclaving the bottles, 0.5 g/L-Cysteine-($HCl*H_2O$) was added in each one as a reducing agent to consume any residual oxygen. By means of a syringe through the polymeric septum, the substrates of interest (e.g. glucose, xylose, glycerol or any other substrate from sterile anoxic stock solutions, concentration about 5-10 g/l) could be also added to the solution (Figure 7.2b).

a) b)

Figure 7.1. Deionized water (DI) with resazurin takes on a blue color (a); fluxing nitrogen turns it to transparent, except in the presence of interfering organic elements that make the solution taking on a yellowish color. Injection in serum bottle (b).

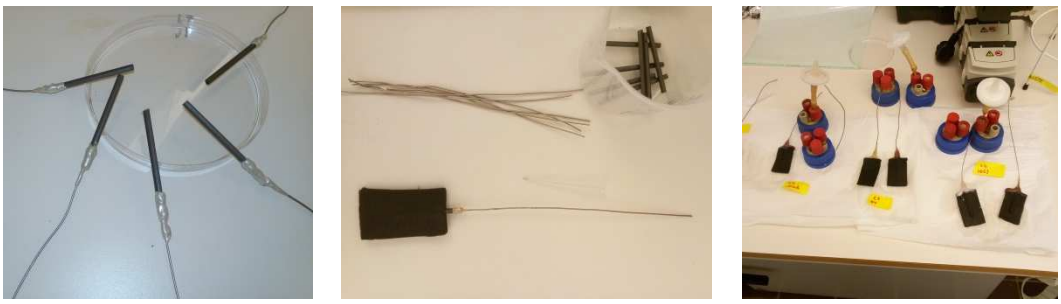
Clostridium spp. were grown prior to inoculation in the MEC reactors. Working in strictly sterile and anaerobic condition (Figure 7.2), *Clostridium spp.* (biosafety Level 1), were prepared and cryopreserved (i.e. frozen in liquid nitrogen at -196°C in the presence of glycerol as stabilizing agents). Than, *C. chromiireducens* and *C. beijerinckii* samples were used inoculating a small amount in the serum bottles prepared. The bottles, inoculate, were incubated at 30°C with stirring speed 120 rmp for 1-2 d, and finally transferred into the M3C reactors under defined feed gas (e.g. N_2 or CO_2 sparging) and nutrient conditions.



a) b)

Figure 7.2. Serum bottles handling in laminar flow hood (a); inocula comes in an ampule lyophilized cultures, under dry conditions. When break the ampoule, with culture very sensible to oxygen, the operator must work in an anaerobic environment (b).

To study the effect of cathodic current evolution, dual-chambers H-type BESs were assembled (Xafenias et al., 2015a). M3C reactors consisted of glassware, ion exchange membranes, and electrodes mainly made of carbon materials (Figure 7.3). Borosilicate reactors (290 mL working volume, each one) were separated by a pretreated PEM (Nafion®117; Ion Power Inc., USA). As described in Xafenias et al. (2015), graphite felt electrodes (SIGRATHERM; SGL Carbon Ltd., UK), 38 cm^2 projected surface area, were used both as working (WE) and counter (CE) electrodes. Electrical power was externally supplied with a potentiostat (MLab 200, Bank Elektronik), that contolled and monitored the whole system, too (Figure 7.4).



a) b) c)

Figure 7.3. Electrode preparation.

a) b)

Figure 7.4. Dual-chambers H-type (a); monitoring by a commercial potentiostat (MLab 200, Bank Elektronik) (b).

Whether required, the pH in the systems was corrected using acid (HCl) or base (NaOH). A redox mediator (e.g. humic acid analogue anthraquinone and neutral red) was also added in some tests.

Small amounts of liquid and gaseous chemicals, including, as an example, organic acids and hydrogen, were produced during the process. These compounds were analyzed using HPLC technique (Ultimate 3000).

Sampling was performed using sterile syringes and needles (Figure 7.5). Aliquots (1 ml) were periodically collected into plastic vials to measure:

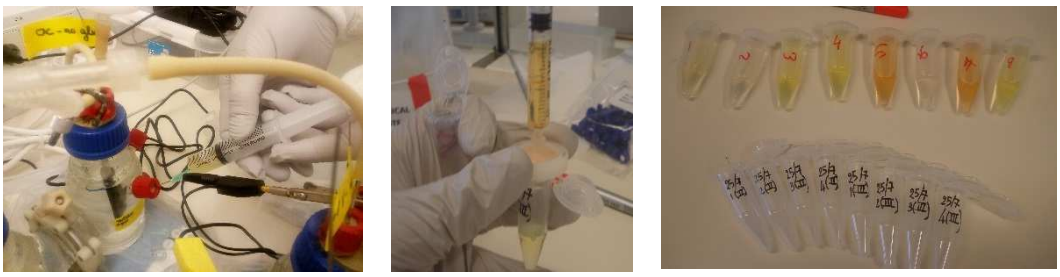
- pH, directly in the vials;
- OD600 (Optical Density at 600 nm by means of spectrophotometers; instrument reads between 0.01 - 0.3);

- produced metabolites (after sample preparation, 0.3 ml were injected in HPLC) (Table 7.2).

.

Compounds	Retention time (min)	standard	type	Cal.Type	Peak Type
Glucose	7,425	External	Area	Linear	Auto
Xylose	7,870	External	Area	Linear	Auto
Succinate	9,030	External	Area	Linear	Auto
Lactate	9,950	External	Area	Linear	Auto
Glycerol	10,600	External	Area	Linear	Auto
Acetate	11,450	External	Area	Linear	Auto
Butyrate	13,550	External	Area	Linear	Auto
1,3-PDO	15,600	External	Area	Linear	Auto
Acetone	15,700	External	Area	Linear	Auto
Ethanol	16,400	External	Area	Linear	Auto
isoprolanol	18,200	External	Area	Linear	Auto
2-butanol	22,880	External	Area	Linear	Auto
1-butanol	25,700	External	Area	Linear	Auto

Table 7.2. Analytes analized during tests. Summary Retention factor is independent of some key variable factors including small flow rate variations and column dimensions. Therefore, it is a useful parameter when comparing retention of chromatographic peaks obtained using different HPLC systems.



a) b) c)

Figure 7.5. Sampling (a); filtration step (b) to otbtain sample for HPLC (c).

For investigating potential electron donors for *Clostridium spp.*, Cyclic Voltammetry (CV) experiments were also performed at least at the system set-up, prior inoculation, and then following inoculation.
All the CVs were carried out in still conditions to rely only on diffusion as transport mechanism for the chemical species in the reactor, i.e. nutrients, etc. are not continuously brought on the electrode surface and products taken away by mixing, and CV results are not influenced by mixing conditions, stirring speed, etc... Furthermore, gas flux was stopped during CVs.

As summarized in Table 7.3, different experimental set-ups have been realized; each test lasted on average one week. Some set-up and tests showed interesting performances in terms of biomass growth and reaction products.

Set up n.	Duration (h)	Culture	Cathodic compartment	Test	Added substances	Potential (V) vs Ag/AgCl
1	120	*C.C.*	MM CO_2 flux	1	G	-1.1
				2		-1.1
				3	G	OC
				4		OC
2	120	*C.C.*	MM CO_2 flux	5	G	10 mA
				6		10 mA
				7	G	OC
				8		OC
3	120	*C.C.*	RM CO_2 flux	9		-1.2
				10		10 mA
				11		0
				12		OC
4	200	*C.B.*	RM with Glucose (10 g/l). N_2 flux	13	A	-1.2
				14	A	-1.1
				15	A	0
				16	A	OC
5	120	*C.B.*	MM with Glucose (10 g/l). N_2 flux	17-18		-1.2
				19-20		OC
6	140	*C.B.*	RM with Glucose (10 g/l) N_2 flux	21-22		OC
				23-24		-1.2
7	125	*C.B.*	RM with Glucose (10 g/l) N_2 flux	25-27	NR	-1.2
				28		-1.2
				29-30	NR	OC
8	120	*C.B.*	RM with Glycerol (10 g/l). N_2 flux	31-34		-1.2
				35-36		OC
9	120	*C.B.*	RM with Xylose (10 g/l) N_2 flux	37-40		-1.2
				41-42		-1.2

Table 7.3. Summary of set-ups and tests. *Clostridium chromiireducens (C.C.), Clostridium beijerinckii (C.B.);* "Minimal Medium" (MM) or "Rich Medium" (RM) are used in tests; CO_2 or N_2 was continuously sparged with bubble in cathodic chamber. Addition at the beginning of the test: G (Glucose; 10 g/l); NR (Neutral Red; 0,1 Mm). Addition during the test: A (Anthraquinone; 0,1 Mm).
Set potential: OC (Open Circuit), 10 mA when potentiostat was made working as a galvanostat to provide 10 mA circulating current.

7.3 Results and discussion

The electrosynthesis tests with *Clostridium chromiireducens* (Set up n. 1-3) did not show significant results; at the cathode, *C. chromiireducens* did not show ability to create EAB. Neither setting a -1.1 V or -1.2 V vs Ag/AgCl as cathodic potentials nor forcing a reductive current (Set up n. 2) improve bacterial growth. Moreover, varying the mineral medium, with the addition of glucose or the use of a rich medium, give no increase in the OD600 in comparison to the OC control.
However, focusing on production of products, some aspects could be evaluate (Table 7.4).

	Test 1			**Test 2**			**Test 3**			**Test 4**		
t	**G**	**GY**	**PDO**	**G**	**GY**	**PDO**	**G**	**GY**	**PDO**	**G**	**GY**	**PDO**
h	g/l	mg/l	mg/l	g/l	mg/l	mg/l	g/l	mg/l	mg/l	g/l	mg/l	mg/l
0	9,8	37,2	351,6	DL	14,2	DL	10,4	11,1	DL	DL	DL	313,7
24	9,0	DL	DL	DL	51,1	331,7	10,0	DL	DL	DL	14,6	232,1
48	8,6	39,2	DL	DL	DL	DL	9,7	DL	DL	DL	DL	DL
72	8,6	41,4	931,6	DL	28,8	DL	9,6	19,4	42,2	DL	DL	29,9
96	7,6	57,6	1013,3	DL	37,8	1038,2	8,7	18,6	347,3	DL	15,7	DL

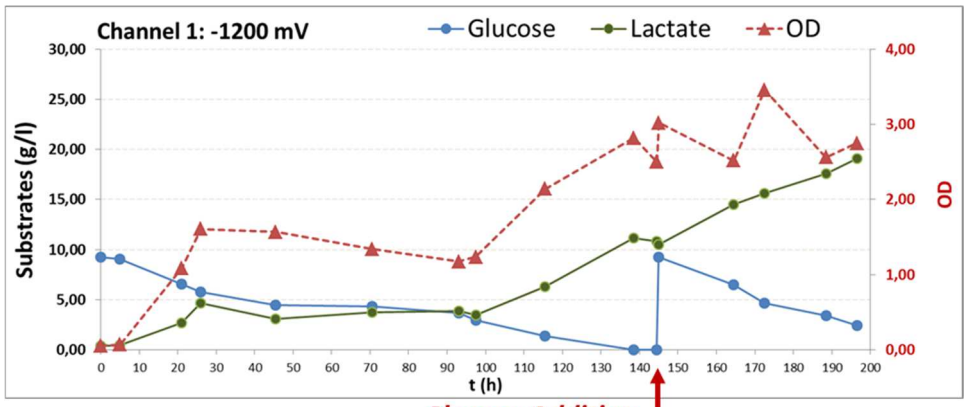
Table 7.4. Set UP 1; G (Glucose), GY (Glycerol), PDO (1,3-Propanediol) concentration during the test.

The concentrations of glycerol and PDO produced during the test appear to be significantly higher in systems with cathodic potential (Test 1-2) than the relative OC. The low byproducts concentrations, close to the DL and below the calibration curve used, and the low circulating currents are not very satisfactory in a synthesis process. Conversely, the production of organic matter from dissolved carbonates could guarantee interesting prospective for groundwater remediaton; the comparison between tests 1 and 2 clarifies how the presence of high organic substance (glucose) added at the beginning of the test does not lead to significant variations in the analyzed by-products.
An analysis of *C. chromiireducens* ability to contribute to cathodic Cr(VI) reduction can be further explored, also with regard to the characteristics of chromium-resistance, with a connection to the induced bioremediation technology maybe amplifiated through a BES.

Electrofermentation tests (set-up n. 4-9) showed different results in relation to the selected mineral medium and to the added substrate.
In tests 13, 24, 28 at the same set potential and substrate (glucose), the production of lactate is almost double in comparison to OC tests 16, 21, 22 (e.g. in Figure 7.6). In all the tests, higher concentrations of lactate are present in the reactors with poised cathode potential.
The effect of a redox mediator, neutral red on substrate consumption and lactate production was also investigated. By comparing tests 13, 24, 28 without the mediator

to the ones where mediator was added at the beginning of the test (25, 26, 27, 29, 30) no significant difference in glucose consumption and lactate production were observed. In Tests 13, 24, 28 circulating current was on average 5-10 mA (Figure 7.7); a similar range was also observed in test with Neutral Red (25, 26, 27).

Test 13



Test 16

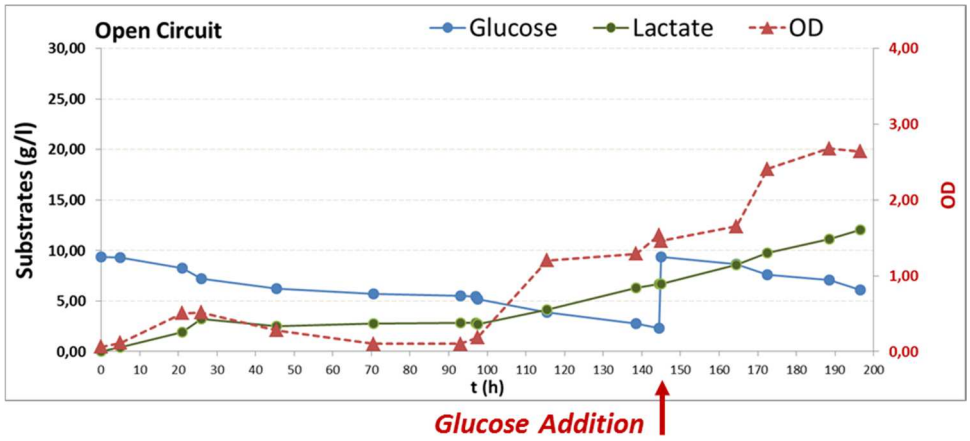
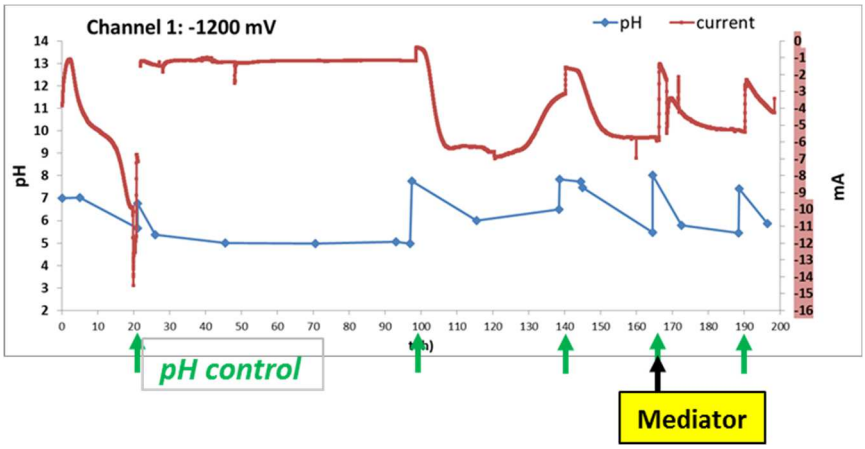


Figure 7.6. Glucose and lactate concentration over time during M3Cs test; -1.2 V vs Ag/AgCl poised biocathode vs OC. OD600 is plotted on the secondary axis.

Test 13



Test 16

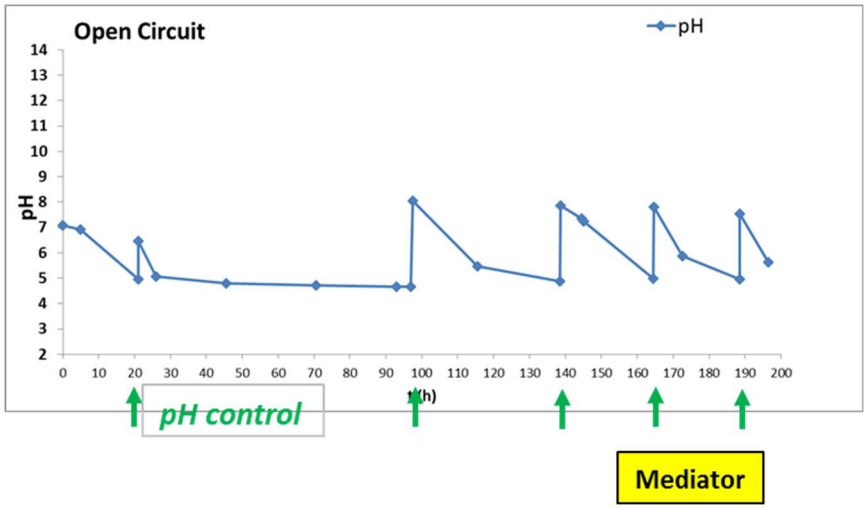
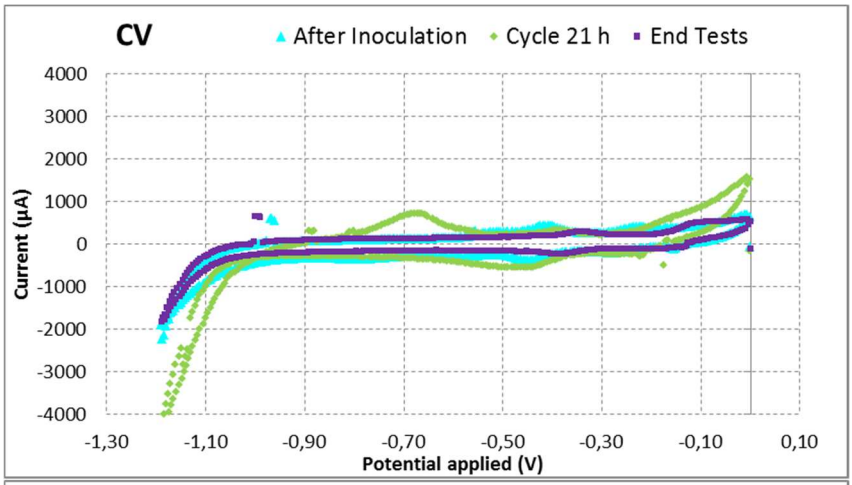


Figure 7.7. pH control over time during MEC test is plotted; biocathode poised vs OC. Cathodic current is also influenced by pH value.

When the pH is brought to almost neutral values from acidic condition, negative current quite immediately go down. This is an incentive to bacterial growth and, therefore, it allows a new increase in current. Moreover, when glucose is almost finished, pH could increase due to lack of consume.
In Tests 14, at -1.1 V vs AgAgCl poised biocathode, it is possible to notice how this sglight difference in the applied potential (only a 100 mV to the previous test) caused a significant reduction in the circulating current, with peak values not exceeding 1 mA. Set-up n. 8 and 9 were similar to the set-up n.6, but different substrates, xylose and glycerol, were added. With such substrates, slower substrate consumption rates where observed with consequent reduced production of metabolites and limited biomass growth. The availability of attractive substrates for specific species is relevant in terms of BESs productivity, as in any biological system. In environments with mixed coltures, like an aquifer, the presence of many species determines the development of some with respect to others.

Cyclic Voltammetry is a potential dynamic electrochemical measurement technique; in figure 7.8, as example, are reported CV of two tests. it is possible to note that there are no evident oxidative/reductive peaks. In fact, there are no significant differences between the CV conducted at the beginning of the test, during and at the end of the experiment for the test 13. In the case of the addition of a mediator (test 26), at the beginning of the test the reduction peak of the added neutral red is clearly visible. Being a reversible reaction, the corresponding peak on the opposite side is also outlined.

Test 13



Test 26

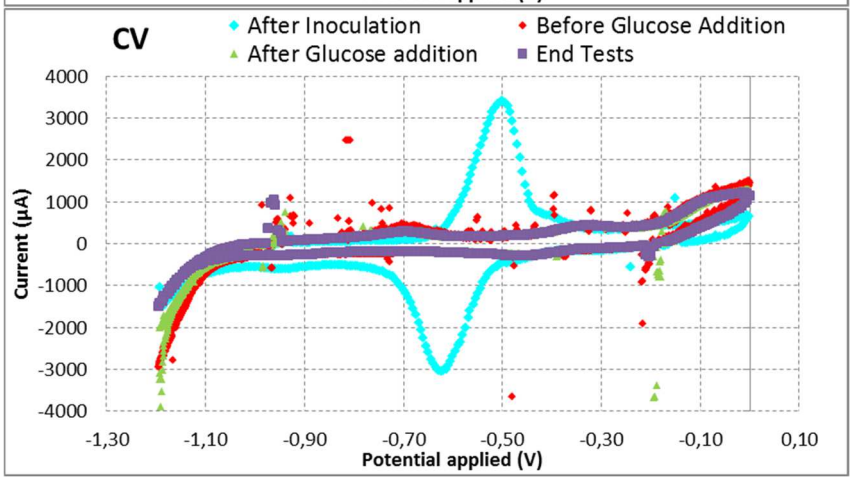


Figure 7.8. Cyclic voltammetric curve, circulating current (mA) as a function of electrode potential (V).

Thanks to these experience it is possible to conclude that *C. beijerinckii* exibits electroactive capability, as evidence of current production that not depend only on glucose consumption; these results differ from previously published data reporting electroactivity of *C. beijerinckii* only in the presence of a mediator (Jiang et al., 2019). During lactate fermentation, glucose is converted to lactic acid. Lactate is one of the derived chemicals that can be recovered from organic containing waste streams. These tests evaluated the potential of a biocathode to enhance lactate production using *C. beijerinckii*. Lactate production was improved at cathode applying −1 V vs SHE. Further research is however needed to understand the mechanisms to fully exploit BES potential, including the selection of microorganisms. Anaerobic processes and principles could be applied for water treatment and recovery of resources.

7.4 Conclusion

The research aimed to support the development of a BES to produce chemicals that could be of particular interest for wastewater treatment, but possibly may find applications also for in-situ groundwater remediation.

Although the experiences with *C. chromiireducens* were not satisfactory, cathodic bioelectrochemical production of acetate from inorganic carbon sources, which could then be used to promote heterotrophic Cr(VI) reduction could be an interesting option for bioremediation. The possibility of promoting in-situ generation of acetate or other simple organic substrates in aquifers and the potential of autochthonous microorganisms should be evaluated. Bioelectrochemical production of organics from inorganic carbon in cathodes could be used to heterotrophically reduce Cr(VI) with the aid of a cathode. Relying on carbonates availability in aquifers, it would be possible to bioelectrically promote in-situ production of organic substares for biological Cr(VI) reduction, as described in the previous chapters, with no chemicals injection in the subsurface.

The EF process, in an anaerobic environment obtained via nitrogen sparging and supplemented with glucose for growth at the cathode, was stimulated through a fixed potential -1 V (vs SHE). Lactate production in BES increase compared to the one obtained in the open circuit control. Addition of mediator, neutral red or anthraquinone, at the beginning or during the tests, did not offer any significant advantages to the electroactivity of the already developed biofilm. This study evaluated the potential of using *C. beijerinckii*, that show EAB properties, to enhance fermentation. The work highlights the feasibility of biocathode to stimulate fermentation products deriving from organics and give chance to promote bio-induced remediation.

8

HEXAVALENT CHROMIUM REDUCTION IN BES

8.1 Introduction

This activity aimed to explore the possibility of Cr(VI) contaminated groundwater treatment in biolectrochemical systems, based on the information from the review of available research for the treatment of chromium-contaminated water with BESs in chapter 4.

In some preliminary tests, chromium reduction was observed in BES where a fixed voltage between the electrodes was imposed; however, in these systems, there was little control over how the reactions take place and the real contribution of the EAB developed on the electrode was not clear.
Enrichment of different inocula was conducted in order to evaluate the development of potentially suitable microorganisms to be chromium resistant/reductive or well suited within a BES system, developing characteristics similar to EAB.
With a mature EAB were conducted, using a non-commercial potentiostat (Attachment B), some tests in a M3Cs systems. To approach real groundwater conditions, differently from previous studies (Huang et al., 2015; Song et al., 2016; Wu et al., 2015), no organic substance was added in the working chamber, dosing carbonates as the sole carbon source; lower Cr(VI) concentrations than wastewater were investigated.

8.2 Materials and methods

8.2.1 Reactor design and setup

Experiments were conducted in dual-chamber H-shaped reactors (Figure 8.1), made of 2 borosilicate-glass bottles (1.2 L working volume each one) separated by

4.52 cm^2 CEM (Membranes International Inc., USA) or PEM (Nafion®117; Ion Power Inc., USA). A frange closure with PTFE o-rings (Ø = 4.5 cm) allowed to place the membrane between the two bottles and seal the reactor.
Graphite cylinders (ATAL Grafiti, Italy, 6 cm length, 1 cm diameter, geometric area 18.85 cm^2) or, in few tests, for comparison, graphite felts (Ouzheng carbon, China) (approximately 128 cm^2 apparent surface area) were used as electrodes (Figure 8.2).

Figure 8.1. Dual-chamber H-shaped reactors (2C-MFC).

a) b)

Figure 8.2. Graphite cylinders (a) and graphite felts (b).

Stainless steel or titanium wires (1 mm diameter) fixed in the centre of the graphite cylinders or inserted into the felt served as current collectors and to keep the electrode suspended into the electrolyte. The wires were covered with heat-shrinkable olytetrafluorethylene (PTFE) tubes (Sigma-Aldrich) to prevent corrosion.

Before use, the CEM was soaked in 5% NaCl solution for at least 24 h as described by Daghio et al. (2016). PEM pre-treatment consisted of sequential boiling and cooling-

down in H_2O_2 5% solution, deionized water (DI), H_2SO_4 1 M solution and finally again in DI. Each step lasted approximately 30 minutes (Casalegno et al., 2014). After this treatment, the damp membrane was air-dried overnight, and subsequently stored in DI. By means of a multimeter, it was always verified that the overall resistance of the whole electrode (including the current collector) fell below 10 Ω.

The anodic and cathodic chambers were filled with sterile minimal medium (MM1: 7 g/l $NaH_2PO_4 \cdot 12H_2O$, 3 g/l KH_2PO_4, 1 g/l NH_4Cl and 0.5 g/l NaCl or MM2: 1238 mg/l Na_2HPO_4, 153 mg/l NaH_2PO_4, 200 mg/l NH_4Cl, 260 mg/l KCl) (autoclaved twice at 120°C for 30 min). MM2 has lower salt concentrations (conductivity 2570 µS/cm) than MM1 (11170 µS/cm), making it more representative of natural conditions (Molognoni et al., 2017). Despite the reduced phosphates content, MM2 medium showed sufficient buffer capacity to keep the pH in the neutral range throughout the tests.

8.2.2 Preliminary tests with chromium

Several preliminary tests have been performed with the aim of excluding Cr(VI) abiotic losses due to sorption onto the glassware, electrodes and membranes (Figure 8.3) or chemical reactions of Cr(VI) with the minimal media uses as electrolytes and even analytical interferences of the electrolyte in Cr(VI) analysis.
Adding potassium dichromate ($K_2Cr_2O_7$), the system was kept in constant mixing for the duration of the tests to favor the spread of hexavalent chromium, even within the pores of graphite. The reactors were kept at a constant temperature (18 °C). Cr(VI) concentration in this control reactors was monitored for the different tests conducted in 10-40 days.

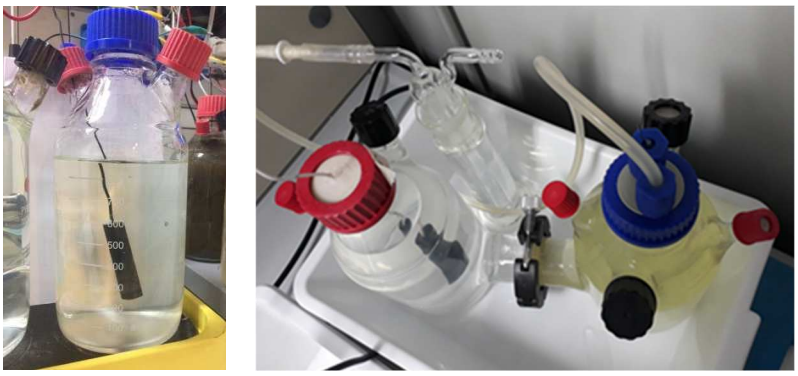


Figure 8.3. Adsorption tests; materials are the same as those used in the Set-up related to reduction tests (Paragraph 8.2.4).

Electrochemical reactions involving Cr(VI) were explored, by imposing a constant voltage (1.5, 3 or 9 V) between the electrodes in completely abiotic systems.
Table 8.1 summarizes the different preliminary tests that have been performed.

Tests	General Information	Cathode Inoculum	ΔV (V)	Initial Cr(VI) concentration (mg/l)
Cr(VI) sorption on materials (glass, graphite, membrane)	MM1	Abiotic	-	10
Interference with medium	MM1 or MM2, 2 g/l di HCO_3^-	Abiotic	-	1-10
Applied voltage (Abiotic remediation cell)	MM1	Abiotic	1.5, 3, 9	10

Table 8.1. Preliminary Tests.

8.2.3 Sources and enrichment of inocula

8.2.3.1 Aerobic conditions

Operating under aerobic conditions in the presence of chromium, the selection of a resistant chromium biomass was evaluated, starting from different inocula, including:

- An enrichment culture selected from the previous bio-induced reduction tests conducted in the microcosms (B5P culture);
- Activated sludge sample deriving from Livescia plant in Fino Mornasco (CO), (FF culture).

The inocula have been enriched in 1 l continuously stirred Pyrex bottles, at room temperature, monitoring with the MARTINA biosensor the temperature and dissolved oxygen trend. To ensure aerobic conditions throughout the test, an aerator with a porous ceramic diffuser was used to maintain DO concentration constantly above 2 mg/l. Periodical measurements of optical density (OD600) and Cr(VI) concentration in the solutions were carried out.
As regards the B5P culture, the initial OD600 was equal to 0.05 and sodium acetate was added, in concentration up to 2 g/l, as carbon source for bacterial growth. For the FF biomass, deriving from 1:20 diluted activated sludge in mineral medium solution, no sodium acetate was initially provided to let the biomass to degrade the residual organic load for a period of about 48 h. At the end of this initial step, sodium acetate, 2 g/l, was added to the solution.
In addition, respirometric tests have been carried out for both the cultures, to evaluate the oxygen uptake rate and link it to the consumption of acetate. Following the addition of sodium acetate to the solution, aeration was turned off until OD decreased to values up to 2 mg/l; once this low threshold was reached, the aeration was switched on, to increase the OD to the initial values (generally up to 6-7 mg/l).

Biological removal of Cr(VI) was also evaluated in a BES, exploiting an inoculum that is characterized by the presence of a chromium-reducing bacteria community. Under aerobic conditions, as already described in Chapter 4.1, purely electrochemical Cr(VI) reduction is less favored than oxygen reduction; according to Nernst's law, oxygen is characterized by higher reduction potential than the different forms of Cr(VI) at the typical environmental conditions (neutral pH). For the preparation of the

inoculum to be used at the cathode, a biomass washing procedure was carried out on the FF culture. The solution was centrifuged three times at 4000 rpm for 10 minutes, to separate the biomass from the solution; the supernatant was removed and disposed off, while the biomass was resuspended and mixed with new mineral medium MM1; the use of Vortex allows a homogeneous distribution of the resuspended biomass. After system set-up, by an external power supply a 500 mV tension between anode and cathode was applied. The system was left open to air to allow O_2 to diffuse inside the reactor. In parallel to the BES system, an open circuit (OC) control was set up: a solution was prepared with the same composition as that present at the cathode of the BES system; a graphite electrode was immersed inside the solution, wrapped with stainless steel wire, prepared in the same way as previously described. The presence of OC control allows distinguishing the reduction linked to a purely electrochemical process from that associated with bacterial activity. In fact, a possible decrease in concentration of hexavalent chromium within the OC system is to be attributed exclusively to a biological type process. Both the BES and the OC were kept in continuous mixing, thanks to a magnetic stirrer, and at 25 °C for the entire duration of the test. In order to monitor the conditions inside the reactors, pH measurements were performed periodically (using a bench-top pH meter) and, through the MARTINA biosensor, the DO and temperature values at the cathode were continuously recorded. In order to monitor the current circulating in the BES, a 1000 Ω resistor was inserted along the anode-cathode connection electric circuit and, using a multimeter, the potential difference at the ends of the resistance was continuously recorded; through the simple application of Ohm's law, it is possible to derive the current circulating within the system.

Table 8.2 summarizes the different tests that have been performed.

Tests	**General Information**	**Inoculum**	**ΔV (V)**	**Initial Cr(VI) concentration (mg/l)**
Inoculum cultivation under aerobic conditions	MM1, 2 g/l $C_2H_3NaO_2$	B5P, FF	No	10
BES cathode under aerobic conditions	MM1; AA; Graphite cylinder electrode; CEM membrane; 2 g/l Sodium acetate (Cathode)	FF	0.5 V	10
Under aerobic conditions	MM1; AA; Graphite cylinder electrode; CEM membrane; 2 g/l Sodium acetate (Cathode)	FF	OC	10

Table 8.2. Aerobic Tests. B5P: from Microcosms test with soil type B, 5 mg/l initial Cr(VI) concentration and ultrafiltration permeate of cheese whey as organic substrate (Chapter 6.2); FF: activated sludge derived from Fino Mornasco Wastewater treatment plant; AA: Abiotic Anode.

8.2.3.2 EAB developement in MFC

Operating in a dual-chamber MFC (2C-MFC) (Figure 8.4) a resistor (500-1000 Ω) was used in the external circuit connecting anode and cathode; a multimeter verified the overall resistance. In the anodic chamber, 0.24 l sludge from an anaerobic digester (SAD) of a wastewater treatment plant in Cremona (Italy) was used as an inoculum; also sludge for agricultural reuse was tested (AS). Sodium acetate, up to 1 g/l, served as the sole carbon source for bacterial growth. Acetate was periodically spiked (50-100 mg/l) into the medium with a syringe every time the current density dropped below 0.5 mA/m^2. In order to establish anaerobic conditions, after inoculation the anodic chamber was flushed for 15 min with sterile-filtered N_2. Finally, the chamber was sealed by a screw cap with PTFE coated silicone septum; the cathode chamber was, instead, kept open to air to let oxygen diffuse into the solution. Samples were periodically withdrawn from the chamber using a syringe, for optical density at 600 nm (OD600).

Samples for COD (chemical oxygen demand) quantification, about 3 ml, were periodically collected and spectrophotometrically analyzed with cuvette tests (Hach Lange LCK314 and spectrophotometer Lange DR360000TM), according to ISO 15705 (2002). COD measurements were used to evaluate the amount of acetate microbially oxidized with time. By considering, in fact, the complete oxidation of acetate, a 1.08 $gO_2/gC_2H_3O_2^-$ conversion factor is estimated to relate the COD concentration to the residual acetate at any time.

In a second series of tests, some MFCs have also been prepared with both chambers inoculated, keeping it in anaerobic conditions, dosing Cr(VI) at the cathode as a possible electron acceptor in place of oxygen, in order to be able to evaluate the reduction in MFC and to select electro-active biomass already chromium resistant. Cr(VI) was injected with concentration ranging from 1 to 5 mg/L. An inorganic carbon source is provided, $KHCO_3$ with concentrations 2 g/l, to force the selection of autotrophics microbial communities able to exploit the electrode as an electron donor. The behavior of the electroactive biofilm was also investigated in systems with sulphate and nitrate, species commonly present in groundwater, as different terminal electron acceptors in the cathodic chambers. 1 ml volume of nitrate solution was withdrawn on a periodic basis for kit (LCK339, range: 0.23 to 13.5 mg/L NO_3^--N). Similar procedure was applied to sulfate analysis (Hach Lange Kit, LCK339), but with 5 ml sample volume solution being taken each time.

Following the addition of substrate (sodium acetate) at the anode:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (19)$$

electroactive bacteria in the system started oxidizing the substate and transferring the resulting electrons to the anode. The electrons flow from the anode to the cathode through the external circuit where reduction reactions occur:

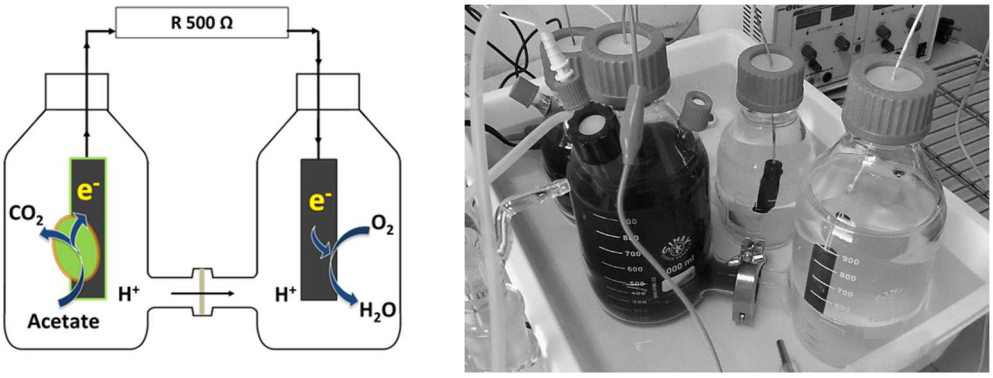
$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (20)$$

$$NO_3^- + 6H^+ + 6e^- \rightarrow 0.5N_2 + 3H_2O \quad (21)$$

$$SO_4^{2-} + 8H^+ + 8e^- \rightarrow S^{2-} + 4H_2O \quad (22)$$

The MFCs operated in a batch-fed mode at a constant temperature (18 ± 0.5 °C) and pH (7.4± 0.1). For ensuring a continuous mixing, the 2C-MFC was set over magnet stirrer. DO and ORP probes (or MARTINA analyzer) were inserted in the cathodic chamber to monitoring the parameters.
For the whole test, the voltage output across the external resistor was continuously recorded using a data logger (Picolog 1012, Pico Technology Ltd., UK, 12 channels); this allowed to monitor the produced current density with time.
The tests lasted about 15-30 days until stable maximum output voltage increased up to 220 mV (around 200-250 mA/m^2), which indicated bacteria have colonized the electrodes. The renewals allow a constant development of the selected biomasses; the best performing ones have been constantly monitored and renewed since over 2 years. Cyclic voltammetry, under a potentiostatic control, was done at the scan rate of 5mV/s and having a window below -0.6 and 0.6.

As summarized in Table 8.3, different experimental "set up" have been realized; each with duration monitoring time. Some Set UPs showed interesting performances in terms of biomass growth.



a) b)

Figure 8.4. MFC scheme (a) and set-up (b) used for experimental work. On the left is the anodic chamber containing inoculum; while on the right, it's the cathodic compartment with an electrode in an electrolytic solution.

Set up	General Information	Inoculum (anode)	Cathode
1	Graphite cylinder locked on stainless steel/titanium wire, MM1	AS	Abiotic, open to air (O_2)
2	Graphite cylinder locked on stainless steel/titanium wire, MM1.	SAD	Abiotic Open (O_2)
3	Graphite cylinder locked on titanium wire; "¼" MM1;	AS	SAD with Cr(VI), 1-5 mg/l. $KHCO_3$, 2g/l
4	Graphite cylinder locked on titanium wire; "¼" MM1;	AS	SI with Cr(VI), 1-5 mg/l. $KHCO_3$, 2g/l
5	Graphite felt on titanium wire, MM2	SAD	Abiotic with NO_3^- 100 mg/l
6	Graphite felt on titanium wire, MM2.	SAD	Abiotic with SO_4^{2-}, 150 mg/l
7	Graphite felt on titanium wire, MM2	SAD	Abiotic with aerator

Table 8.3. Set up.
SAD: Sludge from anaerobic digester (20% volume), AS: agricultural sludge, SI: inoculum derived from chromium contaminated soil;
"¼" MM1 – Minimal Medium concentration reduced by 75% to get closer to more representative conditions of real matrix.

The bioeletroactive anodes producted were used in Cr(VI)-reducing tests. When electrodes were used, at the same time, clean electrodes replaced those removed and the initial volume in MFC was re-established with fresh medium. As reported by Wu et al., (2018c), the acclimatization of the electrodes to the anode of an MFC is a practice of new experimentation, which allows the use of the electroactive inoculum in the cathode compartment.

8.2.3.3 Microbiology

To characterize the microbial communities, samples of the different inocula (SAD, AS and soil) and the anodic solution of the MFC were filtered on 0.45 µm sterile paper filters. The genomic DNA from filters was extracted using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The V5-V6 hypervariable regions of the 16S rRNA gene were PCR-amplified using the 783F and 1046R primers (Huber et al., 2007; Wang and Qian, 2009). The bacterial PCR was performed in 20 µL volume reactions with GoTaq® Green Master Mix (Promega Corporation, Madison, WI) and 1 µM of each primer. After the amplification, DNA quality was evaluated spectrophotometrically and DNA was quantified using Qubit® (Life Technologies, Carlsbad, CA). The sequencing was carried out at Consorzio per il Centro di Biomedicina Molecolare (CBM) Parco Tecnologico Padano (Lodi, Italy). Bioinformatics elaborations have been performed as previously reported (Palma et al., 2018). Classification of the representative sequences of each OTU was done using the RDP classifier (≥80% confidence) (Wang et al., 2007a).

A quality filter was applied to the sequencing results, removing those sequences that appear only once in the entire data set and instead of maintaining the sequences without alignment errors in the two readings (forward-reverse). The remaining

sequences have been grouped into Operational Taxonomic Units (OTUs); each OTU was made up of 97% identical sequences. For each cluster of sequences, a single representative sequence was identified, which, through RDP classifier, was categorized according to the taxonomic units (Phylum, Class, Order, Family, Genus). The relative abundances of each representative sequence and, therefore, of the belonging OTU on the total of the representative sequences of each sample were then calculated.

8.2.3.4 Analyses and calculations

Current was calculated as $I = \Delta V/R$, where ΔV [V] is the measured voltage across the external resistor, R [Ω] (Bruce E Logan et al., 2006). Current density [mA/m^2] was calculated as the ratio of the current recorded I [A] and the electrode's surface area.
MFC performance was evaluated by the Coulombic efficiency (CE) showing the fraction of the electrons obtained from oxidizable substrates, which are recovered at the anode, indicating the efficiency of the conversion of a substrate into electrical current. CE of the MFC, therefore, indicates the microbial conversion efficiency of the substrate into electric current. CE was calculated by assuming the dosed acetate as the only oxidizable substrate and sole electron source in the reactors:

$$CE = (M \cdot I \cdot \Delta t)/(F \cdot V_{an} \cdot b \cdot \Delta S) \quad (23)$$

where M (59 g mol^{-1}) is the molecular weight of acetate (CH_3COO^-), I [A] is the current recorded within time Δt [s], F the Faraday's constant (96485.3 Coulombs/mol of electrons), b is the stoichiometric factor (8 moles of electrons per mole of acetate), V_{an} [l] is the working volume of the anodic compartment, and ΔS is the substrate removed [g/l] within time. The consumed substrate was calculated by assuming the complete oxidation of acetate everytime recorded current was below 0.5 mA m^{-2}.

Tests were carried out to trace the polarization curve and the power curve, observing how the cell voltage (ΔE) and the power density (P) varies in function of the current density. The curves are useful to quantify the internal resistances of the system and also to evaluate the optimal value of the external resistance that maximizes the electric power produced by the MFC. The acquisition of the data was carried out under non-limiting substrate conditions, using resistances varying between 1 and 10000 Ω.
The developed biofilms were able to generate electric signals through oxidation of acetate. The performance of the bio-anode in reducing electron acceptors was assessed in term of current density, the coulombic efficiency as well as the rate of reduction of the final electron acceptors (oxygen, chromium, nitrate and sulfate).

8.2.4 Batch tests with poised biocathode and heterotrophic pre-enriched culture

In the tests, Cr(VI) removal was assessed in a 1,2 l biocathodic chamber of a double-chamber M3C.

An Ag/AgCl reference electrode (Hanna Instruments, 0.2 V vs. standard hydrogen electrode, SHE) was connected to the cathode using a Haber-Lugging capillary filled with saturated 3M KCl (+0.205 V vs. SHE). All measured potentials were relative to this reference, and then converted to SHE.
The cathodic solution, instead, was composed of MM1 or MM2; the anodic solution was the same as for the cathodic chamber in order to balance the charge.
An inorganic carbon source is provided, $KHCO_3$ with concentrations 1-2 g/l, to force the selection of autotrophics microbial communities able to exploit the electrode as an electron donor. Therefore, cathode function as the sole electron donor, selecting and exploiting only autotrophic microorganisms. The counter electrode chamber was filled up with the same volume of $KHCO_3$.
As summarized in Table 8.4, different experimental "Set up" have been evaluated; some tests showed interesting performances in terms of chromium removal.

SET UP	General Information	Cathode inoculum	Potential (mV vs SHE)	$K_2Cr_2O_7$ (mg/l), $KHCO_3$ (g/l)
1	MM1; AA; Graphite cylinder electrode	IPC from AS	-300	1, 2
2	MM1; AA; Graphite cylinder electrode	IPC+CEL from SAD	-300	1, 2
3	MM2; AA; Graphite cylinder (cathode) titanium rod (anode)	IPC+CEL from SAD	-300	1, 1
4	MM2; AA; Graphite cylinder (cathode) titanium rod (anode)	IPC from SAD	-300	1, 1
5	MM2; AA; Graphite cylinder (cathode) titanium rod (anode)	CEL from SAD	-300	1, 1
6	MM1; AA; Graphite cylinder electrode	IPC+CEL from SAD	700	1, 2
7	MM1; AA; Graphite cylinder electrode	IPC from AS	OC	1, 2
8	MM1; AA; Graphite cylinder electrode	IPC+CEL from SAD	OC	1, 2
9	MM2; AA; Graphite cylinder (cathode) titanium rod (anode)	IPC+CEL from SAD	OC	1, 1
10	MM2; AA; Graphite cylinder (cathode) titanium rod (anode)	IPC from SAD	OC	1, 1
11	MM2; AA; Graphite cylinder (cathode) titanium rod (anode)	CEL from SAD	OC	1, 1
12	MM1; AA; Graphite cylinder electrode	Abiotic	-300	1, 2
13	MM1; AA; Graphite cylinder electrode	Abiotic	-	1, 2

Table 8.4. M3Cs tests.
AA: Abiotic Anode; IPC Inoculum prepared with planktonic community; EAB: Electroactive Bacteria, previously prepared; CEL: Colonized Electrode, biofilm attached to electrode/graphite; SAD: Sludge from anaerobic digester, AS: sludge for agricultural reuse; carbon source HCO_3^- (1-2 g/l).

Using a acclimatization method similar to the one reported by Wu et al. (2016a), inoculum prepared with planktonic community (IPC) and/or colonized electrodes (CEL) were transferred into the cathodic chamber containing 1000 µg Cr(VI)/l, and operated between -300 mV and +700 mV (vs. SHE) by means of dual channel Arduino based potentiostat (Politecnico di Milano I3N–DICA, 2016 Cariplo-BEvERAGE).

The acclimatization method involves the transfer of a mature bioanode of a MFC to the cathode chamber of a MEC. The electrical inversion of an electrode with an electroactive biofilm without significant reductions in the efficiencies of electron transfer has already been reported in previous studies (Rozendal et al., 2008; Pisciotta et al., 2012, Zaybak et al., 2013). It has also been shown that some microorganisms are able to exchange electrons with the electrode in both directions (Yang et al., 2015; Xafenias et al., 2015).
The acclimatized bio-electrode was immersed in the polarized system working chamber filled with mineral medium, $KHCO_3$ (2 g/l) as the sole source of carbon, and K_2CrO_7 (Cr(VI) 1 mg/l). The working chamber housed an Ag/AgCl reference electrode (Hanna Instruments, 0.2 V vs. standard hydrogen electrode, SHE).
CEL were also used to prepare open circuit system (OC) as control; to compare biological Cr(VI) removal in bioelectrochemical and non bioelectrochemical systems, without the imposition of a WE potential.
In the tests in which CEL is used, the electrode is previously acclimatized as an anode in the MFCs; this operation reduces the period by microorganisms to colonize the electrode (Wu et al., 2015).
In tests in which IPC was added in addition to the biofilm on the electrode, the injected solution, 0.24 l of solution from the anodic chamber of the MFC, is properly prepared. To minimize the amount of dissolved organic carbon, the inoculum was first subjected to a washing procedure, consisting of a three series of centrifuge passage (10 min at 4000 rpm, Thermo Scientific) and resuspension of the pellet in fresh mineral medium. The solution containing the inoculum is added to the cathode chamber, obtaining initial OD600.
In configurations with only IPC or only CEL it was possible to observe different Cr (VI) removal efficiencies.
The OC systems, to assess the biological Cr(VI) reduction, were prepared exactly the same way of polarized systems without connecting the external circuit. Set UP 7-8 were conducted in parallel with reduction tests with imposed potential; the Set UP 9-11 instead were conducted with the same reactor and starting conditions deriving from the respective Set UP 3-5. These OC tests are therefore affected by the conditions changed over time during the previous cycles at set potential.
The abiotic control (Set UP 12) assess the effects of electrochemical reduction of hexavalent chromium. Some pure abiotic control, in which no potential was imposed to assess any absorption phenomena, also operated in parallel (Set UP 13). The configuration of the abiotic controls was the same as the polarized and OC batches; the solutions were sterilized in an autoclave twice (120°C for 30 min) before filling up the reactors.
Cr(VI) removal test and OC lasted respectively about 6 and 12 days, until more than 90% chromium removal was reached. Abiotic tests lasted 12-14 days until no changes in chromium concentrations were observed. So, through these different systems it was possible to compare the reduction of Cr(VI) exclusively by the electrochemical, biological and bio-electrochemical way. At the end of a reduction cycle; during some tests, a subsequent cycle was carried out in which potassium dichromate is re-dosed to

restore the Cr (VI) concentration to the initial value; moreover, 20% of the anodic and cathodic solutions are renewed.
In figure 8.5 M3Cs and related control at the end of the set-up phase.

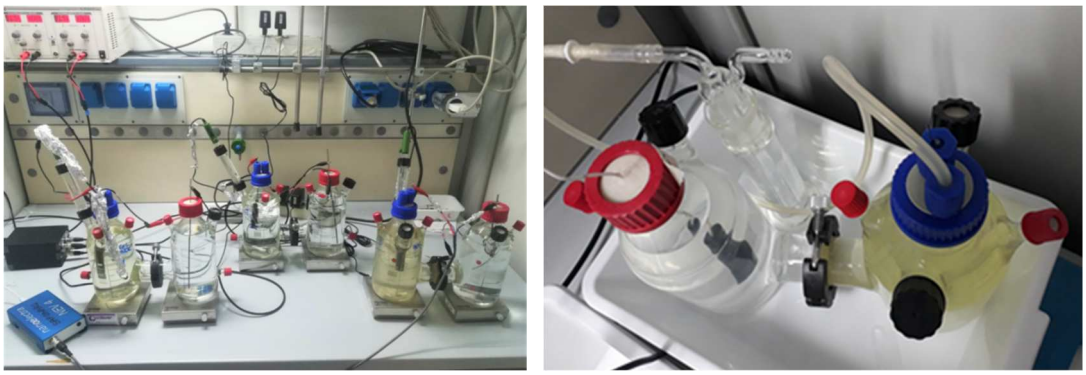


Figure 8.5. M3Cs, in thermostatic cell, at the end of the set-up phase.

To ensure the anaerobic conditions, the cathodic chamber of all the systems was blown with nitrogen for 15 minutes to favor the stripping of the oxygen in solution and in the head space of the reactor. All the reactors were kept in continuous mixing through magnetic stirrers and at a constant temperature of 18 °C in a thermostated room. Experiments were conducted at temperatures 18±0.5 °C.
In all the systems, samples were periodically taken from the cathodic solution for analysis. The sampling took place using syringes, with appropriate needles of sufficient length, through the lateral necks of the reactors equipped with appropriate plugs with pierceable baffles. To maintain the anaerobic conditions, a volume of nitrogen equivalent to the volume of solution taken was blown in each time a sample was taken.
Cr(VI) dissolved concentration was analysed at the beginning, during the experiment and at the end of the experiment. Aliquots were immediately 0.45 µm filtered and Cr(VI) dissolved concentration in all systems was analyzed by spectrophotometric method (APHA 3500-Cr D; C-APAT–IRSA 2003), while the dissolved total chromium was analyzed by ICP-MS (ISO 11083:1994). The amount of soluble Cr(III) was calculated, using a mass balance, as the difference between total soluble chromium concentration and that of hexavalent chromium. Detection limit of spectrophotometric method was 18 µg Cr(VI)/L.
Also OD600 was monitored during tests, to try to evaluate the growth (or decrease) of biomass.
pH and ORP measurements were performed both with the probe and with MARTINA analyzer.
In some tests the concentration of nitrogen compounds was checked: ammonium, nitrates and total nitrogen are quantified using the cuvette test. Instead, nitrites were stimated by the difference between total nitrogen and ammonium and nitrates. The initial ammonium in the systems is due to the presence of NH_4Cl deriving from mineral medium.
The current profiles were recorded using chronoamperometry.

8.2.4.1 Microbiology

At the beginning and at the end of the experiments, the microbial characterization of the bacterial communities enriched on the biocathodes and in the cathodic solutions was performed in collaboration with Milano Bicocca University. The biofilms attached to the polarized electrodes and OC graphite were scraped with a sterile scalpel obtaining graphite powder and biofilm. DNA from filters (0.45 µm sterile paper) was extracted by means of FastDNA® SPIN Kit for Soil (MP) and, subsequently, amplified by PCR using bacterial primers for the 16S rRNA gene with adapters for the Illumina platform (Illumina Miseq).
A quality filter was applied to the sequencing results and the remaining sequences have been grouped into Operational Taxonomic Units (OTUs); each OTU was made up of 97% identical sequences. For each cluster of sequences, a single representative sequence was identified, which, through RDP classifier, was categorized according to the taxonomic unit (Order). The relative abundances of each OTUs were calculated on the total number of OTUs of each sample.

To evaluate the electroactivity of biofilms already present or developed on the electrodes and the effects of the presence of Cr (VI), CV were conducted at various times of the tests. The experiment was done under a potentiostatic control, wherein three electrodes were used: a working, an Ag/AgCl reference and a counter electrode. Both working and reference electrodes were placed very close to each other in the anodic chamber. While, the counter was inserted in the counterpart. The first CVs were performed before and after the addition of Cr (VI) and were used as a reference for those at the end of the reduction cycles. Cyclic voltammetry experiment was done at the scan rate of 1-50 mV/s and having a window between -800 mV and +800 mV (vs Ag/AgCl). During the CVs the systems were kept in non-shaking conditions to avoid losing information on diffusion phenomena near the electrode.

8.2.5 Continuous flow test with poised bioelectrode and heterotrophic pre-enriched culture

The final phase of the experiment investigated a M3C system, similar to those previously batch-tested, except for the continuous feeding of the working electrode compartment, with the aim of stepping forward towards the complexities of real scale applications (Figure 8.6, 8.7).
To this purpose, through conductivity tests, a synthetic medium was selected. From these tests, the MM2 medium was reduced in ammonium amount to make it correspond to representative concentration for groundwater; a minimal medium with the following characteristics was used: 1238 mg/l Na_2HPO_4, 476 mg/l NaH_2PO_4, 81.5 mg/l KNO_3, 250 mg/l KCl, 210 mg/l NaCl (autoclaved twice at 120°C for 30 min).
The cathode chamber was inoculated with the biomass (both planktonic and the electrodic biofilm) from the anodic compartment of the MFC. Subsequently, through the potentiostat, it was possible to set the potential of the working electrode to –300 mV vs. SHE, and to monitor the circulating current. Reduction of Cr(VI) with time was checked by periodic sampling and analysis. The sampling of the solution is

conducted by a tap on the outlet tube, which connects the reactor to the collection tank. Under conditions of continuous flow, without recirculation, it is possible to wash away the biomass from the reactor; a three-way valve has therefore been set up on the delivery tube to perform a possible re-introduction of biomass.
A pump (Watson Marlow 313s) provided a continuous feed of synthetic Cr(VI) polluted solution to the system by continuously drawing water from a reservoir, consisting of a collapsible 2 l Tedlar bag. As regards the power supply, the system can be considered a semi-continuous, as it provides an anodic chamber in batch conditions and a work chamber fed in continuous flow.
In the tests, a hydraulic retention time of 3.5 days was imposed, and based on the geometric characteristics of the system, a flow rate of 0.120 mL/min was selected. This retention time allows operating with flow rates and speeds that not only come close to the typical conditions of the groundwater flow but also to the conditions used in previous experiences, reported in the literature, on continuous fed BES. The flow rate typically use ranged between 0.01-5 mL/min, depending on the type of contaminant and the configuration. For studies using double chamber Cr(VI) reduction (Li et al., 2017), the inlet flow rates vary between 0.04 and 0.117 mL/min, with HRT from 2 to 6 hours, since the reactor has very small size and a high ratio between the surface of the working electrode and the volume of the chamber.

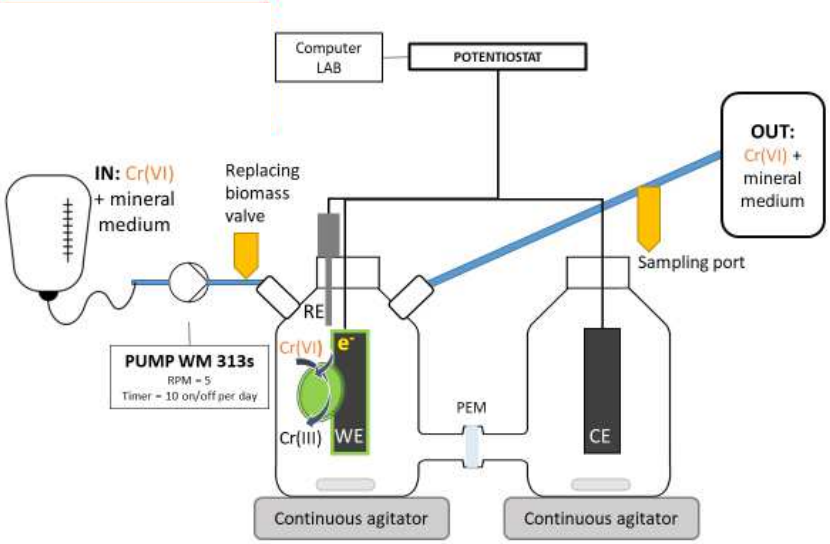


Figure 8.6. Continuous flow scheme. The configuration includes a feed pump positioned upstream of the cathode chamber between the inlet tank, consisting of a Tedlar bag, which contains 1 l of solution, and the reactor.

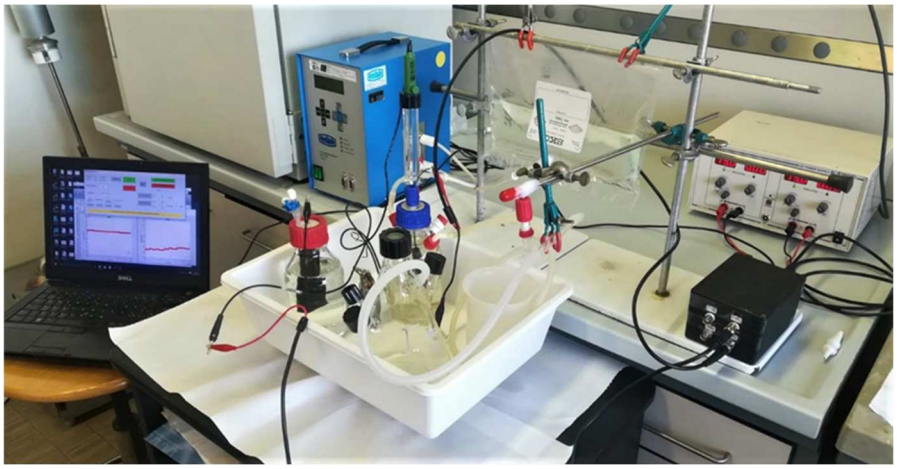


Figure 8.7. Continuous flow set up.

8.3 Results and discussions

8.3.1 Preliminary tests with chromium

In all the sorption and interference tests maximum deviation in chromium concentrations did not exceed 5%, largely within the margin of error of the analytical method, which is approximately 10%. Therefore, significant decrease in Cr(VI) concentration, due to the adsorption on the glassware and/or on the surface of the electrode and chemical reaction with mineral medium can be screened out.

In tests with applied voltage, the pH value diverged in few hours, reaching values as low as 2 in the anodic compartment, and above 11 at the cathode. Partial Cr(VI) reduction (about 30%) was observed. High voltage values brought to fast graphite oxidation and the anode disruption.
These observations highlighted electrochemical reduction is to some extent possible, but, unless pH control is performed, purely electrochemical removal is not characterized by high efficiency.

8.3.2 Aerobic conditions

From the results of the inoculum cultivation under aerobic conditions (about 30 days), it was evident that the bacterial activity was linked to the consumption of acetate, especially for the FF culture, whereas for the B5P culture, despite the addition of acetate at high concentrations, no significant aerobic activity, in term of DO consumption, was recorded. Furthermore, at the end of the tests the OD600 is only significantly increased, with values at 15 d and 30 d respectively equal to 0.055 and 0.095. Finally, the reduction in the concentration of Cr(VI) was no significant.
Enrichment with activated sludge, FF, showed aerobic bacterial activity and significant Cr(VI) reduction. Following two different chromium spikes (10 mg/l), the

residual concentration, within 15 d, was equal to 6-7 mg/l. Part of Cr(VI) maybe underwent a complexation process on the activated sludge flakes in the solution, so, the decrease with time of Cr(VI) concentration in 45 µm filtrate may not be univocally linked to effective Cr(VI) microbial reduction. Nevertheless, microbial activity observed in the respirometric tests confirmed the presence of a chromium-resistant community.

Therefore, the biomass from the FF enrichment was inoculated in BES batch tests under aerobic conditions and the associated OC control. The tests (Figure 8.8) resulted in a reduction of Cr(VI) concentration in time both in the BES system and in the OC; from the initial 10 mg/l Cr(VI), Cr(VI) was reduced in 20 days to about 4 mg/l and 5.7 mg/l, in BES and OC respectively.

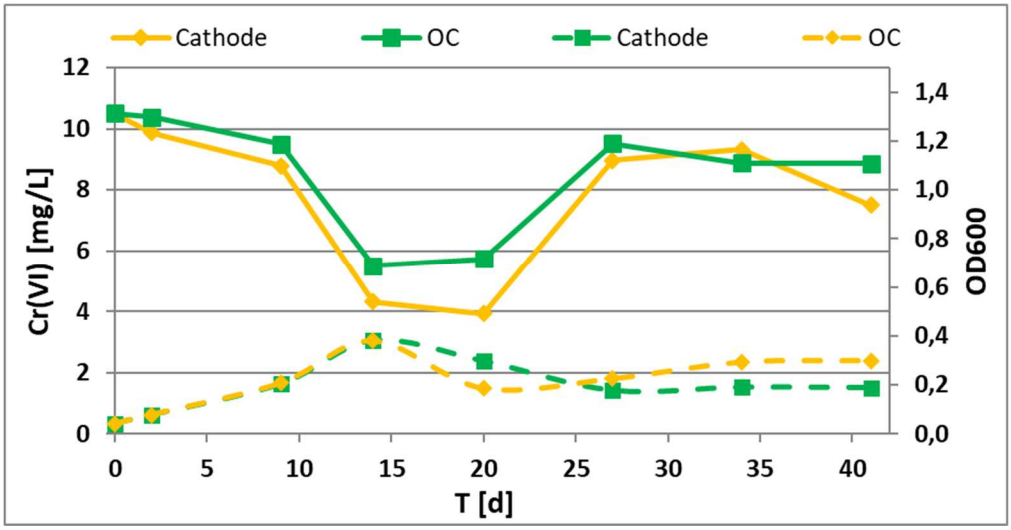


Figure 8.8. Chromium concentration trends (continuous line) and OD600 (dashed line), for the BES (in yellow) and OC system (in green). After 20 days, mineral medium was renewed and Cr(VI) up to 10 mg/L added; and ignition of the ventilation.

The greater removal in the BES in comparison to the OC system is probably linked to the electrochemical Cr(VI) reduction, not active in the OC system, summing up to the effects of the microbial activity. The biological activity is evident from the DO profile showing a fast decrease below the 2 mg/l threshold because of aerobic oxidation of the acetate in the mineral medium, and diffusion of atmospheric O_2 into the system was not sufficient to ensure aerobic conditions throughout the test.
Increase with time of OD600 is linked to the growth of the biomass in both the BES and OC. Similar tests under forced aeration showed very low reduction efficiencies. The reduction shown in BES is therefore more attributable to fermentation processes that may have occurred during the acetate consumption phases during which very low DO levels were recorded. This means that the reduction of Cr(VI) occurred only when anaerobic conditions were locally reached within the systems. This conclusion leads to a BES that contributes with the electrochemical action rather than the EAB biomass

action. Furthermore, there does not seem to be a correlation between the reduction of Cr (VI) and the production of current that remains very low and constant during tests (1-20 µA). This BES system therefore approaches a biological induced system, with the advantages, however, of greater local control than the injection and dosage in the aquifer. The presence of organic substance remains indispensable in order to promote the reduction of chromium in appropriate conditions and to develope the biomass at the cathode. In similar tests, as part of that shown in the figure, it can be observed that the development of biomasses in the cathodic compartment is rather slow. At the new chrome spike and with aeration, biomass growth is slowed.
Another factor that influences the process, and in particular the batch test is the pH increase: in a basic environment the presence of the hexavalent form is more favored.

Therefore, in strictly aerobic conditions, the bacteria use oxygen as a final electron acceptor and, at the cathode, there is no evidence of a significant reduction in the concentration of Cr (VI); the slight removal of Cr (VI) recorded both in the BES and in the open circuit control is probably due to complexation within the biomass. For these reasons, given the difficulties in selecting a biomass characterized by chromium-reducing bacteria, it was decided to set up tests in anaerobic conditions, trying to select a more EAB, capable of exchanging electrons with the electrode and using Cr (VI) as final electron acceptor, thus favoring its reduction to Cr (III). Moreover, fixing a proper set potential of the working electrode can positively influence the biofilm, by regulating the energy available for bacterial growth (Luo et al., 2014).

8.3.3 EAB developement in MFC

A relationship between substrate availability and current production was observed, as rapid increase in the circulating current was recorded after acetate addition, carried out anytime the current density has dropped below 0.5 mA/m^2, corresponding to an almost complete depletion of the substrate. Coulombic Efficiency (CE) trend suggests that an electroactive biofilm, capable of oxidizing acetate and transferring electrons to the anode, is attached and developed on the electrode.

In Set UP 1-2, after an initial phase of a few days, the voltage increases and, through consecutive acetate spikes, a maximum of 178 mV (0.36 mA) and 220 mV (0.44 mA) were respectively reached, indicating the formation of a biofilm on the anode (Figure 8.9). In Set UP 2 tests current density in the 2C-MFC ranged between 0.5 and 237 mA/m^2. Following the first two additions of acetate, current densities were recorded with peak values of 160 and 38 mA/m^2, respectively. Instead, in the two following cycles current density reached a maximum of 227 and 232 mA/m^2 respectively. In the cycles following the transfer of the bioeletroactive anodes producted, with part of the anodic solution renewed (usually 20-40 % of the analyte volume), the maximum current density varied from a minimum of 229 mA/m^2 in the fifth cycle to a maximum of 237 mA m^{-2} in the last cycle. In the first two cycles, unlike subsequent cycles, the peak values of current density were recorded only for short periods (10-15 min). In subsequent cycles, instead, there has been a rapid increase in the current, which has reached and maintained its maximum value for a period varying from a minimum of 1 day (in the fifth cycle, after bioanodes transfer the MECs) to 4.5

days (third cycle). This difference in the trend of the recorded currents suggests that an EAB has attached and developed on the electrodes, capable of transferring electrons from the oxidation of the acetate directly to the electrode. This hypothesis is supported by the calculation of the CE in each cycle, which shows a marked increase; starting from negligible CE at the beginning of the test, during the enrichment period there was an increase in both Set UP; efficiencies of 28% and 18% are achieved respectively for Set UP 1 and Set UP 2. In Set UP 2 CE shows a marked increase between the second (0.5%) and third cycles (18%). Between the fourth and fifth cycles a reduction in the coulombic efficiency is observed, from 16.5% to 5%, respectively, due to the renewal of 40% of the medium. It is possible to notice that the coulombic efficiencies can decrease over time, during the following peaks, as long as acetate avaibility for the biofilm on the electrode is reduced by developing alectroactive microorganisms in solution. Generally, part of the electrons, released during the acetate oxidation reaction, has been used by non-electro-active species, which do not increase the circulating current, and therefore with a negative effect on CE. For example, it is known that methane is the natural end product of microbial activity in most anaerobic environments and therefore methanogenesis represents an important microbiological process to be considered to evaluate the performance of BES, and MFCs in particular. Methanogens, that is microorganisms that convert organic material into methane, compete with EAB for the organic substrates. Thus, unless the methane formed can somehow be reoxidized and then reused by electroactives, the methanogenic activity reduces the amount of electrons available to the anode and therefore the current produced. Recently, it has been shown that EAB in an anode can overwhelm the methanogens using acetate as electron donor, while the use of different substrates, for example glucose, tends to increase the production of methane (Rozendal et al., 2008).

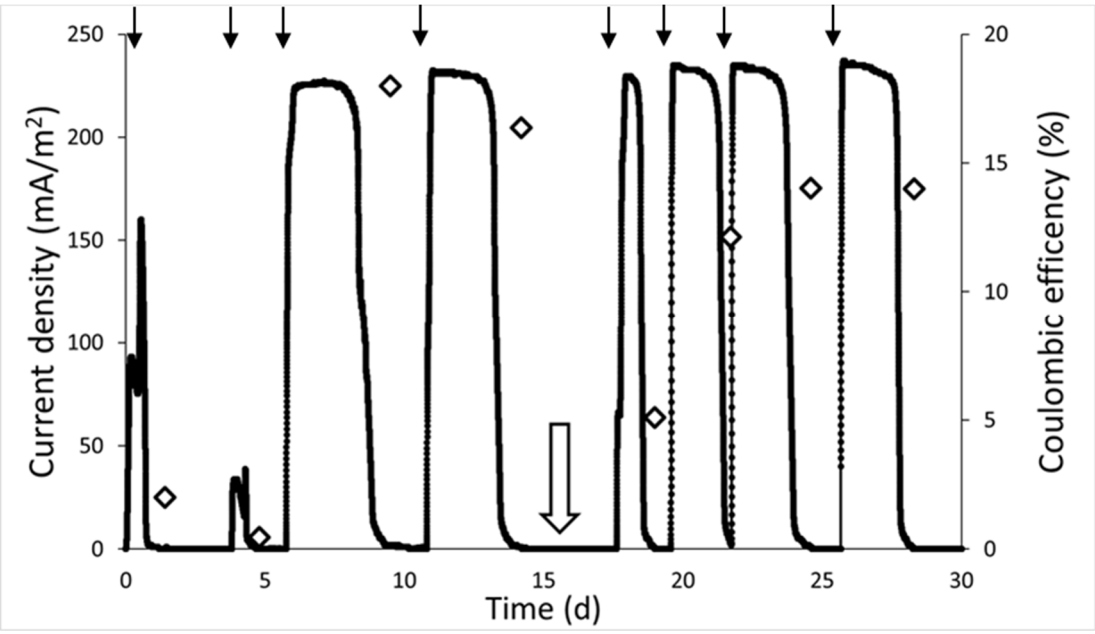


Figure 8.9. Current density and Coulombic efficiency during the anode acclimation phase in 2C-MFC, Set UP 2. Current density (black line) produced by electroactive

biofilm after periodic acetate addition (black arrows) until the solution was renewed (white arrows) (40% replacement of anolyte) and two of the three electrodes used for chromium tests. The CE (empty diamond) was calculated at the end of each current peak.

For Set UP 2 the polarization curve (Figure 8.10) identify an OCV equal to 0.45 V, which, due to internal losses, is lower than the theoretical value of the E_{emf}^{0}, equal to 1.4 V. With the increase of circulating current, the distance between the theoretical value and the recorded one, increase too (Korneel Rabaey and Keller, 2014). For linear polarization curves, the value of the internal resistance of the MFC is equal to the angular coefficient (Logan et al., 2006); in the test as example, assuming a linear trend, the value of the internal resistance would be equal to 940 Ω. The optimal system for the acclimatization of electro-active biomass on graphite cylinder electrodes, must operate by imposing resistances between 500-1000 Ω.

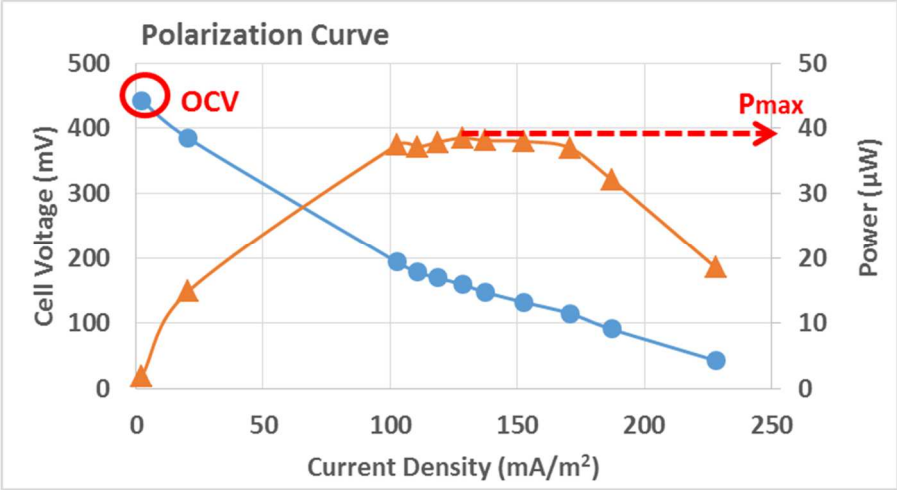
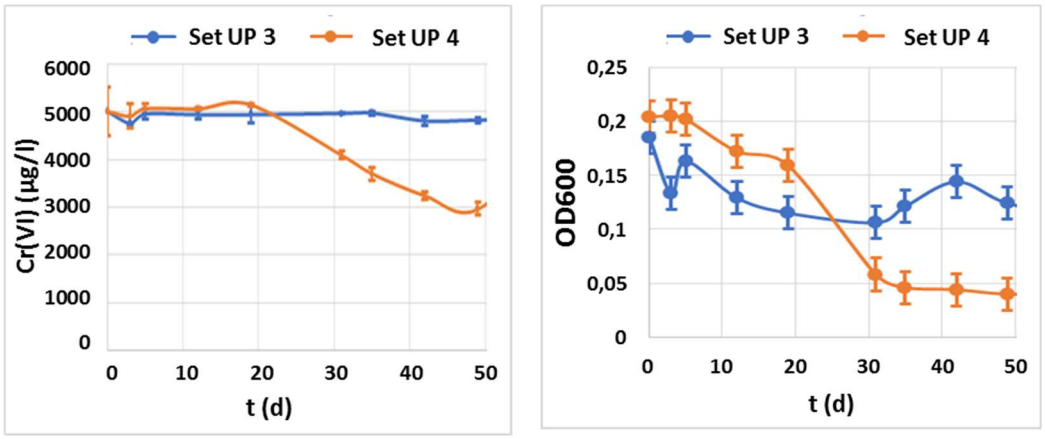


Figure 8.10. Polarization Curve realized for MFC, Set UP 2.

No significant voltage variations were recorded in the tests carried out with MFC and chromium-containing cathodes (Set UP 3-4); therefore it was not possible to calculate the CE at the anode. It can be concluded that no significant development of EAB were observed, not only able to reduce hexavalent chromium but also to grow up.

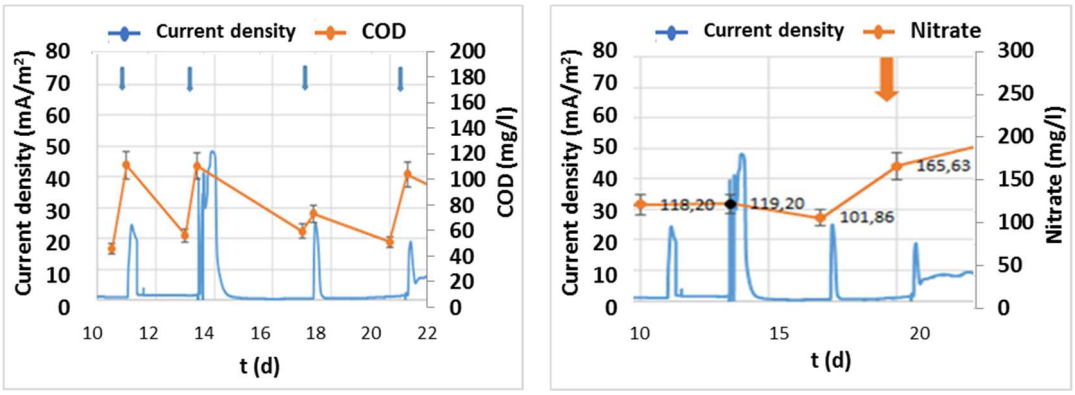
Variable and limited 3-40% chromium removals were observed in 50 days of monitoring starting from initial concentrations of 1-5 mg/l and using two different inoculum (SAD, SI) (Figure 8.11). A reduction in OD600 in the cathode chamber at the was observed during a test conducted with an initial concentration of 5 mg/l. Redox potential also showed a fluctuating trend: a negative peak of the redox potential (-350 mV vs AgAgCl).



a b

Figure 8.11. Cr (VI) concentration (a) and optical density trends in MFC systems during 50 days of monitoring.

In the case of the system with nitrates as terminal electron acceptor (Set UP 5), the current generated during cycles was relatively low. Unlike the second cycle, there is a high current density being recorded (Figure 8.12), reaching a maximum of 49 mA/m^2 in spite of the same of amount of acetate addition (50 mg/l).
In spite the significant amount of electrons released to the cathodic compartment of nitrate's system, their influence in reducing nitrate was limited.

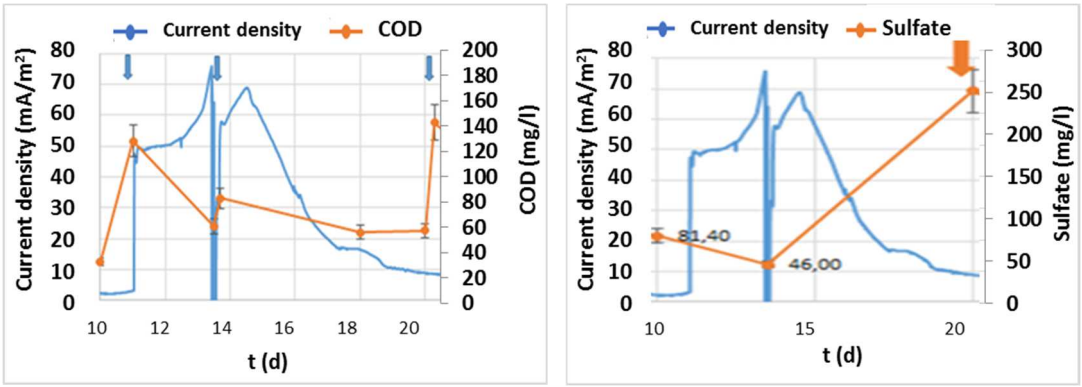


a b

Figure 8.12. Set UP 5, Current density and COD (a), nitrate concentration (b) during tests. Blue arrows (acetate spike); red arrow (nitrate spike).

The system with sulfate (Set UP 6) produced higher current densities (Figure 8.13), with 78 mA/cm^2 peak value. The current density did not reach zero in this phase of the experiment because the concentration of acetate was comparatively high. Because of this, fewer additions (three) of acetate were done on the system.

The concentration of sulfate decreased during cycles. With addition of sulfate through a spike in the BES, was observed that concentration reduced as the current density increased.



a b

Figure 8.13. Set UP 6, Current density and COD (a), Sulphate (b) during tests. Blue arrows (acetate spike); red arrow (sulfate spike).

To discuss a comparison, the first cycle of the various systems is considered (Figure 8.14); there is a high level of current generation in all system. This production of current density varied in the different system when compared. The system of sulfate, nitrate and oxygen produced the currnet density of 47.5 mA/m^2, 47.2 mA/m^2 and 19 mA/m^2 , respectively. This could be linked to the contration of different oxidants in each cathodic chamber since the system of sulfate, nitrate and oxygen was initially 150 mg/l, 100 mg/l and 6 mg/l, respectively.

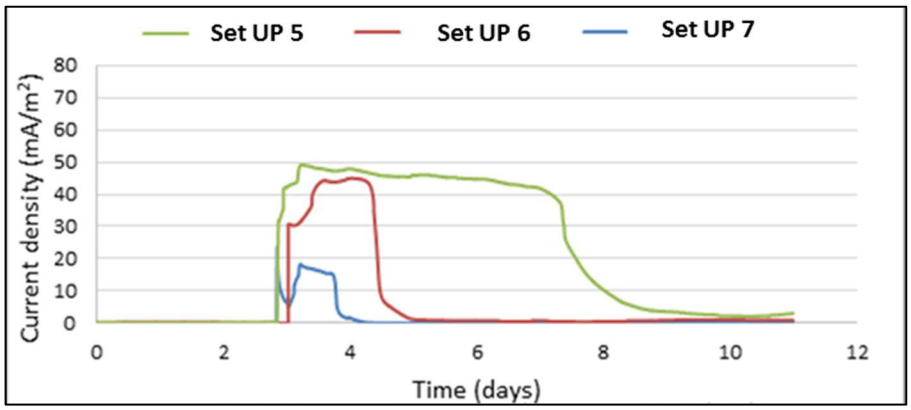


Figure 8.14. Current density trend with different electron acceptors. Comparison of the generatted current density during the first cycle of the different systems.

DO at 1mg/l could be the limiting factor since the influence of oxidant (oxygen) concentration on the performance of MFCs depends on the current density. This is due to the fact that DO becomes a limiting factor.

8.3.3.1 Microbiology

The results of the microbiological analyses showed an evolution from anaerobic digester community compared to the communities founded after 16 d during Set UP 2 (Figure 8.15, 8.16).

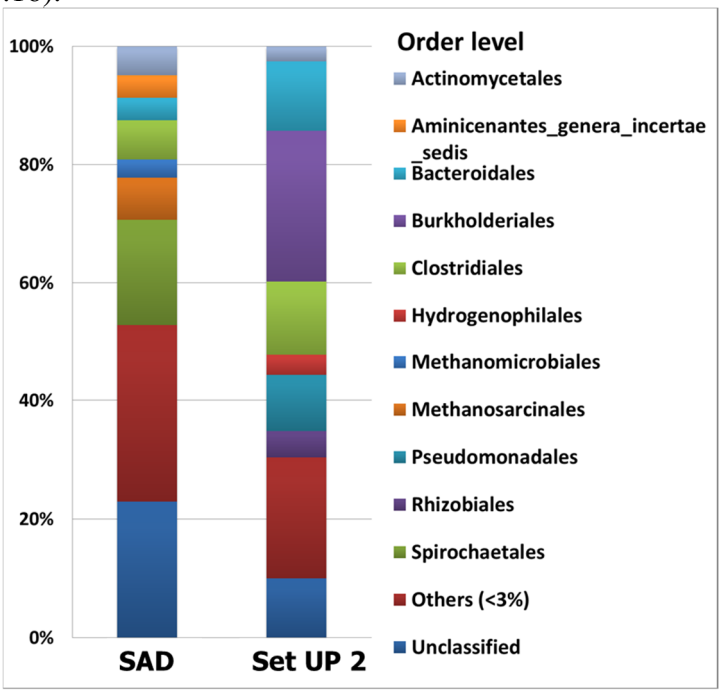


Figure 8.15. Anaerobic digestor's community (SAD) and electroactive anode selection during Set UP 2 tests. Relative abundance at the order level. "Others < 3%" indicated strains with abundance less than 3%.

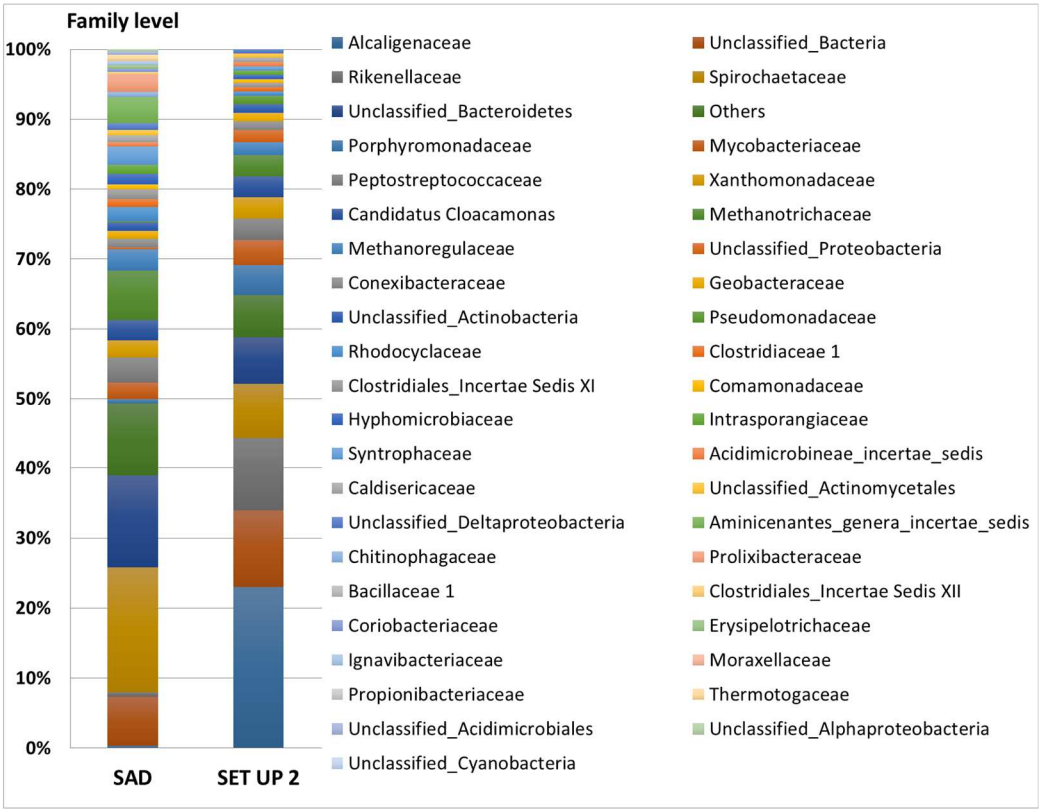


Figure 8.16. Anaerobic digestor's community (SAD) and electroactive anode selection during Set UP 2 tests. Relative abundance at the family level.

It is possible to notice an increase in the relative abundance of bacteria belonging to the orders *Burkholderiales* (about from 1 to 25%), *Bacteroidales* (from 3.1 to 11.7%), *Pseudomonadales* (from 1 to 9.4%).
Representatives of the *Burkholderiales* (*Alcaligenaceae, Comamonadaceae), Bacteroidales (Porphyromonadaceae,), Pseudomonadales (Pseudomonadaceae)* orders have been previously described as bacteria able to perform electrochemical interactions with the anode (Joicy et al., 2019; Read et al., 2010; Saratale et al., 2017; Sotres et al., 2015). In particular *Alcaligenaceae,* that grow up more than 120%, have been previously described as bacteria capable of transferring electrons to the anode (Barbosa et al. 2018; Kumar et al. 2017b; Lu et al. 2017). This confirms that the increase in CE over time has been promoted by the selection of an EAB community. The availability of substrate and the possibility of exploiting the anode as a final electron acceptor favored EAB; conversely other heterotrophic microorganisms reduced or remained unchanged in the solution, such as the order of the *Clostridiales* (from 10.4 to 12.6%).
Conversely almost complete reduction in terms of relative abundance was observed fot bacteria belonging to the orders *Spirochaetales, Methanosarcinales* and *Methanomicrobiales.* Bacteria belonging to the order Spirochaetales have already been observed within heterotrophic anode communities and seem to be related to efficiency

losses (Rimboud et al. 2015). The reduction of methanigens is synonymous with the reduced development of secondary reactions such as methanogenesis.

8.3.4 Tests handled with poised cathode and heterotrophic pre-enriched culture

The following figure 8.17 shows the trends of residual concentrations in the first week of sampling for different Set UPs considered.

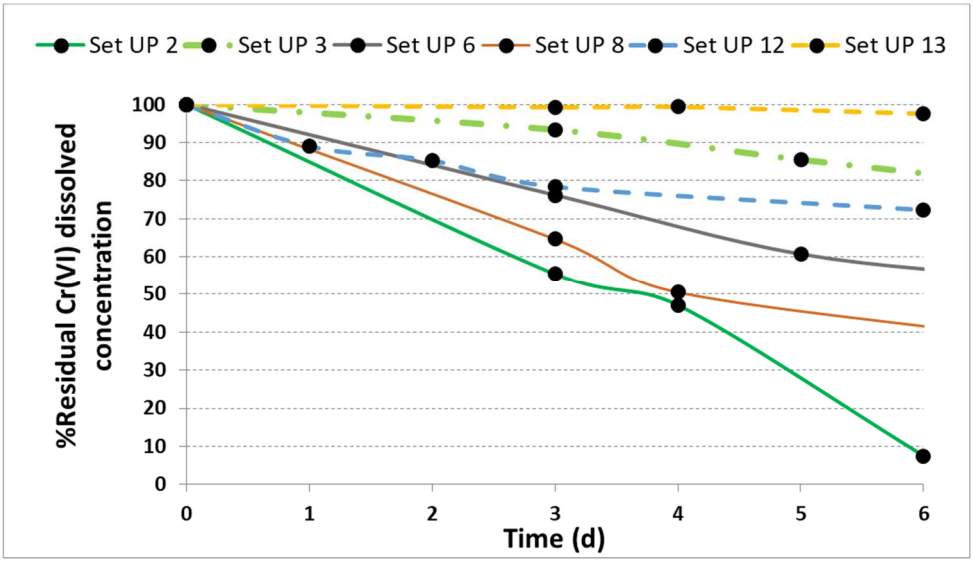


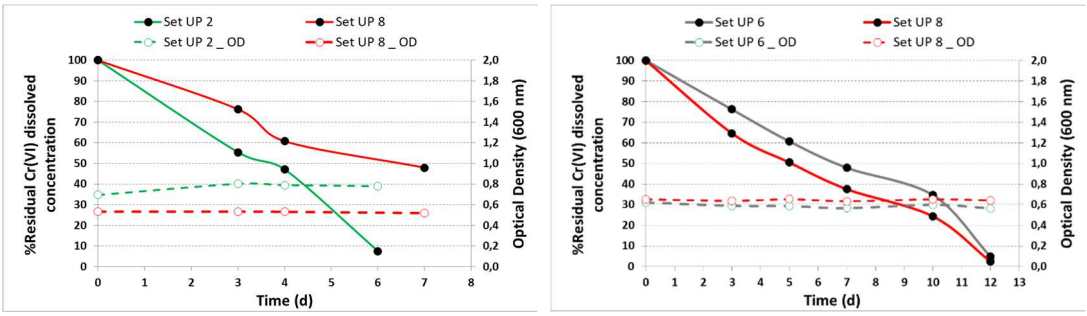
Figure 8.17. Cr(VI) residual concentration (%) for different Set UP tests during first 6 days.

The main considerations are:

- best efficiencies (Set UP 2) are related to a BES compared to OC or abiotic systems.
- efficiencies can be greatly reduced when changing the conditions of the mineral medium used for the test (Set UP 2 vs Set UP 3). The variability of the redox and chemical conditions of an aquifer can therefore influence the efficiency of the process.
- the purely electrochemical reduction of Cr (VI) (Set UP 12) showed an important decrease in the chromium concentration; however, the Cr (VI) reduction stopped at about 30%. In fact the reduction is less thermodynamically favored at neutral pH, due to lower potential and slowing down the reaction rates. The precipitation of Cr (III) on the electrode, as reported in literature (Li et al., 2008), may passivate the electrode, preventing further reduction of the dissolved Cr (VI).
- operating with a suitable potential at the cathode is decisive; the cathodic potential influenced Cr (VI) removal efficiency (Set UP 2 vs Set UP 6).

- purely biological reduction conditions can guarantee significant reductions (Set UP 2 vs Set UP 8).

The most rapid decrease in Cr(VI) concentration was observed at the end of the Set UP 2, with -300 mV vs SHE polarized biocathode. In fact, after only six days, the residual chromium concentration was equal to 7% of the initial concentration (92.1±13.2 µg/l). In the same time, in the OC control (Set UP 8) the dissolved chromium concentration was around 45% (Figure 8.18a). The high Cr(VI) removal efficiency was probably ascribed to the selection of a bacterial community containing electro-active and/or Cr(VI) reducing/resistant bacteria.

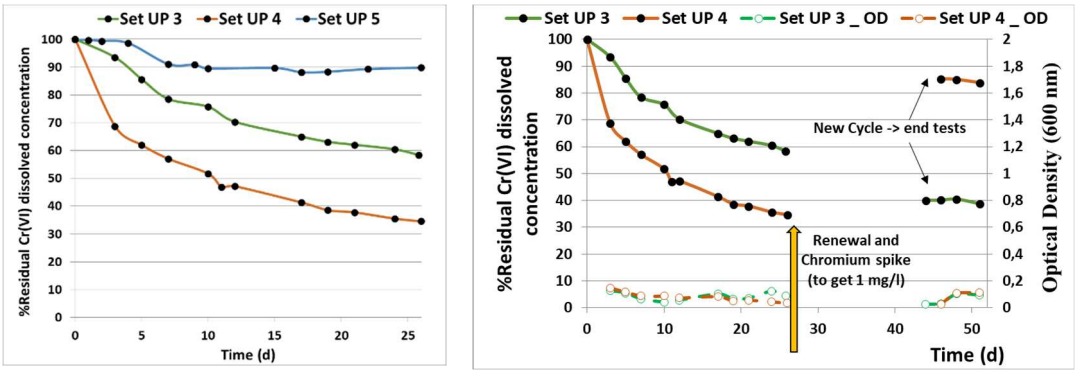


a b

Figure 8.18. Cr(VI) concentration (black symbols) and optical density trend (white symbols).

The cathodic potential influenced the microbial community structure, affecting Cr(VI) removal efficiency. From the beginning of the test until about the tenth day, a more rapid removal of the Cr(VI) was observed in the OC than in the M3C with biocathode poised at 700 mV vs SHE (Figure 8.18b). Residual chromium dissolved concentration at ten days were, in fact, 38% and 55% in OC and M3C, respectively. In the last sampling, at twelve days, however, the percentage of residual dissolved chromium is practically the same in both systems (5%). In the abiotic control (Set UP 13), no significant changes were observed after 12 days of test.
In the OC control, the optical density is almost stable from the beginning at the end of the tests. In the biocathode poised at 700 mV vs SHE, a sensible reduction in OD600 was observed over 12 days, from the initial value of 0.66 to the final value of 0.57; while a slight increase was observed in the M3C with the more efficient poised cathode, between 0.66 and 0.78. This trend suggests that the biocathode set at -300 mV has enhanced microbial growth more than in the OC.

From the comparison between Set UP 3-5 (Figure 8.19), conducted with MM2 and a reduced biomass it is observed that the best efficiencies are obtained in the presence of both IPC and CEL.



a b

Figure 8.19. Cr(VI) concentration (black symbols) and optical density trend (white symbols).

With the presence of the only cathode colonized the microorganisms required an initial acclimatization period for significant reductions in chromium. With batch conditions and less buffer capacity of the solution in the test pH values increase up to values around 9. The system (Set UP 5) is most affected by pH changes because it has no biomass in the reactor. These conditions correspond to an environment less adapt to the reduction of chromium. The M3C with only IPC vice versa, has high efficiency in the first cycle, even higher than Set UP 3. The higher efficiency, equal to 65% in 25 days, may be due to the simultaneous presence of purely electrochemical mechanisms to the electrode. Possible electrode inefficiencies such as lack of biofilm development or passivation, however, result in a reduced removal for the systems at the end of the new reduction cycle (Figure 8.19b). In general, for all the systems the efficiency of removal of hexavalent chromium tends to decrease over time; among the causes nutrient depletion and the progressive increase of the pH in such batch tests conducted with reduced buffer capacity.
The OD600 is also reduced in the tests conducted in the Set UP 3, however, the bioelectrode makes up for this loss.
In the Set UP 9-11 no significant reductions in the concentration of hexavalent chromium were observed. The removal of Cr (VI) by biological route is a minority component; in these tests the pH remained almost constant with values around 8, so a lower tendency for reduction processes. The optical density in the Set UP 9-10 showed a decreasing trend, in line with what was observed in the potential-imposed tests.

In systems, weakly negative currents in the order of 10^1-10^3 µA have generally been observed. The recorded current data are often difficult to evaluate, as the low values are disturbed by numerous environmental interferences (PCs, lights, stirrer) that prevent appreciating variations or significant trends over time.

In BES systems with the presence of ammoniacal nitrogen in mineral medium the development of nitrifying biomass is possible. Looking, for example, at the graphs relating to Set UP 3, it can be seen that there is clear nitrate formation over time (Figure 8.20). The peak, positive for ammonium and negative for nitrates, at the end of the first cycle is due to the renewal of 20% of the volume of the cathodic solution. The

nitrification process can be conducted biologically from genera of bacteria such as *nitrosomonas* and *nitrobacter*; the first oxidize ammonium to nitrites, the second nitrites to nitrates (Anthonisen et al., 2014). Once the oxidation of ammonium is complete, nitrates begin to be used as electron acceptors and therefore to be reduced. In light of these results it has been hypothesized that the oxidation of ammonium by the ammonium-oxidant bacteria may have favored the reduction of Cr (VI). Due to the high concentration of initial ammonium it is possible the reduction of Cr (VI) can also take place indirectly as well as via bioelectrochemistry, ie by exploiting the electrons supplied by the ammonium oxidation.

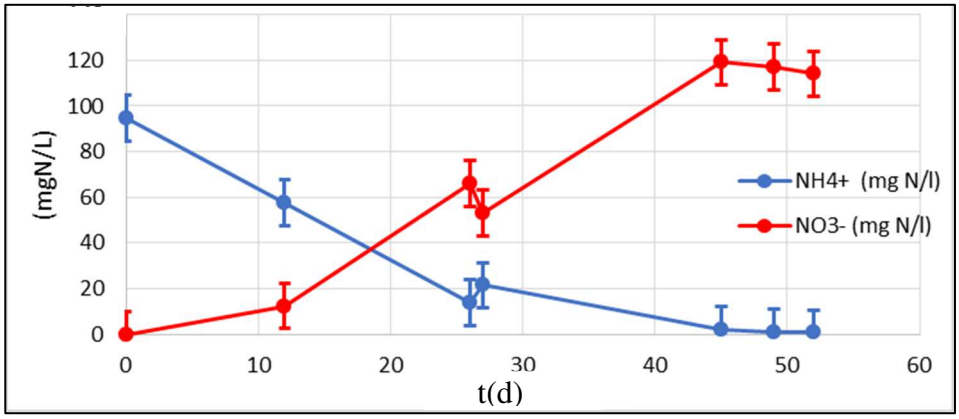
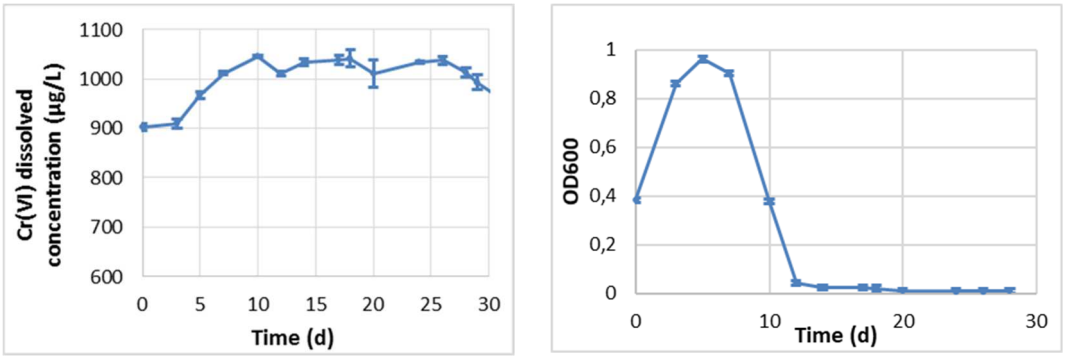


Figure 8.20. Ammonium and nitrate trend during a test (Set UP 3).

The monitoring of cathodic currents and the results of chemical analyzes, during the continuous flow test, did not allow to observe a significant bioelectrochemical removal of Cr (VI) (Figure 8.21). However, this system has provided useful information to the development and research on this technology, also in consideration of the limited number of continuous BES studies for the removal of Cr (VI) reported in the literature.



a b

Figure 8.21. Cr(VI) concentration and optical density trend during the test.

From the analysis, the continuous system has not shown an ability to appreciably remove Cr (VI) in the reactor. Throughout the continuous test, the concentration values remained constant around the value of 1 mg/l, with minimal variations mainly related to the analytical uncertainties and the dosage of Cr (VI) in the inlet tank. It cannot be excluded that the incomplete mixing of the reactor may have led to the creation of preferential flow paths. During the tests, there was a drop in the optical density mainly due to the loss of biomass in the outflow. The analyzes showed very high initial OD600 values due to the high loss of biomass. The decrease in biomass was also visible on the electrode, with a loss of the biofilm. The pH measurements performed on the samples have always provided values of 7.8±0.1. This result confirms the choice of the mineral medium, which showed a good buffer capacity.

8.3.4.1 Microbiology

The microbial communities, from the beginning to the end of the M3C tests, are reported for some Set UP (Figure 8.22, 8.23).

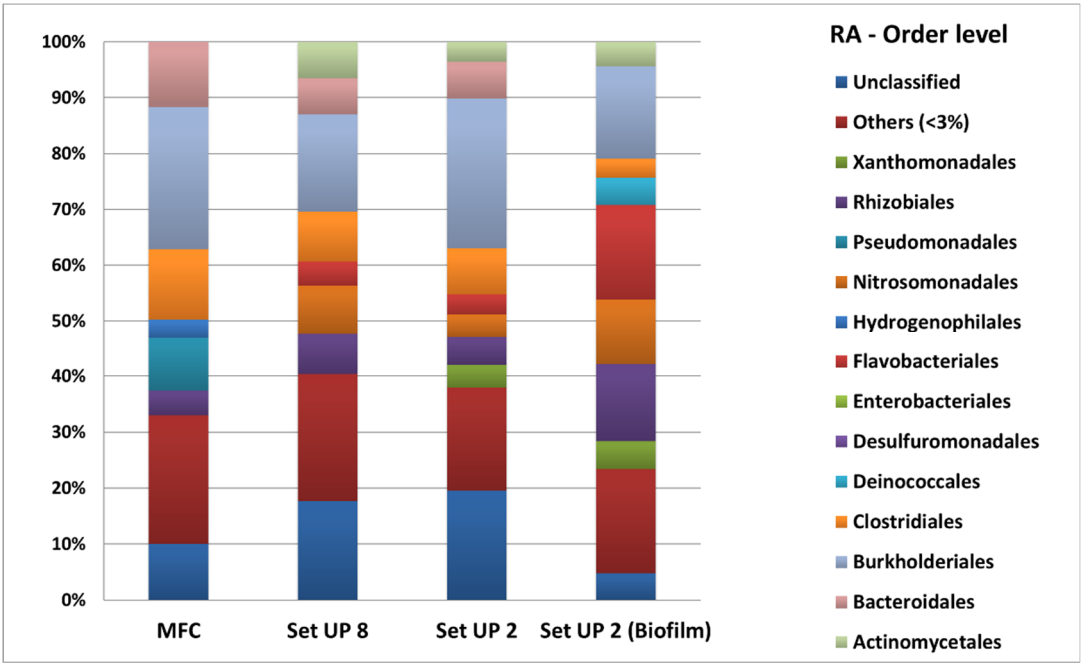


Figure 8.22. Bacterial community structure at the order level. "Other orders < 3%" indicated strains with abundance less than 3%.

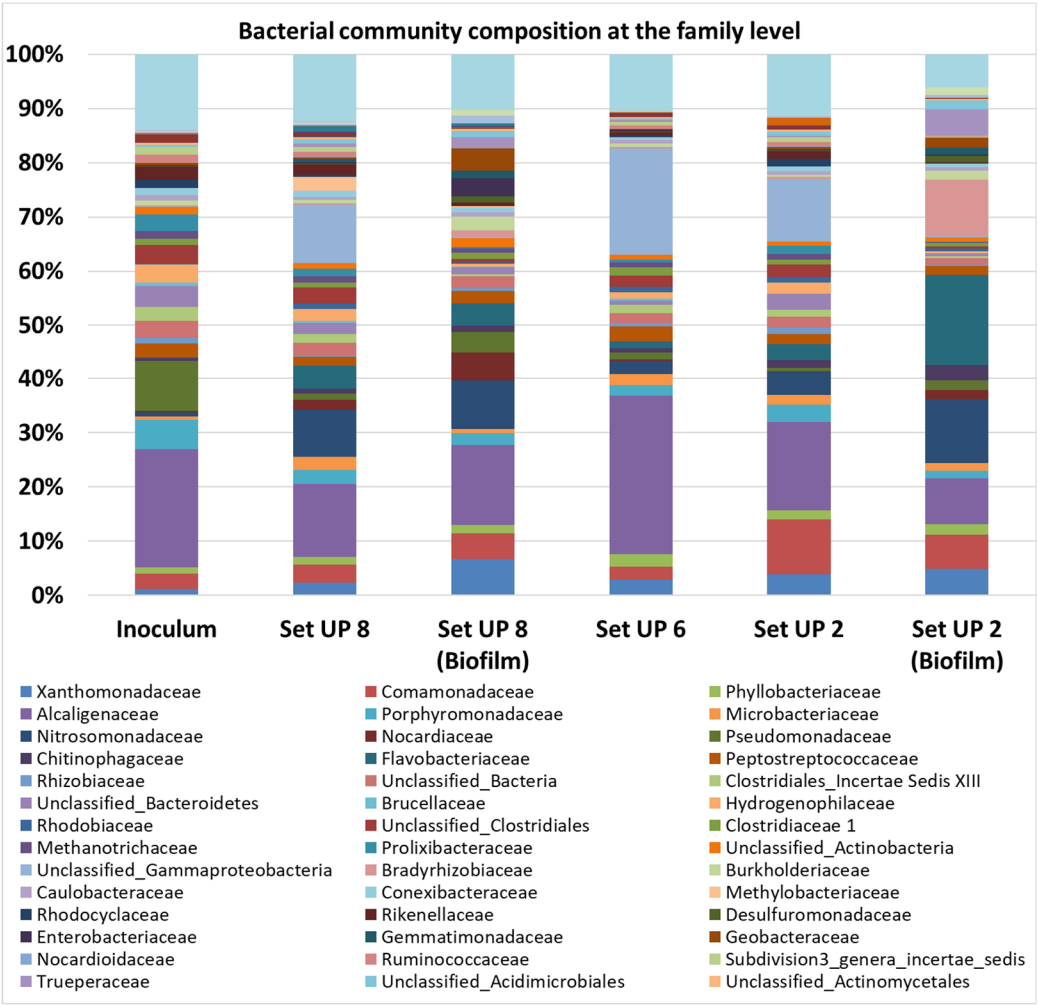


Figure 8.23. Bacterial community structure at the family level. Relative abundance at the order level.

The EAB community used in 2C-M3C tests was found to be dominated by Proteobacteria (26% *Burkholderiales*, 9% *Pseudomonadales* and 4% *Rhizobiales*), Firmicutes (13% *Clostridiales*) and Bacteroidetes (12% *Bacteroidales*). The addition of Cr (VI) and carbonates, as the sole carbon source, influenced the suspended microbial community.

Compared to the inoculum derived from MFC anode, both in polarized (Set UP 2) and in OC systems (Set UP 8), *Pseudomonadales*, *Bacteroidales* and *Clostridiales* reduced their relative abundances, by about 80%, 50% and 30% respectively. Viceversa, *Flavobacteriales* order showed relative abundance increase, from 0.3% to, respectively, 3.6% and 4.4% for polarized and OC systems.

In OC, an increase in the relative abundance of the *Nitrosomonadales*, from <1.5% to 8.8%, was observed.

On the other hand, in polarized systems there was a slight increase in the orders *Burkholderiales* due to a net increase in the population of *Comamonadaceae*, from

2.8% to 10%. In the same system also relative abundance of *Xanthomonadales* increased respect to the inoculum.
Bacteria belonging to *Burkholderiales* order (*Comamonadaceae* and *Alcaligenaceae* families) are already reported in other studies as EAB, chromium tolerant/resistant and autotrophic microrganisms (Fernandez et al., 2009; Im et al., 2018; Paiva et al., 2015). Furthermore, bacteria belonging to these two families have been observed in BESs both for the removal of inorganic compounds, such as H_2S and NO_3^- under autotrophic or mixotrophic conditions (Im et al., 2018; Khanongnuch et al., 2018) and for Cr(VI) bioreduction (Morel et al., 2016; Thatoi et al., 2014).
The lack of organic electron donors, such as acetate, has influenced the structure of the suspended bacterial community, suggesting a possible advantage for autotrophic microorganisms (*Nitrosomonadales* and *Flavobacteriales*) able to use carbonates and ammonium in the mineral medium as carbon source and inorganic electron donor (Herrmann et al., 2017).

The results of 16S rRNA gene sequencing of the biofilms developed were compared and it was deepened on a genus level (Table 8.5).

Order Genus	OC graphite (%)	POL -0.3 V electrode (%)
Burkholderiales	22.1	16.5
Advenella	14.2	8.1
Ralstonia	2.6	1.6
Polaromonas	1.5	< 1.5
Flavobacteriales	4.1	16.9
Moheibacter	3.9	16.6
Nitrosomonadales	9.0	11.8
Nitrosomonas	8.8	11.8
Rhizobiales	5.0	13.7
Nitrobacter	< 1.5	9.8
Actinomycetales	9.0	4.4
Rhodococcus	4.8	1.5
Xanthomonadales	6.8	5.0
Stenotrophomonas	2.9	3.1
Clostridiales	5.0	3.4
Clostridium XI	1.7	1.8
Desulfuromonadales	5.2	2.9
Geobacter	4.1	1.8
Bacteroidales	3.1	1.7
Petrimonas	1.6	< 1.5
Pseudomonadales	4.1	1.9
Pseudomonas	2.9	< 1.5
Sphingobacteriales	1.7	2.9
Acidimicrobiales	< 1.5	1.7
Deinococcales	1.7	4.9
Truepera	1.7	4.9
Enterobacteriales	3.4	< 1.5
Escherichia/shigella	3.4	< 1.5
Other orders < 1.5%	11.7	7.8
Other genera < 1.5%	29.2	22.7
Unclassified order	8.0	4.6
Unclassified genus	16.4	16.3

Table 8.5. Relative abundance at order and genus level of the biofilm developed on graphite of the OC system (Set UP 8) and on polarized electrode (Set UP 2).

On the polarized bioelectrode bacteria belonging to the orders *Flavobacteriales* (16.9% compared to 4.1% in OC graphite), *Rhizobiales* (13.7% compared to 5% in OC graphite), and *Deinococcales* (4.9% compared to 1.7% in OC graphite) were more abundant; instead the same were almost absent in the planktonic communities (*Flavobacteriales* 3.6-4.4%, *Rhizobiales* <1.5%, *Deinococcales* <1.5%).
The only genus detected within the *Flavobacteriales* order was *Moheibacter*. The sharp increase in relative abundances of this genus on the polarized bioelectrode, as well as in other electroactive biocathodic communities as reported in the literature

(Liao et al., 2018; Sun et al., 2018), suggest this genus is involved in the transfer of electrons from an electrode. The only genus detected within the *Rhizobiales* order was *Nitrobacter*. Bacteria belonging to the *Nitrobacter* genus are known for oxidizing nitrite to nitrate and were previously observed in autotrophic biocathodes for nitrate removal (Huang et al., 2013; Xiao et al., 2015). The whole *Deinococcales* order was constituted by *Truepera* genus. This genus has been previously described in electroactive cathodic communities also consisting of microorganisms belonging to the genera *Moheibacter* and *Nitrosomonas* (Sun et al., 2018; Liao et al., 2018). Although an ecological relationship has not yet been defined, the abundance of these genera on the polarized bioelectrode suggests they were advantaged compared to OC system.

CV on the BESs reported (Figures 8.24, 8.25) are those carried out at scanning speeds of 5-10 mV/s, as they were more significant and less affected by interference in the case of low-speed scans or rough approximations in the opposite case. In fact, at high speeds the representative peaks of the reactions may be less evident. The graphs were obtained by averaging the data of several cycles through a moving average; generally, the second and third cycles. The first cycle, of practice, is discarded as it is more affected by errors.

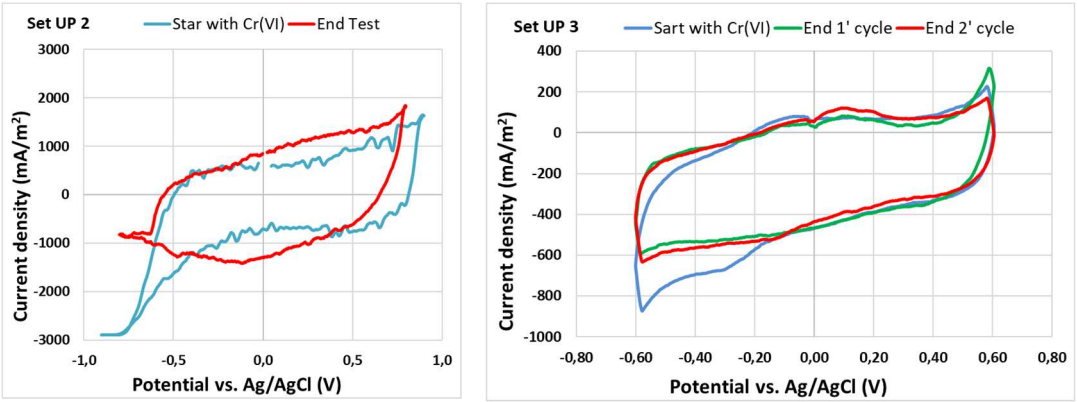


Figure 8.24. Comparison between CV at the initial time and at the end of the chromium reduction cycles, the scanning speed is 10 mV/s for the Set UP 2 and 5 mV/s for the Set UP 3.

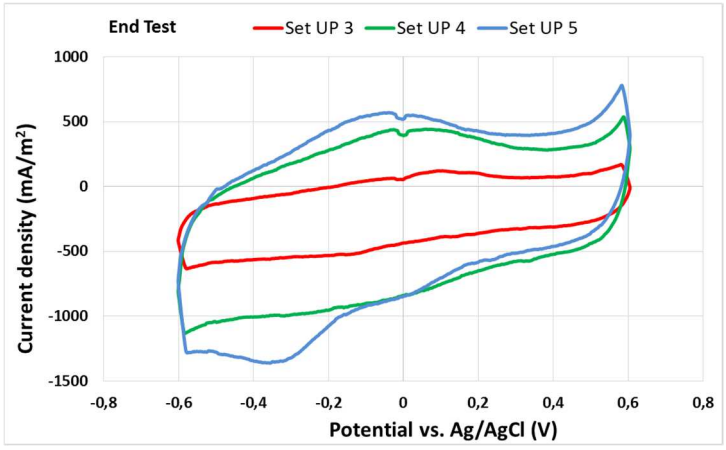


Figure 8.25. Comparison of CV at the end of the reduction cycles for the Set UP considered, scanning speed 5 mV/s.

From the graphs shown, and in general from what emerged in the studies conducted on CVs, some considerations:

- peaks of between -600 and -400 mV (vs Ag/AgCl) can be associated with the presence and reduction of chromium which generates a cathodic current; this aspect is described in similar CV reported in the literature (Xafenias et al., 2013). The presence of a not particularly marked peak can be linked to the low residual concentration of hexavalent chromium at the end of the test compared to the initial condition in the reactor.
- in order to observe representative peaks of the redox conditions of the reduction tests it was excluded the identification of reactions, such as the hydrolysis of water peak that can be highlighted around -700 mV vs AgAgCl.
- from the comparison of the scans at different speeds there is an increase in the currents as the scanning speed increases, as foreseen by the theoretical principles relating to CVs; the increase in scanning speed, from 5 mV/s to 10 mV/s, does not seem to provide further information about the reactions taking place within the analyzed systems.
- the tests can provide useful information about the actual presence of EAB species. In fact, in the CVs of the Set UPs conducted with the presence of biomass, greater currents have been recorded, compared to the abiotic control; the electro-active biomass, draws electrons from the cathode or uses the cathode as an electron donor, while in the abiotic system the passage of current is exclusively related to chemical factors.
- by comparing the CVs made at the end of the tests relating to the Set UP 4-5 it is possible to appreciate significant differences in currents produced also as a function of the residual chromium; the planktonic biomass in the solution does not seem to affect the trend of the CV which is more related to the operating electrode.

- a greater cathodic background current, compared to the initial scan, can be attributed to the growth of the EAB community, as evidenced by corresponding OD600 measurements.

8.4 Conclusion

Results reinforce earlier works (Huang et al., 2011b; Lovley, 2011; Xafenias et al., 2013) that suggest the potential of biocathode to stimulate Cr(VI) reduction in contaminated waters. The chance of using Cr(VI) as a terminal electron acceptor for a biocathode in a BES has been investigated, at the laboratory scale. Moreover, this experimental work exploring the possibility of effective reduction in groundwater at lower Cr(VI) concentrations than wastewater so far investigated (Chapter 4).
In some preliminary tests chromium reductions have been observed in BES where inocula were used directly at the cathode. Efforts were concentrated in the investigation of the possible mechanisms of a biocatode which operates with an active role with respect to a reduction deriving only from a bio-induced process.
Due to relatively long time for the EAB to colonize the electrode, an acclimation phase at the anode of MFCs was performed. Then, Cr (VI) reduction was investigated using a BES in M3C configuration; the biocathode inoculum derived from MFCs, transferring the planktonic solution and/or directly an electrode with the developed biofilm. $KHCO_3$ was provided with 1-2 g/l concentrations, as inorganic carbon source. Tests were carried out under anaerobic conditions with biocathode poised between -300 to +700 mV vs SHE.
The fastest chromium removal was observed in M3C poised at -300 mV vs SHE compared to other potential and purely electrochemical and biological control. The purely electrochemical reduction stopped at about 30%; the precipitation of Cr(III) may passivate the electrode, preventing further reduction of the dissolved Cr(VI).
Thanks to the presence of an electroactive biofilm it was possible to observe more than 90% hexavalent chromium reduction at neutral pH. Conversely, in previous studies, the electrochemical reduction of Cr(VI) was found to be strongly dependent on the pH of the cathodic solution (An et al., 2014; Wang et al., 2008).
High Cr(VI) removal efficiency in the M3C-300 was probably ascribed to the selection of a bacterial community containing electro-active and/or Cr(VI) reducing/resistant bacteria. Community analysis suggested that known EAB families were able to adapt under M3C conditions; autotrophs like *Alcaligenaceae* appear to be favored. *Bradyrhizobiaceae, Trueperaceae* and *Flavobacteriaceae* were selectively enriched on the polarized cathode biofilm; the microbial consortium lends a contribution for high removal of chromium to the biocathode.
Although BESs require further laboratory testing and scale up, the use of bioelectrochemical systems for removing hexavalent chromium is a new, sustainable and promising approach for remediation of water polluted with Cr(VI).

CONCLUSIONS

Groundwater resources are highly vulnerable to hexavalent chromium contamination. The main causes of the presence of Cr(VI) in soil and groundwater are related to industrial activities and incorrect waste management.

Alternatives to the complete removal of Cr(VI) contaminated soil or groundwater aim to decrease the environmental and health risks by reducing Cr(VI) to Cr(III). One available option, in-situ bioremediation, relies on the capabilities of Cr(VI) redaction of several microrganisms. Bioremediaton technologies for Cr(VI) include bio-induced reduction, that consists in the stimulation of autochthonous microbial activity by injection of readily biodegradable organic substrates to promote reducing conditions conducive to Cr(VI) reduction and precipitation.

The possibility of using by-products of the food industry as substrates for bacterial growth has been investigated. Lab scale tests under bio-induced conditions, carried out in batch systems, were performed to select proper organic substrates, to study the kinetics of the process and to evaluate iron involvement in chromium precipitation. The highest Cr(VI) abatements were obtained in microcosms containing waste from the brewing process and soil which had the higher total heterotrophic bacteria concentration. Fe(II) availability was also a key factor in Cr(III) co-precipitation. It was necessary to reach stable redox potentials below -200 mV vs SHE to observe a reduction in the Cr (VI) concentration. The use of inexpensive byproducts in comparison with registered brand reagents can be an interesting alternative approach. In particular, the features of selected substrates are injectability, non-toxicity and non-recalcitrance. Interesting prospects could be related to reduced costs and overall environmental impacts; therefore, injection of this substrate into real aquifer could potentially be applicable. Further tests under anaerobic conditions should be carried out for a comparison with registered brand reagents, to understand their possible placement within the remediation technologies market.

Injection, however, has peculiar limitations, such as the difficulty of appropriately defining dosages and frequency of injection according to site-specific conditions. Furthermore, with bio-induced reduction, chromium precipitates throughout the whole contaminated area with no concrete chance of recovering the metal, which possibly undergoes partial re-oxidation with time. Acceptance, by the institutions and decision-makers, of the diffusion of the by-products in the aquifer is another item of discussion that could prevent the diffusion of the technology if not adequately studied.

Bioelectrochemical systems, investigated for over a decade principally for wastewater treatment, have been recently proposed as an option for groundwater remediation as well. By using electrodes as virtually inexhaustible electron donors and acceptors to

promote microbial oxidation-reduction reactions, BESs should offer the advantage in in-situ remediation of avoiding the addition of the substrates. Electrosynthesis tests conducted for the production of organics from inorganic carbon, propose a new path, to be investigated further, for the use of cathodes to heterotrophically promote in-situ Cr(VI) reduction. Moreover, local control of the processes can also allow the recovery of the contaminated matrix.
Batch tests with poised electrode and heterotrophic pre-enriched culture, to shorten the time required for EAB development, have been performed. The effectiveness of this system was tested in comparison to pure microbial and pure electrochemical control, an initial concentration of Cr(VI) of 1 mg/L was applied and no organic substance was added during the chromium removal phase. Tests, operating in M3Cs, showed a decrease in Cr(VI) both in the polarized reactors and in the control systems. The M3C operating at -300 mV vs SHE with the bioelectrode polarized, showed a faster rate than other systems with a removal efficiency over 90% in 6 d. The characterization of the microbiological communities, carried out using molecular techniques, suggested that known EAB families were able to adapt at M3C conditions. The results showed that the bacterial community was affected by the polarization, and there were differences from the biomass developed in the open circuit control.
BES treatment looks like a sustainable approach in terms of environmental impact, management and costs. Even though positive results have been reported with abiotic cathode systems, biocathodes have been shown to offer advantages in Cr(VI) reduction from the perspective of groundwater remediation, above all in terms of their effectiveness in the typical soil/groundwater pH range and the exploitation of microbial catalysis, limiting cathode passivation due to Cr(III) precipitation.

In conclusion, with the aim of improving cost-effectiveness and sustainability in hexavalent chromium-contaminated groundwater remediation, biological mechanisms are interesting. At the lab-scale, technologies tested were competent in reducing Cr(VI) with initial concentrations 1-10 mg/l. Bio-induced processes, more consolidated in engineering terms reaching full scale implementation, provide electron donors to ensure bacterial metabolism and reduction of chromium. The criteria proposed do not recommend the technology when the presence of iron in the soil matrix is scarce. Moreover, the injection is not effective in the presence of different lithologies and heterogeneity limits. In similar conditions, BESs could be a possible alternative, taking into account the possible application through PRB. Applying the criteria, BES technology may not be suggested only in the presence of high concentrations of chromium. Until now, BES testing at pilot plant level is still scarce; for the technology scaling-up, long term studies and modelling are required in order to explore all the open issues and identify feasible approaches to full potential BES exploitation in groundwater remediation. It is expected that evaluated in situ technologies can be integrated into remediation projects for the resolution of groundwater contamination. Often P&T remain in function for a long period, without the possibility of favorably closing the administrative procedure, with consequent cost increases and the necessity of continuous monitoring.

ACKNOWLEDGMENTS

This work was financially supported by

- Eupolis (Institute for research, statistics and training of the Lombardy Region) - Analisi e promozione di nuove tecnologie di bonifica e di caratterizzazione dei siti contaminati. Codice: ter 13010/001, 2015.
- Fondazione Cariplo in the framework of the project BEvERAGE-BioElEctrochemical RemediAtion of Groundwater plumes (2015-0195).

BIBLIOGRAPHY

Aachib M., Mbonimpa M., Aubertin M. (2004). Measurement and prediction of the oxygen diffusion coefficient in unsatured media, with applications to soil covers. Water, air, soil pollution 156: 163-193. Kluwer Academic Publishers.

Accornero M., Marini L., Lelli M. (2010). "Prediction of the thermodynamic properties of metal-chromate aqueous complexes to high temperatures and pressures and implications for the speciation of hexavalent chromium in some natural waters". Applied Geochemistry. 25: 242–260.

Ackerley D.F., Gonzalez C.F., Park C.H., Ii R.B., Matin A., Keyhan M. (2004). Chromate-Reducing Properties of Soluble Flavoproteins from Pseudomonas putida and Escherichia coli. Am Soc Microbiol. 70(2):873–82.

Agenzia per la Protezione dell'Ambiente e per i Servizi Tecnici (APAT) (2006), Manuale per le indagini ambientali nei siti contaminati.

Agenzia per la Protezione dell'Ambiente e per i Servizi Tecnici (APAT) (2008), Criteri metodologici per l'applicazione dell'analisi assoluta di rischio ai siti contaminati.

Ahn, J. S., Chon, C. M., Moon, H. S., & Kim, K. W. (2003). Arsenic removal using steel manufacturing byproducts as permeable reactive materials in mine tailing containment systems. Water Research, 37(10), 2478–2488. http://doi.org/10.1016/S0043-1354(02)00637-1.

An Z, Zhang H, Wen Q, Chen Z, Du M. (2014). Desalination combined with hexavalent chromium reduction in a microbial desalination cell. Desalination. Elsevier B.V.; 354:181–8.

Anelli S., Bellotti M., Saponaro S., Sezenna E., Trozzi A. (2013). Relative risk assessment for prioritization of contaminated sites at regional scale, Proceedings of AquaConSoil 2013 — 12th International UFZ-Deltares Conference on Groundwater-Soil-Systems and Water Resource Management, 16—19 April 2013 Barcelona, Spain.

Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S., & Srinath, E. G. (2014). Inhibition of nitrification and nitrous acid compounds. Water Pollution Control Federation, 48(5), 835–852.

Apte, A.D., Verma S., Tare V., and Bose P. (2005). Oxidation of Cr (III) in tannery sludge to Cr(VI): field observations and theoretical assessment. Journal of Hazardous Materials. 121: 215-222. doi:10.1016/j.jhazmat.2005.02.010.

ARPA Piemonte, 2012. Activities in the management of groundwater monitoring network - Monitoring period 2009-2011: Proposal for classification of the state of quality of groundwater bodies as requested in the Decree 260/2010, Agenzia regionale Protezione Ambiente del Piemonte.

Ashraf, M.A.; Hussain, I.; Rasheed, R.; Iqbal, M.; Riaz, M.; Arif, M. (2017). Advances in Microbe-Assisted Reclamation of Heavy Metal Contaminated Soils over the Last Decade: A Review. J. Environ. Manage. 198, 132–43.

ASTM Committee (2012). Standard guide for Direct-Push groundwater sampling for environmental site characterization. D6001-05. ASTM International: West Conshohocken, PA, USA.

ASTM Committee (2013). Standard guide for development of groundwater monitoring wells in granular aquifers. D5521-05. ASTM International: West Conshohocken, PA, USA.

Aulenta F., Catervi A., Majone M., Panero S., Reale P., Rossetti S. (2007). Electron transfer from a solid-state electrode assisted by methyl viologen sustains efficient microbial reductive dechlorination of TCE. Environmental Science & Technology 41(7): 2554–2559.

Aulenta F., Majone M., Tandoi V. (2006). Enhanced anaerobic bioremediation of chlorinated solvents: environmental factors influencing microbial activity and their relevance under field conditions, J. Chem. Technol. Biotechnol. 81(9):1463–1474.

Aulenta F., Tocca L., Verdini R., Reale P., Majone M. (2011). Dechlorination of trichloroethene in a continuous-flow bioelectrochemical reactor: effect of cathode potential on rate, selectivity, and electron transfer mechanisms, Env. Sci. Tech. 45(19): 8444-8451.

Aulenta, F., Canosa, A., Reale, P., Rossetti, S., Panero, S., Majone, M. (2009). Microbial reductive dechlorination of trichloroethene to ethene with electrodes serving as electron donors without the external addition of redox mediators. Biotechnology and Bioengineering 103(1): 85–91.

Aulenta, F., Harnisch, F., (2017). Editorial Microbial electrochemical technologies: maturing but not mature 10–11. https://doi.org/10.1111/1751-7915.13045.

Aulenta, F., Lai, A., Simone, M., Majone, M., Verdini, R., (2016). Bioelectrochemical CAHs dechlorination. N. Biotechnol. 33, S132–S133. https://doi.org/10.1016/j.nbt.2016.06.1183.

Azzellino, A., Colombo, L., Lombi, S., Marchesi, V., Piana, A., Merri, A., Alberti, L., (2019). Groundwater diffuse pollution in functional urban areas: The need to

define anthropogenic diffuse pollution background levels. Sci. Total Environ. 656, 1207–1222. https://doi.org/10.1016/j.scitotenv.2018.11.416.

Bae W.C., Lee H.K., Choe Y.C., Jahng D.J., Lee S.H., Kim S.J., Jeong B.C. (2005) Purification and characterization of NADPH-dependent Cr(VI) reductase from Escherichia coli ATCC 33456, J. Microbiol. 43(1):21–27.

Bajracharya S, (2016). Microbial Electrosynthesis of Biochemicals. Innovations on biocatalysts, electrodes and ion-exchange for CO2 supply, chemicals production and separation. PhD thesis, Wageningen University, Wageningen, NL. ISBN 978-94-6257-853-1.

Baldi F., Barbieri P. (2008). Microrganismi e metalli, microbiologia ambientale ed elementi di ecologia microbica, Milano - ita, casa editrice ambrosiana, vol. unico, pp. 181-205. (ISBN 9788808184344), 10278/22405.

Barbosa, S.G., Peixoto, L., Soares, O.S.G.P., Pereira, M.F.R., Heijne, A. Ter, Kuntke, P., Alves, M.M., Pereira, M.A., (2018). Influence of carbon anode properties on performance and microbiome of Microbial Electrolysis Cells operated on urine. Electrochim. Acta 267, 122–132. https://doi.org/10.1016/j.electacta.2018.02.083.

Bardiya N., Bae J.H., (2011). Dissimilatory perchlorate reduction: A review. Microbiol. Res. 166, 237–254. https://doi.org/10.1016/j.micres.2010.11.005.

Barrera-Díaz C.E., Lugo-Lugo V., Bilyeu B. (2012). A review of chemical, electrochemical and biological methods for aqueous Cr(VI) reduction. Journal of Hazardous Materials Elsevier; Jul 15, p. 1–12.

Bartlett R.J. (1991). Chromium Cycling in Soils and Water: Links, Gaps, and Methods. Environmental Health Perspectives Vol. 92, pp. 17-24.

Bartlett, R.J., B.R. James, J.O. Nriagu, and E. Nieboer. (1988). "Mobility and Bioavailability of Chromium in Soils." In J.O. Nriagu and E. Nieboer (Eds.), Chromium in the Natural and Human Environments, pp. 267–303. John Wiley & Sons, New York.

Battaglia-Brunet F, Foucher S, Denamur A, Ignatiadis I, Michel C, Morin D. (2002). Reduction of chromate by fixed films of sulfate-reducing bacteria using hydrogen as an electron source. J Ind Microbiol Biotechnol. Mar;28(3):154–9.

Bekele T.C., Bucklin K., Burger S., Martinez L., Parnass B., Renzi E., Rodriguez M., Wong, P. (2011). Investigation and Remediation of Plating Facilities. Technical Resource Guide. USEPA, Department of Toxic Substances Control (DTSC). Sacramento, CA, USA.

Belchik S.M., Kennedy D.W., Dohnalkova A.C., Wang Y., Sevinc P.C., Wu H., (2011). Extracellular reduction of hexavalent chromium by cytochromes MtrC and OmcA of Shewanella oneidensis MR-1. Appl Environ Microbiol. 277(12):4035–41.

Bencheikh-Latmani R. Global transcriptional profiling of Shewanella oneidensis MR-1 during Cr (VI) and U (VI) reduction. (2005). Appl Environ Microbiol. 71(11):7453–60.

Benova D., Hadjidekova V., Hristova R., Nikolova T., Boulanova M., Georgieva I., Grigorova M., Popov T., Panev T., Georgieva R., Natarajan A.T., Darroudi F., Nilsson R. (2002), Cytogenetic effects of hexavalent chromium in Bulgarian chromium platers. Mutation Research, 2002, 514**:** 29-38.

Beretta G.P., Pellegrini R. (2006) Linea Guida 14 del trattamento chimico in Situ dei terreni e delle acque sotterranee. Technical Report. Linea Guida 14, pubblicazione frutto della convenzione tra la provincia di Milano e l'università degli studi di Milano.

Beretta G., Mastorgio A.F., Pedrali L., Saponaro S., Sezenna E., (2019). The effects of electric, magnetic and electromagnetic fields on microorganisms in the perspective of bioremediation. Reviews in Environmental Science and Biotechnology. Springer Netherlands. https://doi.org/10.1007/s11157-018-09491-9.

Bertoldi M. (2007), Possibilità di utilizzo di fertilizzanti di nuova formulazione derivanti da sottoprodotti del ciclo conciario. Università Cattolica del Sacro Cuore, XIX ciclo, a.a. 2007/08, Piacenza. http://hdl.handle.net/10280/637.

Beukes J.P, Pienaar J.J, Lachmann G, Giesekke E.W. (1999). The reduction of hexavalent chromium by sulphite in wastewater. Water, 25(3), 363. Available online: http://www.wrc.org.za (accessed on 15th July 2018).

Beverskog B, Puigdomenech I. Revised Pourbaix diagrams for Chromium at 25-300°C. (1997). Corros Sci.; 39(1):43–57.

Bianco Prevot, A., Ginepro M., Peracaciolo E., Zelano V., De Luca D.A., (2018). Chemical vs bio-mediated reduction of hexavalent chromium. An in-vitro study for soil and deep waters remediation. Geoderma 312, 17–23. https://doi.org/10.1016/j.geoderma.2017.09.032.

Brodie E.L., Joyner D.C., Faybishenko B., Conrad M.E., Rios-Velazquez C., Malave J., Martinez R., Mork B., Willett A., Koenigsberg S., Herman D.J., Firestone M.K., Hazen T.C. (2011). Microbial community response to addition of polylactate compounds to stimulate hexavalent chromium reduction in groundwater, Chemosphere 85: 660–665.

Brungesh K, Nagabhushana B, Raveendra R, Krishna K, Prashantha P, Nagabhushana H. (2015) Adsorption of Cr(VI) from Aqueous Solution onto a Mesoporous Carbonaceous Material Prepared from Naturally Occurring Pongamia pinnata Seeds. Environ Anal Toxicol. OMICS International; 3;5(6):1–7.

Buerge I.J., Hug S.J. (1997) Kinetics and pH Dependence of Chromium(VI) reduction by iron(II). Technical Resource Guide. Swiss Federal Institute for Environmental Science and Technology (EAWAG). 3, 1-7.

Butler EC, Chen L, Hansel CM, Krumholz LR, Elwood Madden AS, Lan Y, et al. Biological versus mineralogical chromium reduction: potential for reoxidation by manganese oxide. (2015). Environ Sci Process Impacts.; 17(11):1930–40.

Camargo FAO, Bento FM, Okeke BC, Frankenberger W. T. (2004). Hexavalent chromium reduction by an actinomycete, Arthrobacter crystallopoietes ES 32. Biol Trace Elem Res.;97(2):183–94.

Caputo D., De Gennaro B., Pansini M., (1999). Chromium removal from water by ion exchange using zeolites and solidification of the resulting sludge in a cement matrix. In: Kiricsi I, Nagy JB, Karge HG, Palyi G, editors. Porous Materials in Environmentally Friendly Processes Studies in Surface Science and Catalysis. Elsevier Science; p. 723–30.

Careghini A., Saponaro S., Sezenna E. (2013). Biobarriers for groundwater treatment: A review. Water Sci. Technol. 67(3), 453–468. doi:10.2166/wst.2012.599.

Casalegno A., Bresciani F., Di Noto V., Casari C.S., Li Bassi A., Negro E., Di Fonzo F. (2014). Nanostructured Pd barrier for low methanol crossover DMFC. International Journal of Hydrogen Energy, vol. 39, no. 6, pp. 2801–2811.

CCME (1999). Chromium. Technical Resource Guide. Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health. Canadian Council of Ministers of the Environment. In: Canadian environmental quality guidelines. Winnipeg, MB, Canada.

Cecconet D., Devecseri M., Callegari A., Capodaglio A.G. (2018). Effects of process operating conditions on the autotrophic denitrification of nitrate-contaminated groundwater using bioelectrochemical systems. Sci Total Environ. Elsevier; Feb 1;613–614:663–71.

Chacon-Carrera R.A., Lopez-Ortiz A., Collins-Martınez V., Melendez-Zaragoza M.J., Salinas-Gutierrez J., Espinoza-Hicks J.C., Ramos-Sanchez V.H., (2019). Assessment of two ionic exchange membranes in a bioelectrochemical system for wastewater treatment and hydrogen production. International Journal of hydrogen energy, 44, 12339-12345. https://doi.org/10.1016/j.ijhydene.2018.10.153.

Chai L., Huang S., Yang Z. et al. (2009). Cr (VI) Remediation by Indigenous Bacteria in Soils Contaminated by Chromium-Containing Slag. Journal of Hazardous Materials, 167(1–3), 516–22.

Chao W., Huan D., Feng Z. (2016). The Remediation of Chromium (VI)-Contaminated Soils Using Microbial Fuel Cells, Soil and Sediment Contamination: An International Journal, 25:1, 1-12. DOI: 10.1080/15320383.2016.1085833.

Chen D., Wang H., Yang K., (2016). Effective biodegradation of nitrate, Cr(VI) and p-fluoronitrobenzene by a novel three dimensional bioelectrochemical system. Bioresour. Technol. 203, 370–373. https://doi.org/10.1016/j.biortech.2015.12.059.

Cheng C.J., Lin, T.H., Chen, C.P., Juang, K.W., Lee, D.Y. (2009). The effectiveness of ferrous iron and sodium dithionite for decreasing resin-extractable Cr(VI) in Cr(VI)-spiked alkaline soils. Journal of Hazardous Materials, 164(2-3), 510–516. http://doi.org/10.1016/j.jhazmat.2008.08.037.

Cheung K.H., Gu J. (2007). Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: A review. International Biodeterioration & Biodegradation 59, 8-15.

Chirwa E.M., Molokwane P.P., (2011). Biological Cr(VI) reduction: Microbial diversity, kinetics and biotechnological solutions to pollution. In: Sofo A, editor. Biodiversity. Rijeka, Croatia: InTech; p. 75–100.

Choi C., Hu N., Lim B. (2014). Cadmium recovery by coupling double microbial fuel cells. Bioresour Technol. Elsevier; Oct 1;170:361–9.

Christoph W., Eggenberger U., Kurz D., Zink S., Mäder U. (2012). A chromate-contaminated site in southern Switzerland – Part 1: Site characterization and the use of Cr isotopes to delineate fate and transport. Applied Geochemistry. 27, 644–654.

Chrysochoou M., Ferreira D., Johnston C.P. (2010). Calcium polysulfide treatment of Cr contaminated soil, Journal of Hazardous Materials, 179, 650–657. https://doi.org/10.1016/j.jhazmat.2010.03.052.

Chrysochoou M., Johnston C.P., Dahal G. (2012). A comparative evaluation of hexavalent chromium treatment in contaminated soil by calcium polysulfide and green-tea nanoscale zero-valent iron, Journal of Hazardous Materials, 201– 202, 33– 42.

Chrysochoou M., Ting A. (2011) A Kinetic Study of Cr(VI) Reduction by Calcium Polysulfide. Sci. Total. Environ. 409(19), 4072–77.

Chrysochoou, M., Theologou, E., Bompoti, N., Dermatas, D., Panagiotakis, I. (2016). Occurrence, origin and transformation processes of geogenic chromium in soils and sediments. Curr. Pollut. Rep., 2, 224–235.

Chung J, Nerenberg R, Rittmann BE. (2006). Bio-reduction of soluble chromate using a hydrogen-based membrane biofilm reactor. Water Res. Pergamon; May 1;40(8):1634–42.

Cissoko N., Zhang Z., Zhang J., Xu X. (2009). Removal of Cr(VI) from simulative contaminated groundwater by iron metal. Process Safety and Environmental Protection 87, 395-400.

CLAIRE (2009). Electrokinetic Ferric Ion Remediation and Stabilisation (FIRS) of Hexavalent Chromium Contaminated Soils: An Ex Situ Field Scale Demonstration. Contaminated Land: Applications in Real Environments, Available on line.

Clauwaert P., Rabaey K., Aelterman P. (2007). Biological denitrification in microbial fuel cells. Environmental Science & Technology 41(9): 3354–3360.

Clauwaert P., Aelterman P., Pham T.H., De Schamphelaire L., Carballa M., Rabaey K., Verstraete W., (2008). Minimizing losses in bio-electrochemical systems: The road to applications. Appl. Microbiol. Biotechnol. 79, 901–913. https://doi.org/10.1007/s00253-008-1522-2.

Clauwaert P., Desloover J., Shea C., Nerenberg R., Boon N., Verstraete W., (2009). Enhanced nitrogen removal in bio-electrochemical systems by pH control. Biotechnol. Lett. 31, 1537–1543. https://doi.org/10.1007/s10529-009-0048-8.

Colin V.L., Villegas L.B., Abate C.M. (2012). Indigenous microorganisms as potential bioremediators for environments contaminated with heavy metals. International Biodeterioration & Biodegradation 69, 28-37.

Coma M., Puig S., Pous N., Balaguer M.D., Colprim J., (2013). Biocatalysed sulphate removal in a BES cathode. Bioresour. Technol. 130, 218–223. https://doi.org/10.1016/j.biortech.2012.12.050.

Daghio M., Aulenta F., Vaiopoulou E., Franzetti A., Arends J.B.A., Sherry A., (2017). Electrobioremediation of oil spills. Water Research Elsevier Ltd; p. 351–70.

Daghio M, Espinoza Tofalos A., Leoni B., Cristiani P., Papacchini M., Jalilnejad E., (2018). Bioelectrochemical BTEX removal at different voltages: assessment of the degradation and characterization of the microbial communities. J Hazard Mater. Elsevier; 341:120–7.

Daghio, M., Vaiopoulou, E., Patil, S.A., Suárez-Suárez, A., Head, I.M., Franzetti, A., Rabaey, K., 2016. Anodes stimulate anaerobic toluene degradation via sulfur cycling in marine sediments. Appl. Environ. Microbiol. 82. https://doi.org/10.1128/AEM.02250-15.

Das S., Mishra J., Das S.K., Pandey S., Srinivas Rao D., Chakraborty A., Sudarshan M., Das N., Thatoi H. (2014). Investigation on mechanism of Cr(VI) reduction and removal by Bacillus amyloliquefaciens, a novel chromate tolerant bacterium isolated from chromite mine soil. Chemosphere 96, 112-121.

Dayan A.D., Paine A.J. (2001). Mechanisms of chromium toxicity, carcinogenicity and allergenicity: Review of the literature from 1985 to 2000. Human & Experimental Toxicology, 20, 439-451.

De Flora S. (2000), Threshold mechanism and site specificity in chromium(VI) carcinogenesis. Carcinogenesis, vol. 21 no. 4 pp. 533-541.

Deng B, Stone AT. (1996). Surface-Catalyzed Chromium(VI) Reduction: The TiO2−CrVI−Mandelic Acid System. Environ Sci Technol. American Chemical Society; Jan;30(2):463–72.

Dermatas D., Panagiotakis I., Theologou E., Chrysochoou M., Toskos T. (2013). Remediation Technologies For Hexavalent Chromium Contaminated Aquifers. ISWA 2013: Proceedings of the International Solid Waste Association World Congress, 7-11 October, Vienna 7 pp, 2013.

Dhal B., Thatoi H., Das N., Pandey B.D. (2010). Reduction of hexavalent chromium by Bacillus sp. isolated from chromite mine soils and characterization of reduced product. Published online in Wiley Online Library: 25 June 2010.

Dhal B., Thatoi H.N., Das N.N. et al. (2013). Chemical and Microbial Remediation of Hexavalent Chromium from Contaminated Soil and Mining/metallurgical Solid Waste: A Review. Journal of Hazardous Materials 250–251, 272–91.

Di Battista A., Verdini R., Rossetti S., Pietrangeli B., Majone M., Aulenta F. (2012). CARD-FISH analysis of a TCE-dechlorinating biocathode operated at different set potentials. N. Biotechnol. 30: 33–38.

Di Palma L., D'Onorfi R., Gueye M. T., Petrucci E. (2013). Experimental assessment of chromium immobilization in contaminated soil by in situ chemical reduction, Proceedings of the 13th International Conference on Environmental Science and Technology Athens, Greece, 5-7 September 2013.

Drees K.P., Abbaszadegan M., Maier R.M. (2003). Comparative electrochemical inactivation of bacteria and bacteriophage, Water Res. 37:2291-2300.

Dresel P.E., Wellman D.M., Cantrell K.J., Truex, M.J. (2011). Review: Technical and policy challenges in deep vadose zone remediation of metals and radionuclides. Environ. Sci. Technol. 45(10), 4207–4216.

Duca, M., Koper, M.T.M., (2012). Powering denitrification: the perspectives of electrocatalytic nitrate reduction. Energy Environ. Sci. 5, 9726. https://doi.org/10.1039/c2ee23062c.

Dutta PK, Keller J, Yuan Z, Rozendal RA, Rabaey K. Role of sulfur during acetate oxidation in biological anodes. Environ Sci Technol. 2009 May;43(10):3839–45.

ECHA, (2014). Chemical Safety Report, CSR-PI-5.5.2. https://echa.europa.eu/documents.

Elangovan R., Philip L., Chandraraj K. (2010). Hexavalent chromium reduction by free and immobilized cell-free extract of Arthrobacter rhombi-RE, Appl. Biochem. Biotechnol. 160:81-97.

Elgrishi, N., Rountree, K.J., McCarthy, B.D., Rountree, E.S., Eisenhart, T.T., Dempsey, J.L., 2018. A Practical Beginner's Guide to Cyclic Voltammetry. J. Chem. Educ. 95, 197–206. https://doi.org/10.1021/acs.jchemed.7b00361.

Elkateb T., Chalaturnyk R., Robertson P.K. (2003). An Overview of Soil Heterogeneity: Quantification and Implications on Geotechnical Field Problems. Can. Geotech. J. 40(1), 1–15.

ElMekawy, A., Hegab, H.M., Dominguez-Benetton, X., Pant, D., (2013). Internal resistance of microfluidic microbial fuel cell: Challenges and potential opportunities. Bioresour. Technol. 142, 672–682. https://doi.org/10.1016/j.biortech.2013.05.061

Erdem M., Gür F., Tümen F. (2004), Cr(VI) reduction in aqueous solutions by siderite. Journal of Hazardous Materials B113 (2004) 217–222.

Escapa A., Mateos R., Martínez E.J., Blanes J. (2016). Microbial electrolysis cells: An emerging technology for wastewater treatment and energy recovery. from laboratory to pilot plant and beyond. Renew Sustain Energy Rev. Elsevier; 55:942–56.

Espinoza Tofalos et al. (2018). Anaerobic toluene degradation and exoelectrogenic activity by *Cupriavidus metallidurans* CH34 in Bioelectrochemical Systems. 4th European meeting of the International Society for Microbial Electrochemisty and Technology, EU-ISMET 4 Newcastle, UK 2018/09/12-14.

Èupolis Lombardia (2015), Analisi e promozione di nuove tecnologie di bonifica e di caratterizzazione dei siti contaminati - CODICE: TER 13010/001.

European Commission, 2013. Science for Environment Policy: Future Brief on Bioelectrochemical systems.

European Commission, 2016. Limiting the presence of total chromium in fertilising products.

European Commission, 2017. European achievements in soil remediation and brownfield redevelopment. Technical Resource Guide. A report of the European Information and Observation Network's National Reference Centres for Soil.

European Commission, 2018. Proposal for a Directive on the quality of water intended for human consumption. Http://Ec.Europa.Eu/Environment/ Water/Water-Drink/Review_En.Html 332, 02/03/2018.

Faisal A.A., Rawaa J.M. (2018). Performance of zero-valent iron barrier through the migration of lead-contaminated groundwater. Ass. Arab. Universities J. Engineering Sci. 25, 132-144.

Fan Y., Sharbrough E., Liu H., (2008). Quantification of the internal resistance distribution of microbial fuel cells. Environ. Sci. Technol. 42, 8101–8107.

FCEE (2004), Principles and practices of enhanced anaerobic bioremediation of chlorinated solvents, Prepared for Air Force Center for Environmental Excellence, Brooks City-Base, Texas, September 2004.

Field E.K., Gerlach R., Viamajala S., Jennings L.K., Peyton B.M., Apel W.A. (2012). Hexavalent chromium reduction by Cellulomonas sp. strain ES6: the influence of carbon source, iron minerals, and electron shuttling compounds. Biodegradation, 24:437-450.

Focardi S, Pepi M, Landi G, Gasperini S, Ruta M, Di Biasio P, et al. (2012). Hexavalent chromium reduction by whole cells and cell free extract of the moderate halophilic bacterial strain Halomonas sp. TA-04. Int Biodeterior Biodegradation; 66:63–70.

Focardi S., Pepi M., Focardi S. E. (2013). Microbial Reduction of Hexavalent Chromium as a Mechanism of Detoxification and Possible Bioremediation Applications. In R. Chamy (Ed.), Biodegradation - Life of Science. InTech.

Franco D.V., Da Silva L.M., Jardim W.F. (2009). Reduction of Hexavalent Chromium in Soil and Ground Water Using Zero-Valent Iron Under Batch and Semi-Batch Conditions. Water, Air & Soil Pollution, 197:49-60.

Franzetti A., Daghio M., Parenti P., Truppi T., Bestetti G., Trasatti S.P., Cristiani P., (2017). Monod Kinetics Degradation of Low Concentration Residual Organics in Membraneless Microbial Fuel Cells. J. Electrochem. Soc. 164. https://doi.org/10.1149/2.0141703jes.

Fricke K., Harnisch F., Schröder U., (2008). On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells. Energy Environ. Sci. 1, 144–147. https://doi.org/10.1039/b802363h.

Fruchter J. (2002a). In situ treatment of chromium-contaminated groundwater. Environ. Sci. Technol. 36, 464A–472A.

Fruchter J. (2002b). "New technologies show promise for removing chromium(VI) pollution at lower cost". Environmental Science & Technology. December 1: 465A-472A.

Fuller S.J., Stewart D.I, Ian T.B. (2013). Chromate Reduction in Highly Alkaline Groundwater by Zerovalent Iron: Implications for Its Use in a Permeable Reactive Barrier. Ind. Eng. Chem. Res. 52(13), 4704–14.

Gambelunghe A., Piccinini R., Ambrogi M., Villarini M., Moretti M., Marchetti C., Abbritti G., Muzi G. (2003). Primary DNA damage in chrome-plating workers. Toxicology, 188: 187-195.

Gangadharan P., Nambi I.M. (2015). Hexavalent chromium reduction and energy recovery by using dual-chambered microbial fuel cell. Water Sci Technol. IWA Publishing;71(3):353–8.

Ghosal D., Ghosh S., Dutta T.K., Ahn Y. (2016). Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01369.

Gorby Y.A., Yanina S., McLean J.S., Rosso K.M., Moyles D., Dohnalkova A., Beveridge T.J., Chang I.S., Kim B.H., Kim K.S., Culley D.E., Reed S.B., Romine M.F., Saffarini D.A., Hill E.A., Shi L., Elias D.A., Kennedy D.W., Pinchuk G., Watanabe K., Ishii S., Logan B., Nealson K.H., Fredrickson J.K. (2006). Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms. Proc Natl Acad Sci USA 103:11358–11363.

Goulhen F., Gloter A., Guyot F., Bruschi M. (2006). Cr(VI) detoxification by Desulfovibrio vulgaris strain Hildenborough: microbe-metal interactions studies. Applied Microbiology and Biotechnology. 71: 892-897.

Gregory K.B., Bond D.R., and Lovley D.R. (2004). Graphite electrodes as electron donors for anaerobic respiration. Environmental Microbiology 6(6): 596–604.

Gregory, K.B., Lovley, D.R., 2005. Remediation and Recovery of Uranium from Contaminated Subsurface Environments with Electrodes. Environ. Sci. Technol. 39, 8943–8947. https://doi.org/10.1021/es050457e.

Grieger K.D., Fjordbøge A., Hartmann N.B., Eriksson E., Bjerg P.L., Baun A. (2010). Environmental benefits and risks of zero-valent iron nanoparticles (nZVI) for in situ remediation: Risk mitigation or trade-off? Journal of Contaminant Hydrology 118, 165-183.

Guan C.Y., Tseng Y.H., Tsang D.C.W., Hu A., Yu C.P., (2019). Wetland plant microbial fuel cells for remediation of hexavalent chromium contaminated soils and electricity production. J. Hazard. Mater. 365, 137–145. https://doi.org/10.1016/j.jhazmat.2018.10.086.

Gude V.G., (2016). Wastewater treatment in microbial fuel cells- an overview, J. Clean. Prod. 122, 287–307.

Gude V.G., Kokabian B., Gadhamshetty V., (2013). Beneficial Bioelectrochemical Systems for Energy, Water, and Biomass Production. J. Microb. Biochem. Technol. https://doi.org/10.4172/1948-5948.S6-005.

Guertin J., Jacobs J.A., Avakian C.P. (2005). Chromium(VI) Handbook. Publisher: Boca Raton, Fla.: Independent Environmental Technical Evaluation Group. CRC Press. 784 pages. ISBN:1566706084 9781566706087. CRC Press: Boca Raton, FL, USA, 2005. 784 p.

Guha H., Jayachandran K., Maurrasse F. (2001). Kinetics of chromium (VI) reduction by a type strain Shewanella alga under different growth conditions. Environmental Pollution 115 (2001) 209-218.

Guiot S.R., Cimpoia R., Kuhn R., and Alaplantive A. (2008). Electrolytic methanogenic-methanotrophic coupling for tetrachloroethylene bioremediation: Proof of concept. Environmental Science & Technology 42(8): 3011–3017.

Guo X., Mei N. (2014). “Assessment of the Toxic Potential of Graphene”. Journal of Food and Drug Analysis 22(1): 105–15.

Gupta S., Yadav A., Verma N. (2017) Simultaneous Cr(VI) reduction and bioelectricity generation using microbial fuel cell based on alumina-nickel nanoparticles-dispersed carbon nanofiber electrode. Chem Eng J. Jan;307:729–38.

Habibul YH, Wang YH, Chen W, Yu HQ, Sheng GP. (2016). Bioelectrochemical Chromium (VI) Removal in Plant-Microbial Fuel Cells Nuzahat Environmental Science & Technology 2016 50 (7), 3882-3889. DOI: 10.1021/acs.est.5b06376.

Harbottle MJ, Lear G, Sills GC, Thompson IP (2009). Enhanced biodegradation of pentachlorophenol in unsaturated soil using reversed field electrokinetics. J Environ Manage 90(5):1893–1900.

Harnisch, F., Aulenta, F., Schröder, U., (2011). Microbial Fuel Cells and Bioelectrochemical Systems: Industrial and Environmental Biotechnologies Based on Extracellular Electron Transfer, Second Edi. ed, Comprehensive Biotechnology, Second Edition. Elsevier B.V. https://doi.org/10.1016/B978-0-08-088504-9.00462-1.

Harnisch, F., Freguia, S., (2012). A basic tutorial on cyclic voltammetry for the investigation of electroactive microbial biofilms. Chem. - An Asian J. 7, 466–475. https://doi.org/10.1002/asia.201100740.

Hashim, M.A., Mukhopadhyay, S., Sahu, J.N., Sengupta, B., (2011). Remediation technologies for heavy metal contaminated groundwater. J. Environ. Manage. 92, 2355–2388. https://doi.org/10.1016/j.jenvman.2011.06.009.

Hassan Z., Ali S., Farid M. et al. (2017). Effect of Chromium (Cr) on the Microbial and Enzymatic Activities in the Soil: A Review. In Biodiversity conservation in changing climate. 305–23.

He F., Zhao D., Liu J., Roberts C. B. (2007). Stabilization of Fe–Pd nanoparticles with sodium carboxymethyl cellulose for enhanced transport and dechlorination of trichloroethylene in soil and groundwater. Industrial & Engineering Chemistry Research, 46(1), 29–34.

He Y. (2003). Chromate reduction and immobilization under high pH and high ionic strength conditions. Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University. 25, 1-238.

He S., Wu, J., (2019). Hydrogeochemical Characteristics, Groundwater Quality, and Health Risks from Hexavalent Chromium and Nitrate in Groundwater of Huanhe Formation in Wuqi County, Northwest China. Expo. Heal. 11, 125–137. https://doi.org/10.1007/s12403-018-0289-7.

Hirose A., Kouzuma A., Watanabe K., (2019). Towards development of electrogenetics using electrochemically active bacteria. Biotechnol. Adv. 0–1. https://doi.org/S0734975019300266.

Höhener P., Ponsin V. (2014). In Situ Vadose Zone Bioremediation. Curr. Opin. Biotechnol, 27, 1–7.

Horitsu H, Futo S, Miyazawa Y, Ogai S, Kawai K. (1987). Enzymatic reduction of hexavalent chromium by hexavalent chromium tolerant *Pseudomonas ambigua* G-1. Agric Biol Chem; 51(9):2417–20.

Hsu (2011). Evaluation of Chromium Remediation in Groundwater: Investigations Using Microbial Fuel Cells and Electrokinetic Systems. Thesis. 2011.

Hsu L, Masuda SA, Nealson KH, Pirbazari M. (2012). Evaluation of microbial fuel cell Shewanella biocathodes for treatment of chromate contamination. RSC Adv. The Royal Society of Chemistry; Jun 13;2(13):5844–55.

Hua B., Deng B. (2003). Influences of Water Vapor on Cr(VI) Reduction by Gaseous Hydrogen Sulfide. Environ. Sci. Technol. 37(20), 4771–77.

Huang L., Chai X., Chen G., Logan B.E., (2011a). Effect of set potential on hexavalent chromium reduction and electricity generation from biocathode microbial fuel cells. Environ. Sci. Technol. 45, 5025–5031. https://doi.org/10.1021/es103875d.

Huang L., Chai X., Cheng S., Chen G., (2011b). Evaluation of carbon-based materials in tubular biocathode microbial fuel cells in terms of hexavalent chromium reduction and electricity generation. Chem. Eng. J. 166, 652–661. https://doi.org/10.1016/j.cej.2010.11.042.

Huang L., Chen J., Quan X., Yang F., (2010). Enhancement of hexavalent chromium reduction and electricity production from a biocathode microbial fuel cell. Bioprocess Biosyst. Eng. 33, 937–945. https://doi.org/10.1007/s00449-010-0417-7.

Huang L., Wang Q., Jiang L., Zhou P., Quan X., Logan B.E., (2015). Adaptively Evolving Bacterial Communities for Complete and Selective Reduction of Cr(VI), Cu(II), and Cd(II) in Biocathode Bioelectrochemical Systems. Environ. Sci. Technol. 49, 9914–9924. https://doi.org/10.1021/acs.est.5b00191.

Huang L., Zhou P., Quan X., Logan B.E., (2018). Removal of binary Cr(VI) and Cd(II) from the catholyte of MFCs and determining their fate in EAB using fluorescence probes. Bioelectrochemistry 122, 61–68. https://doi.org/10.1016/j.bioelechem.2018.02.010.

Hubbard S.S., Williams K., Conrad M.E., Faybishenko B., Peterson J., Chen J., Long P., Hazen T. (2008). Geophysical Monitoring of Hydrological and Biogeochemical Transformations Associated with Cr(VI) Bioremediation. Environmental Science & Technology 2008, 42, 3757-3765.

Huber, J.A., Welch, D.B.M., Morrison, H.G., Huse, S.M., Neal, P.R., Butterfield, D.A., Sogin, M.L., (2007). Microbial population structures in the deep marine biosphere. Science (80). 318, 97–101.

Humphries AC, Nott KP, Hall LD, Macaskie LE. (2005). Reduction of Cr(VI) by immobilized cells of desulfovibrio vulgaris NCIMB 8303 and Microbacterium sp. NCIMB 13776. Biotechnol Bioeng;90(5):589–96.

IARC (2012). IARC Monographs on the evaluation of carcinogenic risks to humans - Chromium (VI) compounds. Monographs, Volume 100C.

Ibbini J., Santharam S., Davis L. C. and Erickson L. E. (2010). Laboratory and Field Scale Bioremediation of Tetrachloroethene (PCE) Contaminated Groundwater, Jordan Journal of Mechanical and Industrial Engineering, Volume 4, Number 1, Pages 35 – 44.

Ieropoulos, I., Greenman, J., and Melhuish, C. (2008). Microbial fuel cells based on carbon veil electrodes: Stack configuration and scalability. International Journal of Energy Research. 32: 1228–1240. Doi: 10.1002/er.1419.

Im C. H., C. Kim, Y. E. Song, S. E. Oh, B. H. Jeon, and J. R. Kim. (2018). Electrochemically enhanced microbial CO conversion to volatile fatty acids using neutral red as an electron mediator. Chemosphere, vol. 191, pp. 166–173.

Inglett KS, Bae HS, Aldrich HC, Hatfield K, Ogram A V. (2011). *Clostridium chromiireducens* sp. nov., isolated from Cr(VI)-contaminated soil. Int J Syst Evol Microbiol;61(11):2626–31.

INSURE, Innovative Sustainable Remediation (2017). EQS limit and guideline values for contaminated sites, Report 2017.

Ishibashi Y, Cervantes C, Silver S. (1990). Chromium reduction in *Pseudomonas putida*. Appl Environ Microbiol;56(7):2268–70.

ISPRA (2012). Annuario dei dati ambientali – Edizione 2012 (Chapter 15). Istituto Superiore per le Protezione e la Ricerca Ambientale, http://annuario.isprambiente.it/sites/default/files/pdf/2012/annuario/15_Pericolosita_antropica.pdf.

ISPRA 2014. Condizioni Ambientali – Atmosfera. Technical Resource Guide.

ISS–INAIL (2018), Documento di supporto alla Banca dati "ISS-INAIL".

ITRC (2011). Permeable Reactive Barrier: Technology Update, PRB-5, Interstate Technology & Regulatory Council, PRB: Technology Update Team. Washington, DC, USA,www.itrcweb.org.

Jabeen G, Rarooq R (2016). Bio-Electrochemical Synthesis of Commodity Chemicals by Autotrophic Acetogens Utilizing CO 2 for Environmental Remediation. J. Biosci. 41(3), September 2016, 367–380.

Jackman SA, Maini G, Sharman AK, Knowles CJ (1999). The effects of direct electric current on the viability and metabolism of acidophilic bacteria. Enzyme Microb Technol 24(5–6):316–324.

Jacobs J., Hardison R., & Rose J. (2001). In situ remediation of heavy metals using sulfur-based treatment technologies. Hydrovisions. Retrieved from http://www.fast-tek.com/Article-HV01-In-Situ-MetalsRemediation.pdf.

Jadhav UU, Hocheng H. A review of recovery of metals from industrial waste. J Achiev Mater Manuf Eng. 2012;54(2):159–67.

Jafarian, A., Jafarian, S., (2017). Geogenic and Anthropogenic Chromium Contamination in Groundwater in an Ophiolitic Area, Northeastern Iran. Univers. J. Geosci. 5, 183–190. https://doi.org/10.13189/ujg.2017.050603.

James B.R., Petura J.C., Vitale R.J., Mussoline G.R. (1997). Oxidation-reduction chemistry of chromium: relevance to the regulation and remediation of chromate-contaminated soils. Journal of Soil Contamination. 6: 569-580. doi:10.1080/15320389709383590.

Jeyasingh J., Philip L. (2005). Bioremediation of chromium contaminated soil: optimization of operating parameters under laboratory conditions. Journal of Hazardous Materials B118, 113-120.

Jeyasingh J., Somasundaram V., Philip L. et al. (2011) Pilot scale studies on the remediation of chromium contaminated aquifer using bio-barrier and reactive zone technologies. Chemical Engineering Journal 167, 206-214.

Jiang Y, May HD, Lu Lu, Liang P, Huang X, Ren ZJ. (2019). Carbon dioxide and organic waste valorization by microbialelectrosynthesis and electro-fermentation. Water Research 149, 42-55.

Jin W, Du H, Zheng S, Zhang Y. (2015). Electrochemical processes for the environmental remediation of toxic Cr(VI): A review. Electrochimica Acta;1044–55.

Joicy, A., Song, Y.-C., Lee, C.-Y., (2019). Electroactive microorganisms enriched from activated sludge remove nitrogen in bioelectrochemical reactor. J. Environ. Manage. 233, 249–257. https://doi.org/10.1016/j.jenvman.2018.12.037

Jørgensen BB. (1982). Mineralization of organic matter in the sea bed – the role of sulphate reduction. Vol. 296, Nature. p. 643–645.

Jung R.K., Cheng S., Oh S.E., Logan B.E., (2007). Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells. Environ. Sci. Technol. 41, 1004–1009. https://doi.org/10.1021/es062202m.

Kadier A, Simayi Y, Abdeshahian P, Azman NF, Chandrasekhar K, Kalil MS. (2016). A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. Alexandria Eng J. Faculty of Engineering, Alexandria University; Mar;55(1):427–43.

Kamaludden S.P.B., Arunkumar K.R., Avudainayagam S., Ramasamy, K. (2003). Bioremediation of chromium contaminated environments. Indian J. Exp. Biol. 41, 972-985.

Kavita B., Keharia H., (2012). Reduction of hexavalent chromium by Ochrobactrum intermedium BCR400 isolated from a chromium-contaminated soil. 3 Biotech 2, 79–87. https://doi.org/10.1007/s13205-011-0038-0

Kazakis N., Kantiranis N., Kalaitzidou K., Kaprara M., Mitrakas M., Frei R., Vargemezis G., Tsourlos P., Zouboulis A., Filippidis A., (2017). Origin of hexavalent chromium in groundwater: The example of Sarigkiol Basin, Northern Greece. Sci. Total Environ. 593-594, 552–566. https://doi.org/10.1016/j.scitotenv.2017.03.128

Kazakis, N., Kantiranis, N., Voudouris, K.S., Mitrakas, M., Kaprara, E., Pavlou, A., 2015. Geogenic Cr oxidation on the surface of mafic minerals and the hydrogeological conditions influencing hexavalent chromium concentrations in groundwater. Sci. Total Environ. 514, 224–238. https://doi.org/10.1016/j.scitotenv.2015.01.080.

Khan F.A., Plus R.W. (2003). In situ abiotic Detoxification and immobilization of hexavalent chromium. Graund Water Monitoring & Remediation. 23(1), 77-84.

Kim C, Lee CR, Song YE, Heo J, Choi SM, Lim D-HH, (2017). Hexavalent chromium as a cathodic electron acceptor in a bipolar membrane microbial fuel cell with the simultaneous treatment of electroplating wastewater. Chem Eng J. Elsevier; Nov 15;328:703–7.

Kim C., Zhou Q., Deng B. et al. (2001). Chromium (VI) Reduction by Hydrogen Sulfide in Aqueous Media: Stoichiometry and Kinetics. Environmental Science & Technology. 35(11), 2219–25.

Kokabian B, Gude VG. (2015). Role of membranes in bioelectrochemical systems. Membr Water Treat;6(1):53–75.

Kozaderova, O.A., (2018). Electrochemical Characterization of an MB-2 Bipolar Membrane Modified by Nanosized Chromium (III) Hydroxide 13, 508–515. https://doi.org/10.1134/S1995078018050075.

Kracke F, Vassilev I, Krömer JO. (2015). Microbial electron transport and energy conservation - the foundation for optimizing bioelectrochemical systems. Front Microbiol. Frontiers Media SA;6:575.

Kumar A., Huan-Hsuan Hsu, L., Kavanagh, P., Barrière, F., Lens, P.N.L., Lapinsonniere L., Leech D. (2017a). The ins and outs of microorganism–electrode electron transfer reactions. Nature Reviews Chemistry, (1), [0024]. https://doi.org/10.1038/s41570-017-0024.

Kumar S., Sandeep K. Malyan, Suddhasatwa Basu, and Narsi R. Bishnoi. (2017b). Syntrophic Association and Performance of Clostridium, Desulfovibrio, Aeromonas and Tetrathiobacter as Anodic Biocatalysts for Bioelectricity Generation in Dual Chamber Microbial Fuel Cell. Environmental Science and Pollution Research 24 (19).

Lai A, Aulenta F, Mingazzini M, Palumbo MT, Papini MP, Verdini R, et al. (2017). Bioelectrochemical approach for reductive and oxidative dechlorination of chlorinated aliphatic hydrocarbons (CAHs). Chemosphere. Elsevier Ltd;169:351–60.

Lai A., Verdini R., Aulenta F., Majone M. (2015). Influence of nitrate and sulphate reduction in the biolectrochemically assisted dechlorination of cis-DCE, Chemosphere 125:147-154

Lai et al. (2018). Chromium (VI) reduction under electro-assisted reductive dechlorination conditions by dechlorinating consortium. 7th European Bioremediation Conference (EBC-VII) and the 11th International Society for Environmental Biotechnology conference (ISEB 2018) Chaina, Creta, Grecia 2018/06/25-28.

Laxmipriya S., Sivakumar M., Anand K., Shanmugam P. (2010). Study on soil bio-remediaton for preventing groundwater contamination of chromium bearing

leather processing waste dumping sites. Proceedings of the International Conference on "Recent Advances in Space Technology Services and Climate Change - 2010", RSTS and CC-2010, p 32-40, 2010.

Lee S.E., Lee J.U., Chon H.T., Lee J.S. (2008). Reduction of Cr(VI) by indigenous bacteria in Cr-contaminated sediment under aerobic condition. Journal of Geochemical Exploration 96, 144-147.

Leitão P, Nouws H, Danko AS, Aulenta F. (2017). Bioelectrochemical Dechlorination of 1,2-DCA with an AQDS-Functionalized Cathode Serving as Electron Donor. Fuel Cells;17(5):612–7.

Li M, Zhou S, Xu Y, Liu Z, Ma F, Zhi L, et al. (2018). Simultaneous Cr(VI) reduction and bioelectricity generation in a dual chamber microbial fuel cell. Chem Eng J. Elsevier;334:1621–9.

Li Q., Yang Z., Chai L., Wang B., Xiong S., Liao Y., Zhang S. (2013). Optimization of Cr(VI) bioremediation in contaminated soil using indigenous bacteria. Journal of Central South University of Technology. 20: 480-487.

Li X, Abu-Reesh I, He Z. (2015). Development of Bioelectrochemical Systems to Promote Sustainable Agriculture. Agriculture;5(3):367–88.

Li X., Lei L., Yang C., Zhang W.X. (2016). Reduction of Cr(VI) by Nanoscale Zero Valent Iron (nZVI): The Reaction Kinetics. Proceeding of 4th International Conference on Bioinformatics and Biomedical Engineering, IWBBIO 2016, Granada, Spain. Springer International Publishing, Switzerland, 2016.

Li Y, Lu A, Ding H, Jin S, Yan Y, Wang C, et al. (2009). Cr(VI) reduction at rutile-catalyzed cathode in microbial fuel cells. Electrochem commun. Elsevier B.V.;11(7):1496–9.

Li Y, Wu Y, Puranik S, Lei Y, Vadas T, Li B. (2014). Metals as electron acceptors in single-chamber microbial fuel cells. J Power Sources. Elsevier B.V;269:430–9.

Li Z., Zhang X., Lei L., (2008). Electricity production during the treatment of real electroplating wastewater containing Cr6+ using microbial fuel cell. Process Biochem. 43, 1352–1358. https://doi.org/10.1016/j.procbio.2008.08.005.

Liang P., Fan M., Cao X., Huang X., (2009). Evaluation of applied cathode potential to enhance biocathode in microbial fuel cells. J. Chem. Technol. Biotechnol. 84, 794–799. https://doi.org/10.1002/jctb.2114

Liao Y., Min X., Yang Z. et al. (2014). Physicochemical and Biological Quality of Soil in Hexavalent Chromium-Contaminated Soils as Affected by Chemical and Microbial Remediation. Environmental Science and Pollution Research 21(1), 379–88.

Lilli M.A., Moraetis D., Nikolaidis N.P.; Karatzas G.P.; Kalogerakis N. (2015). Characterization and mobility of geogenic chromium in soils and riverbed sediments of asopos basin. J. Hazard. Mater. 281, 12–19.

Lin Y.T., Huang C.P. (2008), Reduction of chromium(VI) by pyrite in dilute aqueous solutions. Separation and Purification Technology 63, 191-199.

Liu H, Guo L, Liao S, Wang G. (2012). Reutilization of immobilized fungus Rhizopus sp. LG04 to reduce toxic chromate. J Appl Microbiol;112(4):651–9.

Liu J., Duan C., Zhang X. et al. (2011a). Potential of Leersia Hexandra Swartz for Phytoextraction of Cr from Soil. Journal of Hazardous Materials 188(1–3), 85–91.

Liu L, Yuan Y, Li F, Feng C. (2011b). In-situ Cr(VI) reduction with electrogenerated hydrogen peroxide driven by iron-reducing bacteria. Bioresour Technol. Elsevier Ltd; 102(3):2468–73.

Liu Y., Mou H., Chen L. et al. (2015). Mirza, Z.A.; Liu, L. Cr(VI)-contaminated groundwater remediation with simulated permeable reactive barrier (PRB) filled with natural pyrite as reactive material: Environmental factors and effectiveness. J. Hazard. Mater, 298, 83–90.

Logan B.E. (2009). Exoelectrogenic bacteria that power microbial fuel cells, Nat. Rev. Microbiol. 7(5): 375−381.

Logan BE. Microbial fuel cells. (2008). Wiley. New York.

Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., (2006). Microbial fuel cells: Methodology and technology. Environ. Sci. Technol. 40, 5181–5192. https://doi.org/10.1021/es0605016.

Lohner S.T., Becker D., Mangold K.M., Tiehm A. (2011). Sequential reductive and oxidative biodegradation of chloroethenes stimulated in a coupled bioelectro-process,. Environ. Sci. Technol. 45:6491-6497.

Lotfy S.M., Mostafa A.Z. (2014). Phytoremediation of contaminated soil with cobalt and chromium. Journal of Geochemical Exploration 144 (2014) 367–373.

Lovley DR, Phillips EJP. (1994). Reduction of Chromate by Desulfovibrio-Vulgaris and Its c3 Cytochrome. Appl Environ Microbiol; 60(2):726–8.

Lovley, D.R., (2011). Powering microbes with electricity: direct electron transfer from electrodes to microbes. Environ. Microbiol. Rep. 3, 27–35. https://doi.org/10.1111/j.1758-2229.2010.00211.x

Loyaux-Lawniczak S., Lecomte P., Ehrhardt J.J. (2001). Behavior of Hexavalent Chromium in a Polluted Groundwater: Redox Processes and Immobilization in Soils. Environmental Science & Technology, 35, 1350-1357.

Lu L., Huggins T., Jin S., Zuo Y., Ren Z.J. (2014). Microbial metabolism and community structure in response to bioelectrochemically enhanced remediation of petroleum hydrocarbon-contaminated soil, Environ. Sci. Technol. 48: 4021-4029.

Lu P., Feng Q., Meng Q., Yuan T. (2012). Electrokinetic remediation of chromium- and cadmium-contaminated soil from abandoned industrial site. Separation and Purification Technology 98, 216–220.

Lu Z, Chang D, Ma J, Huang G, Cai L, Zhang L. (2015). Behavior of metal ions in bioelectrochemical systems: A review. Journal of Power Sources. p. 243–60.

Lu M, Shing C, Babanova S, et al. (2017). Long-Term Performance of a 20-L Continuous Flow Microbial Fuel Cell for Treatment of Brewery Wastewater. Journal of Power Sources 356: 274–87.

Ludwig R.D., Su C., Lee T.R. et al. (2007). In Situ Chemical Reduction of Cr(VI) in Groundwater Using a Combination of Ferrous Sulfate and Sodium Dithionite: A Field Investigation. Environmental Science & Technology 41(15), 5299–5305.

Luo, H., Fu, S., Liu, G., Zhang, R., Bai, Y., Luo, X., (2014). Autotrophic biocathode for high efficient sulfate reduction in microbial electrolysis cells. Bioresour. Technol. 167, 462–468. https://doi.org/10.1016/j.biortech.2014.06.058.

Malaviya P, Singh A. (2011). Physicochemical technologies for remediation of chromium-containing waters and wastewaters. Crit Rev Environ Sci Technol; 41(12):1111–72.

Malaviya, P., Singh, A., (2014). Critical Reviews in Microbiology Bioremediation of chromium solutions and chromium containing wastewaters. https://doi.org/10.3109/1040841X.2014.974501.

Marrazzo M. (2009), Applicazione delle barriere permeabili reattive e dell'ossidazione chimica in situ alla bonifica delle acque sotterranee. ISPRA, 2009.

Marsh TL, McInerney MJ. (2001). Relationship of Hydrogen Bioavailability to Chromate Reduction in Aquifer Sediments. Appl Environ Microbiol. American Society for Microbiology; Apr 1;67(4):1517–21.

Massacheleyn, P.H., J.H. Pardue, R.D. De Laune, and W.H. Patrick. (1992). "Chromium redox chemistry in a lower Mississipi Valley bottomland hardwood wetland". Environmental Science and Technology. 26: 1217-1226. doi:10.1021/es50002a611.

McEwan N., Courtney T., Parry R.A., Knupfer P. (2011). Chromite – A cost-effective refractory raw material for refractories in various metallurgical applications. Southern African Pyrometallurgy 2011, R.T. Jones & P. den Hoed.

McGrath S.P. (1995). Chromium and nickel. Heavy Metals in Soils, 2nd edit. – Alloway B.J. – Blackie Academic & Professional, London.

Mclean J, Beveridge TJ. (2001). Chromate Reduction by a Pseudomonad Isolated from a Site Contaminated with Chromated Copper Arsenate. Appl Environ Microbiol; 67(3):1076–84.

McLean JS, Beveridge TJ, Phipps D. (2000). Isolation and characterization of a chromium-reducing bacterium from a chromated copper arsenate-contaminated site. Environ Microbiol; 2(6):611–9.

Medeiros M.G., Rodrigues A.S., Batoreu M.C., Laires A., Rueff J., Zhitkovich A. (2003). Elevated levels of DNA-protein crosslinks and micronuclei in peripheral lymphocytes of tannery workers exposed to trivalent chromium. Mutagenesis, 18: 19-24.

Mohapatra RK, Parhi PK, Thatoi H, Panda CR. (2017). Bioreduction of hexavalent chromium by Exiguobacterium indicum strain MW1 isolated from marine water of Paradip Port, Odisha, India. Chem Ecol; 33(2):114–30.

Molognoni, D., Devecseri, M., Cecconet, D., Capodaglio, A. G. (2017). Cathodic groundwater denitrification with a bioelectrochemical system. Journal of Water Process Engineering. https://doi.org/10.1016/j.jwpe.2017.07.013.

Molokwane, P.E., Meli, K.C., Nkhalambayausi-Chirwa, E.M., (2008). Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading: Brits culture (South Africa). Water Res. 42, 4538–4548. https://doi.org/10.1016/j.watres.2008.07.040.

Morris J.M., Jin S., Crimi B., Pruden A. (2009). Microbial fuel cell in enhancing anaerobic biodegradation of diesel, Chem. Eng. J. 146(2): 161−167.

Moscoviz, R., Toledo-Alarcón, J., Trably, E., Bernet, N., (2016). Electro-Fermentation: How To Drive Fermentation Using Electrochemical Systems. Trends Biotechnol. 34, 856–865. https://doi.org/10.1016/j.tibtech.2016.04.009.

Mueller N.C., Braun J., Bruns J., Černík M., Rissing P., Rickerby D., Nowack B. (2011). Application of nanoscale zero valent iron (NZVI) for groundwater remediation in Europe. Environmental Science and Pollution Research, 19:550-558.

Mukhopadhyay B., Sundquist J., Schmitz R.J. (2007). Removal of Cr(VI) from Cr-contaminated groundwater through electrochemical addition of Fe(II). J. Environ. Manage., 82, 66-76.

Mullet M., Boursiquot S., & Ehrhardt J.-J. (2004). Removal of hexavalent chromium from solutions by mackinawite, tetragonal FeS. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 244(1-3), 77–85. http://doi.org/10.1016/j.colsurfa.2004.06.013.

Nancharaiah Y V, Mohan SV, Lens PNL. (2015). Bioresource Technology Metals removal and recovery in bioelectrochemical systems : A review;195:102–14.

NAVFAC (2004). A Practical Approach to the Design, Monitoring, and Optimization of in Situ MTBE Aerobic Biobarriers, TR-2257-ENV, Naval Facilities Engineering Command: Port Hueneme, CA, USA.

Nayak A.K., Panda S.S, Basu A. Dhal, N.K. (2018). Enhancement of Toxic Cr (VI), Fe, and Other Heavy Metals Phytoremediation by the Synergistic Combination of

Native Bacillus Cereus Strain and Vetiveria Zizanioides L. Int. J. Phytoremediation. 20(7), 682–91.

Němeček J., Lhotský O., Cajthaml T. (2014). Nanoscale Zero-Valent Iron Application for in Situ Reduction of Hexavalent Chromium and Its Effects on Indigenous Microorganism Populations. Science of the Total Environment 485–486(1), 739–47.

Němeček J., Pokorný P., Lhotský O., Knytl V, Najmanová P, Steinová J. (2016). Combined nano-biotechnology for in-situ remediation of mixed contamination of groundwater by hexavalent chromium and chlorinated solvents. Sci. Total. Environ. 563–564, 822–834.

Nevin KP, Hensley SA, Franks AE, Summers ZM, Ou J, Woodard TL (2011). "Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms". Appl.Environ.Microbiol. 77, 2882–2886. doi:10.1128/AEM.02642-10

Nguyen VK, Park Y, Yang H, Yu J, Lee T. Effect of the cathode potential and sulfate ions on nitrate reduction in a microbial electrochemical denitrification system. J Ind Microbiol Biotechnol. Springer Berlin Heidelberg; 2016a;43(6):783–93.

Nguyen VK, Park Y, Yu J, Lee T. Simultaneous arsenite oxidation and nitrate reduction at the electrodes of bioelectrochemical systems. Environ Sci Pollut Res. Springer Berlin Heidelberg; 2016b Oct 20;23(19):19978–88.

NICOLE (2012). How to implement Sustainable Remediation in a contaminated land management project? Technical Resource Guide. Working Group Sustainable Remediation.

NICOLE (2018). Network for Industrially Contaminated Land in Europe. How to implement Sustainable Remediation in a contaminated land management project? Available online: http://www.nicole.org/uploadedfiles/wg-sustainableremediation-finalreport.pdf (accessed on 15th July 2018).

Nitisoravut R., Regmi R., (2017). Plant microbial fuel cells: a promising biosystems engineering, Renew. Sustain. Energy Rev. 76, 81–89.

Nozawa-Inoue M, Jien M, Yang K, Rolston DE, Hristova KR, Scow KM. (2011). Effect of nitrate, acetate, and hydrogen on native perchlorate-reducing microbial communities and their activity in vadose soil. FEMS Microbiol Ecol. NIH Public Access; 76(2):278–88.

Nriagu, J.O. (1988). Production and uses of chromium. In J.O. Nriagu and E. Nieboer (Eds.), Chromium in the Natural and Human Environments, pp. 81–103, John Wiley & Sons, New York.

Obiri-Nyarko F., Grajales-Mesa S.J., Malina G. (2014). An overview of permeable reactive barriers for in situ sustainable groundwater remediation. Chemosphere. 111, 243-259.

Ohio EPA (2005). Use of Direct Push Technologies for Soil and Ground Water Sampling. Technical Resource Guide for Ground Water Investigations. Retrieved from http://www.epa.state.oh.us/ddagw/.

Ohio EPA (2014). Reduction-Oxidation (Redox) Control in Ohio's Ground Water Quality The Technical Series on Ground Water Quality. Technical Resource Guide.

Okoh A.I., Trejo-Hernandez, M. R. (2006). Remediation of petroleum hydrocarbon polluted systems: Exploiting the bioremediation strategies. African Journal of Biotechnology Vol. 5 (25), pp. 2520-2525, 29 December, 2006. Available online at http://www.academicjournals.org/AJBISSN 1684–5315 © 2006 Academic Journals.

Oliver D.S., Brockman FJ, Bowman RS, Kieft TL, (2003). Microbal reduction of hexavalent chromium under vadose zone conditions. J Environ Qual. Jan-Feb;32(1):317-24.

Ontañon O.M., González P.S., Ambrosio L.F., Paisio C.E., Agostini E. (2014). Rhizoremediation of phenol and chromium by the synergistic combination of a native bacterial strain and Brassica napus hairy roots. International Biodeterioration & Biodegradation 88 (2014) 192-198.

OSHA (2009). Hexavalent Chromium. Technical Resource Guide. U.S. Department of Labor Occupational Safety and Health Administration, 3373-10.

Paiva M.C., Ávila M.P., Reis M.P., Costa P.S., Nardi R.M.D., Nascimento A.M.A., (2015). The microbiota and abundance of the class 1 integron-integrase gene in tropical sewage treatment plant influent and activated sludge. PLoS One, vol. 10, no. 6, pp. 1–12.

Pal A, Paul AK. (2004). Aerobic chromate reduction by chromium-resistant bacteria isolated from serpentine soil. Microbiol Res;159(4):347–54.

Palma et al. (2016). "Bioelectrochemical groundwater circulation well": a scalable reactor configuration for in-situ treatment of contaminated groundwater. Poster 3rd European Meeting of the International Society for Microbial Electrochemisty and Technology – EU-ISMET 2016. Roma 2016/09/26-28.

Palma, E., Daghio, M., Espinoza Tofalos, A., Franzetti, A., Cruz Viggi, C., Fazi, S., Petrangeli Papini, M., Aulenta, F., (2018). Anaerobic electrogenic oxidation of toluene in a continuous-flow bioelectrochemical reactor: Process performance, microbial community analysis, and biodegradation pathways. Environ. Sci. Water Res. Technol. 4, 2136–2145. https://doi.org/10.1039/c8ew00666k.

Palma, E., Daghio, M., Franzetti, A., Petrangeli Papini, M., Aulenta, F. (2017). The bioelectric well: a novel approach for in situ treatment of hydrocarbon-contaminated groundwater. Microb Biotechnol 11: 112–118.

Palmer C.D., Puls R.W. (1994). Natural Attenuation of Hexavalent Chromium in Groundwater and Soils. Technical Resource Guide. USEPA Ground Water Issue.

Palmer C.D., Wittbrodt P.R. (1991). Processes Affecting the Remediation of Chromium Contaminated Sites. J. Environ. Health. 92(6), 25–40.

Panagiotakis I., Dermatas D., Vatseris C., Chrysochoou M., Papassiopi N., Xenidis A., Vaxevanidou K. (2015). Forensic investigation of a Chromium (VI) groundwater plume in Thiva, Greece. J. Hazard. Mater. 281, 27–34.

Pang Y, Xie D, Wu B, Lv Z, Zeng X, Wei C, et al. (2013). Conductive polymer-mediated Cr(VI) reduction in a dual-chamber microbial fuel cell under neutral conditions. Synth Met. Elsevier;183:57–62.

Panneerselvam P., Choppala G., Kunhikrishnan A., Bolan N. (2013). Potential of Novel Bacterial Consortium for the Remediation of Chromium Contamination. Water, Air, & Soil Pollution, 224:1716.

Parsons, B.H. (2010). Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation - Final report. ESTCP Project ER-0627.

Patil S.A., Hägerhäll C., Gorton L., (2014). Electron transfer mechanisms between microorganisms and electrodes in bioelectrochemical systems. Bioanal. Rev. 1, 71–129. https://doi.org/10.1007/11663.

Patterson R.R., Fendorf S., Fendorf M. (1997). Reduction of hexavalent chromium by amorphous iron sulfide. Environmental Science and Technology, 31, 2039-2044.

Pham TH, Rabaey K, Aelterman P, Clauwaert P, De Schamphelaire L, Boon N, Verstraete W (2006). Microbial fuel cells in relation to conventional anaerobic digestion technology. Eng Life Sci 6:285–292.

Ping L., Qiyan F., Quingjun M., Tao Y. (2012). Electrokinetic remediation of chromium- and cadmium-contaminated soil from abandoned industrial site. Separation and Purification Technology 98, 216-220.

Pisciotta J. M., Zaybak Z., Call D. F., Nam J. Y., and Logan B. E. (2012). Enrichment of microbial electrolysis cell biocathodes from sediment microbial fuel cell bioanodes, vol. 78, no. 15.

Polti M.A., Atjián M.C., Amoroso M.J., Abate C.M. (2011). Soil chromium bioremediation: Synergic activity of actinobacteria and plants. International Biodeterioration & Biodegradation 65, 1175-1181.

Pous N, Puig S, Coma M, Balaguer MD, Colprim J. (2013). Bioremediation of nitrate-polluted groundwater in a microbial fuel cell. J Chem Technol Biotechnol. Sep;88(9):1690–6.

Pous, N., Balaguer, M.D., Colprim, J., Puig, S., (2018). Opportunities for groundwater microbial electro-remediation. Microb. Biotechnol. 11, 119–135. https://doi.org/10.1111/1751-7915.12866.

Pradhan D., Sukla L.B., Sawyer M. et al. (2017). Recent Bioreduction of Hexavalent Chromium in Wastewater Treatment: A Review. J. Ind. Eng. Chem. 55, 1–20.

Puls R.W., Paul C.J., Powell R.M. (1999). The application of in situ permeable reactive (zero-valent iron) barrier technology for the remediation of chromate-contaminated groundwater: a fiel test. Applied Geochemistry 14, 989–100.

Qu M., Chen J., Huang Q. et al. (2018). Bioremediation of Hexavalent Chromium Contaminated Soil by a Bioleaching System with Weak Magnetic Fields. Int. Biodeterior. Biodegradation. 2018, 128, 41–47.

Rabaey K, Angenent LTT, Schröder U, Keller J, (2009). Bioelectrochemical Systems: From Extracellular Electron Transfer to Biotechnological Application. Vol. 8. London: IWA Publishing; 488 p.

Rabaey K, Rozendal RA (2010). "Microbial electrosynthesis — revisiting the electrical route for microbial production". Nat Rev Microbiol 8:706–16. doi: 10.1038/nrmicro2422.

Rai D, Eary LE, Zachara JM. (1989). Environmental chemistry of chromium. Sci Total Environ. Elsevier; 86(1–2):15–23.

Rajeswari V, Janaki V, Shanthi K, Kamala-Kannan S. (2016). Adsorption and subsequent detoxification of hexavalent chromium in aqueous solution using polypyrrole-bacterial extracellular polysaccharide nanocomposite. Environ Prog Sustain Energy. Wiley-Blackwell; 35(5):1293–7.

Rakoczy J., Feisthauer S., Wasmund K., Bombach P., Neu T.R., Vogt C., Richnow H.H. (2013). Benzene and sulfide removal from groundwater treated in a microbial fuel cell, Biotechnol. Bioeng. 110(12): 3104–3113.

Ramachandriya KD, Kundiyana DK, Wilkins MR, Terrill JB, Atiyeh HK, Huhnke RL (2013). Carbon Dioxide Conversion to Fuels and Chemicals Using a Hybrid Green Process". Applied Energy 112: 289–99.

Ranieri, E.; Petros, G. (2014). Effects of plants for reduction and removal of hexavalent chromium from a contaminated soil. Water Air Soil Pollut. 225, doi:10.1007/s11270-014-1981-2.

Raptis S., Gasparatos D., Economou-Eliopoulos M., Petridis, A., (2018). Chromium uptake by lettuce as affected by the application of organic matter and Cr(VI)-irrigation water: Implications to the land use and water management. Chemosphere 210, 597–606.

Rayment, G.E. and F.R. Higginson. (1992). Australian Laboratory Handbook of Soil and Water Chemical Methods. Vol. 3. Inkata Press, Melbourne, AUS.

Read S.T., Dutta P., Bond P.L., Keller J., Rabaey K., (2010). Initial development and structure of biofilms on microbial fuel cell anodes. BMC Microbiol. 10. https://doi.org/10.1186/1471-2180-10-98.

Regione Lombardia (2001). Acque sotterranee in lombardia, gestione sostenibile di una risorsa strategica.

Regione Lombardia (2016). Programma Regionale di Gestione dei Rifiuti - Piano Regionale di Bonifica delle Aree Inquinate. Programma di monitoraggio integrato PRGR/PRB-VAS.

Ricardo AR, Carvalho G, Velizarov S, Crespo JG, Reis MAM. (2012). Kinetics of nitrate and perchlorate removal and biofilm stratification in an ion exchange membrane bioreactor. Water Res. 46(14):4556–68.

Richard, F.C., and A.C.M. Bourg. (1991). Aqueous geochemistry of chromium: a review. Water Resources. 25: 807–816.

Rimboud M., Desmond-Le Q., Erable B., Bouchez T., Bergel A. (2015). Multi-System Nernst-Michaelis-Menten Model Applied to Bioanodes Formed from Sewage Sludge. Bioresource Technology 195: 162–69.

Rosenbaum, M., Aulenta, F., Villano, M. et al. (2011). Cathodes as electron donors for microbial metabolism: Which extracellular electron transfer mechanisms are involved? Bioresource Technology 102: 324–333.

Rousseau, R., Délia, M.L., Bergel, A., 2014. A theoretical model of transient cyclic voltammetry for electroactive biofilms. Energy Environ. Sci. 7, 1079–1094. https://doi.org/10.1039/c3ee42329h

Rozendal, R.A., Hamelers, H.V.M., Rabaey, K., Keller, J., Buisman, C.J.N., (2008). Towards practical implementation of bioelectrochemical wastewater treatment. Trends Biotechnol. 26, 450–459. https://doi.org/10.1016/j.tibtech.2008.04.008.

Rynk R. (2004), Bioremediation With Cheese Whey, BioCycle, Vol. 45, No. 2, p. 26.

Sadik O.A., Noah N.M., Okello V.A., Sun Z. (2014). Catalytic Reduction of Hexavalent Chromium Using Palladium Nanoparticles: An Undergraduate Nanotechnology Laboratory, dx.doi.org/10.1021/ed300229r,Journal of Chemical Education, 91, 269−273.

Sagar, S., Dwivedi, A., Yadav, S., Tripathi, M., Kaistha, S.D., (2012). Hexavalent chromium reduction and plant growth promotion by Staphylococcus arlettae Strain Cr11. Chemosphere 86, 847–852. https://doi.org/10.1016/j.chemosphere.2011.11.031

Santini, M., Marzorati, S., Fest-Santini, S., Trasatti, S., Cristiani, P., (2017). Carbonate scale deactivating the biocathode in a microbial fuel cell. J. Power Sources 356, 400–407. https://doi.org/10.1016/j.jpowsour.2017.02.088.

Santoro C, Arbizzani C, Erable B, Ieropoulos I. (2017). Microbial fuel cells: From fundamentals to applications. A review. J Power Sources. Elsevier B.V; 356:225–44.

Saratale, G.D., Saratale, R.G., Shahid, M.K., Zhen, G., Kumar, G., Shin, H.S., Choi, Y.G., Kim, S.H., (2017). A comprehensive overview on electro-active biofilms, role of exo-electrogens and their microbial niches in microbial fuel cells (MFCs). Chemosphere 178, 534–547. https://doi.org/10.1016/j.chemosphere.2017.03.066

Sasaki, K., Tsuge, Y., Sasaki, D., Kondo, A., (2014). Increase in lactate yield by growing Corynebacterium glutamicum in a bioelectrochemical reactor. J. Biosci. Bioeng. 117, 598–601. https://doi.org/10.1016/j.jbiosc.2013.10.026.

Savannah River National Laboratory (SRNL). (2011). The Scenarios Approach to Attenuation-Based Remedies for Inorganic and Radionuclide Contaminants, SRNL-STI-2011-00459. Savannah River National Laboratory: Aiken, SC, USA, 2011.

Sawada A., Mori K., Tanaka S., Fukushima M., Tatsumi K. (2004). Removal of Cr(VI) from contaminated soil by electrokinetic remediation. Waste Management 24 (2004) 483-490.

Ščančar, J., and R. Milači. (2014). A critical overview of Cr speciation analysis based on high performance liquid chromatography and spectrometric techniques. Journal of Analytical and Atomic Spectrometry. 29: 427–443.

Scherer M.M., Richter S., Valentine R.L. (2000). Alvarez, P.J. J. Chemistry and Microbiology of Permeable Reactive Barriers for In Situ Groundwater Clean up. Crit. Rev. Environ. Sci. Technol. 30(3), 363-411.

Seaman, J. C., Bertsch, P. M., & Schwallie, L. (1999). In Situ Cr (VI) Reduction within and Aquifer Systems Using Fe (II) Solutions. Environ. Sci. Technol., 33(6), 938–944.

Sedlazeck K.P, Hollen D., Muller P. et al. (2017). Mineralogical and Geochemical Characterization of a Chromium Contamination in an Aquifer-A Combined Analytical and Modeling Approach. Applied Geochemistry. 87, 44–56.

Sethi R., Day S., Di Molfetta A. (2011). Clamshell vs. Backhoe excavation of permeable reactive barriers. Am. J. Environ. Sci. 7(5), 463-467.

Shah P., Strezov V., Nelson P.F. (2008). Speciation of chromium in Australian coals and combustion products. Fuel 102, 1-8.

Shantaram, A., Beyenal, H., Raajan, R. et al. (2005). Wireless Sensors Powered by Microbial Fuel Cells . Environmental Science & Technology 39: 5037–5042. Doi: 10.1021/es0480668.

Sharma S, Adholeya A. (2012). Hexavalent chromium reduction in tannery effluent by bacterial species isolated from tannery effluent contaminated soil. Vol. 5, Journal of Environmental Science and Technology. p. 142–54.

Sharma, P., Bihari, V., Agarwal, S.K., Verma, V., Kesavachandran, C.N., Pangtey, B.S., Mathur, N., Singh, K.P., Srivastava, M., Goel, S.K., (2012). Groundwater Contaminated with Hexavalent Chromium [Cr (VI)]: A Health Survey and Clinical Examination of Community Inhabitants (Kanpur, India). PLoS One 7, 3–9. https://doi.org/10.1371/journal.pone.0047877.

She P, Song B, Xing XH, Loosdrecht M Van, Liu Z (2006). Electrolytic stimulation of bacteria Enterobacter dissolvens by a direct current. Biochem Eng J 28(1):23–29.

Shi J, Zhao W, Liu C, Jiang T, Ding H. (2017). Enhanced performance for treatment of Cr (VI)-containing wastewater by microbial fuel cells with natural pyrrhotite-coated cathode. Water; 9(12):1–11.

Shi L, Dong H, Reguera G, Beyenal H, Lu A, Liu J, et al. (2016). Extracellular electron transfer mechanisms between microorganisms and minerals. Nature Reviews Microbiology Nature Publishing Group; Oct 30, p. 651–62.

Shupack S.I. (1991). The Chemistry of Chromium and Some Resulting Analytical Problems. Environmental Health Perspectives Vol. 92, pp. 7-11, 1991.

Singh R., Misra V., Singh R.P. (2011). Synthesis, characterization and role of zero-valent iron nanoparticle in removal of hexavalent chromium from chromium-spiked soil. Journal of Nanoparticle Research (2011), 13:4063-4073.

Singh R., Misra V., Singh R.P. (2012). Removal of Cr(VI) by Nanoscale Zero-valent Iron (nZVI) From Soil Contaminated with Tannery Wastes. Bulletin of Environmental Contamination and Toxicology (2012) 88:210-214.

Somenahally, A.C., Mosher, J.J., Yuan, T., Podar, M., Phelps, T.J., Brown, S.D., Yang, Z.K., Hazen, T.C., Arkin, A.P., Palumbo, A. V., Van Nostrand, J.D., Zhou, J., Elias, D.A., (2013). Hexavalent chromium reduction under fermentative conditions with lactate stimulated native microbial communities. PLoS One 8, 1–11. https://doi.org/10.1371/journal.pone.0083909

Song, T.T.-S., Jin, Y., Bao, J., Kang, D., Xie, J., shun Song, T., Jin, Y., Bao, J., Kang, D., Xie, J., (2016). Graphene/biofilm composites for enhancement of hexavalent chromium reduction and electricity production in a biocathode microbial fuel cell. J. Hazard. Mater. 317, 73–80. https://doi.org/10.1016/j.jhazmat.2016.05.055.

Sophia AC, Sai S. (2016) Modified microbial fuel cell for Cr(VI) reduction and simultaneous bio-electricity production. J Environ Chem Eng. Elsevier B.V.; 4(2):2402–9.

Sophia AC, Saikant S. (2016). Reduction of chromium(VI) with energy recovery using microbial fuel cell technology. J Water Process Eng. Jun;11:39–45.

Sotres, A., Díaz-Marcos, J., Guivernau, M., Illa, J., Magrí, A., Prenafeta-Boldú, F.X., Bonmatí, A., Viñas, M., (2015). Microbial community dynamics in two-chambered microbial fuel cells: Effect of different ion exchange membranes. J. Chem. Technol. Biotechnol. 90, 1497–1506. https://doi.org/10.1002/jctb.4465.

Squillace, P.J., Scott, J.C., Moran, M.J., Nolan, B.T., Kolpin, D.W., (2002). VOCs, pesticides, nitrate, and their mixtures in groundwater used for drinking water in the United States. Environ. Sci. Technol. 36, 1923–1930.

Srivastava S., Thakur I.S. (2006). Evaluation of bioremediation and detoxification potentiality of Aspergillus niger for removal of hexavalent chromium in soil microcosm. Soil Biology & Biochemistry 38, 1904–1911.

Storch P., Messer A., Palmer D. et al. (2002). Pilot test for in situ geochemical fixation of chromium (VI) using calcium polysulfide. Proceedings of the Third

International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Battelle Press, Monterey (CA).

Strycharz SM, Woodard TL, Johnson JP, et al. (2008). Graphite electrode as a sole electron donor for reductive dechlorination of tetrachlorethene by Geobacter lovleyi. Applied and Environmental Microbiology 74(19): 5943–5947.

Sukumar C., Janaki V., Kamala-Kannan S., Shanthi K. (2014). Biosorption of chromium(VI) using Bacillus subtilis SS-1 isolated from soil samples of electroplating industry. Clean Technologies and Environmental Policy, 16:405-413.

Suthersan S.S., LutesC.C., PalmerP.L., LenzoF., PayneF.C., LilesD.S., BurdickJ. (2002). Final Technical Protocol for Using Soluble Carbohydrates to Enhance Reductive Dechlorination of Chlorinated Aliphatic Hydrocarbons, Submitted to ESTCP and AFCEE under Contract #41624-99-C-8032.

Swietlik R., Milik A., Molenda M., Trojanowska M., Siwiec J. (2010). Chromium (III/VI) speciation in urban aerosol. Atmospheric Environment 45 (2011) 1364-1368.

SWRCB (State Water Resources Control Board), (2014). State Water Resources Control Board Division of Water Quality GAMA Program. GROUNDWATER INFORMATION SHEET Hexavalent Chromium, Revised November 2017.

Tahri Joutey N, Bahafid W, Sayel H, Ananou S, El Ghachtouli N. (2014). Hexavalent chromium removal by a novel Serratia proteamaculans isolated from the bank of Sebou River (Morocco). Environ Sci Pollut Res. 2014;21(4):3060–72.

Tandukar M., Huber S.J., Onodera T., Pavlostathis S.G. (2009). Biological chromium (VI) reduction in the cathode of a microbial fuel cell, Environ. Sci. Technol. 43:8159–8165.

Tandukar, M., Huber, S.J., Onodera, T., Pavlostathis, S.G., (2009a). Biological chromium(VI) reduction in the cathode of a microbial fuel cell. Environ. Sci. Technol. 43, 8159–8165. https://doi.org/10.1021/es9014184

Tandukar, M., Huber, S.J., Onodera, T., Pavlostathis, S.G., Engineering, E., (2009b). Biological Chromium (VI) Reduction in the Cathode of a Microbial Fuel Cell. Environ. Sci. Technol. 43, 8159–8165. https://doi.org/10.1021/es9014184

Tang J., Johannesson K.H. (2002). Speciation of rare earth elements in natural terrestrial waters: Assessing the role of dissolved organic matter from the modeling approach. Geochimica et Cosmochimica Acta, Vol. 67, No. 13, pp. 2321-2339, 2003.

Tang S.C.N., Yin K., Lo I.M.C. (2011). Column study of Cr(VI) removal by cationic hydrogel for in-situ remediation of contaminated groundwater and soil. J. Contam. Hydrol. 125(1–4), 39–46.

Thapa B., Kumar A., Ghimire A. (2012). Review on bioremediation of petroleum hydrocarbon contaminants in soil. Kathmandu university journal of science, engineering and technology Vol. 8, No. I, pp 164-170.

Thatoi H., Das S., Mishra J. et al. (2014). Bacterial Chromate Reductase, a Potential Enzyme for Bioremediation of Hexavalent Chromium: A Review. J. Environ. Manage. 146, 383–99.

Theologou E., Panagiotakis I., Dermatas D., Chrysochoou M., Toskos T., (2013). Remediation Technologies for Hexavalent Chromium Contaminated Aquifers. Proc. 13th Internatonal Conf. Environ. Sci. Technol. 5–7.

Thiruvenkatachari R., Vigneswaran S., Naidu R. (2008), Permeable reactive barrier for groundwater remediation, Journal of Industrial and Engineering Chemistry 14: 145-156.

Thornton E.C., Amonette J.E. (1999). Hydrogen Sulfide Gas Treatment of Cr(VI)-Contaminated Sediment Samples from a Plating-Waste Disposal Sites – Implications for in-Situ Remediation. Environmental Science and Technology 1999, 33, 4096-4101.

Thornton E.C., Gilmore T.J., Olsen K.B. et al. (2007). Treatment of a Chromate-Contaminated Soil Site by in Situ Gaseous Reduction. Ground Water Monit. R. 27(1), 56–64.

Tiwari, A.K., Orioli, S., De Maio, M., (2019). Assessment of groundwater geochemistry and diffusion of hexavalent chromium contamination in an industrial town of Italy. J. Contam. Hydrol. 225, 103503. https://doi.org/10.1016/j.jconhyd.2019.103503.

Tiwary R.K., Dhakate R., Ananda Rao V., Singh V.S. (2005), Assessment and prediction of contaminant migration in ground water from chromite waste dump. Environ Geol, 48: 420-429.

Tremblay P, Zhang T (2015). Electrifying Microbes for the Production of Chemicals. Frontiers in Microbiology. March2015|Volume6|Article201.

Truex M., Brady P., Newell C., Denham M., Vangelas K. (2011). The Scenarios Approach to Attenuation-Based Remedies for Inorganic and Radionuclide Contaminants.

Tseng JK, Bielefeldt AR. Low-Temperature Chromium(VI) Biotransformation in Soil with Varying Electron Acceptors. (2002). J Environ Qual; 31(6):1831–41.

Turick CE, Graves C, Apel WA. (1998). Bioremediation potential of Cr(VI)-contaminated soil using indigenous microorganisms. Bioremediat J; 2(1):1–6.

Unceta N., Séby F., Malherbe J., Donard O.F.X. (2010). Chromium speciation in solid matrices and regulation: a review. Analytical and Bioanalytical Chemistry, 397:1097-1111.

United States Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chromium, (2012). Available online: https://www.atsdr.cdc.gov/toxprofiles.

US Department of Agriculture (2017). In Soil Survey Manual - Handbook No. 18; Soil Science Division Staff. Washington, DC, USA.

US Department of Health (2009). Hexavalent Chromium. US Department of Labor Occupational Safety and Health Administration: Washington DC, USA,.

US Department of Health (2012). Toxicological profile for chromium. Technical Resource Guide. United States Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry.

USDA (2017) Soil survey manual. Technical Resource Guide. Soil Science Division Staff. C. Ditzler, K. Scheffe, and H.C. Monger (eds.). Handbook 18. Government Printing Office, Washington, D.C.

USDE (2011) The Scenarios Approach to Attenuation-Based Remedies for Inorganic and Radionuclide Contaminants. Technical Resource Guide. SRNL-STI-2011-00459.

USEPA (1988). In Situ Permeable Reactive Barrier for Contaminated Groundwater at the U.S. Coast Guard Support Center Elisabeth City, North Carolina. United States Environmental Protection Agency: Washington, DC, USA, 1988.

USEPA (1989). Injection Wells: An Introduction to Their Use, Operation, and Regulation. United States Environmental Protection Agency, Office of Drinking Water: Washington, DC, USA, 1989.

USEPA (1997). Recent Developments for In Situ Treatment of Metal Contaminated Soils.

USEPA (1998a). In situ Permeable Reactive Barrier for Contaminated Groundwater at the U.S. Coast Guard Support Center Elisabeth City, North Carolina. Technical Resource Guide.

USEPA (1998b). Toxicological review of hexavalent chromium. Technical Resource Guide.Washington, DC, USA.

USEPA (1999). Groundwater Cleanup: Overview of Operating Experience at 28 Sites, EPA-542-R-99-006, U.S. Environmental Protection Agency, Washington, DC, USA.

USEPA (2000). In Situ Treatment of Soil and Groundwater Contaminated with Chromium. Technical Resource Guide. National Risk Management Research Laboratory, EPA625/R-00/004, 1–82. Washington, DC, USA.

USEPA (2002). Injection Wells. An introduction to their use, operation, and regulation. Technical Resource Guide. Groundwater Protection Council.

USEPA (2007). Treatment technologies for site cleanup: annual status report (Twelfth Edition), EPA-542-R-07–012, U.S. Environmental Protection Agency, Washington, DC, USA.

USEPA (2008). Proven Technologies and Remedies Guidance–Remediation of Metals in Soil. California Environmental Protection Agency, Department of Toxic Substances Control: Sacramento, CA, USA.

USEPA (2009). Interim Advisory for Green Remediation. Technical Resource Guide. Department of Toxic Substances Control.

USEPA (2011). Remediation of Metals in Soil. Technical Resource Guide. Department of Toxic Substances Control, Proven Technologies And Remedies Guidance.

USEPA (2012). Introduction to In Situ Bioremediation of Groundwater. Technical Resource Guide. Office of Solid Waste and Emergency Response, 542-R-13-018, 1–63. Office of Solid Waste and Emergency Response: Cincinnati, OH, USA.

USEPA (2017a). A Citizen's guide to Permeable Reactive Barriers. Technical Resource Guide, EPA 542-F-12-017.

USEPA (2017b). Chromium in Drinking Water. Last updated on april 24, 2017. https://www.epa.gov/dwstandardsregulations/chromium-drinking-water#if-decide.

Uzielli M., Simonini P., Cola S. (2008). Statistical identification of homogeneous soil units for Venice lagoon soils. Proceedings of the 3rd International Conference on Site Characterization, Taiwan. 1209–16.

Vanbroekhoven K., Vermoortel Y., Diels L., Gemoets J. (2007). Stimulation of in situ bioprecipitation for the removal of hexavalent chromium from contaminated groundwater. IMWA Symposium 2007: Water in Mining Environments, R. Cidu & F. Frau (Eds), 27th - 31st May 2007, Cagliari, Italy.

Varadharajan C, Beller HR, Bill M, Brodie EL, Conrad ME, Han R, et al. (2017). Reoxidation of Chromium(III) Products Formed under Different Biogeochemical Regimes. Environ Sci Technol. American Chemical Society;51(9):4918–27.

Varjani, S.J., Upasani, V.N., (2017). A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. Int. Biodeterior. Biodegrad. 120, 71–83. https://doi.org/10.1016/j.ibiod.2017.02.006.

Vengosh, A., Coyte, R., Karr, J., Harkness, J.S., Kondash, A.J., Ruhl, L.S., Merola, R.B., Dywer, G.S., (2016). Origin of Hexavalent Chromium in Drinking Water Wells from the Piedmont Aquifers of North Carolina. Environ. Sci. Technol. Lett. 3, 409–414. https://doi.org/10.1021/acs.estlett.6b00342.

Vesselinov V, Katzman D, Broxton D, Birdsell K, Reneau S, Vaniman D, Longmire P, Fabryka-Martin J, Heikoop J, M.D., (2013). Data and Model-Driven Decision Support for Environmental Management of a Chromium Plume at Los Alamos National Laboratory – 13264. WM2013 Conference, February 24 – 28, 2013,

Phoenix, Arizona USA.

Virdis B., Dominguez-Benetton X. (2018). Pracal Aspects of Cyclic voltammetry Basic principles of CV Nonturnover vs turnover CV. Proceeding of EU-ISMET.

Viti C, Marchi E, Decorosi F, Giovannetti L. (2004). Molecular mechanisms of Cr(VI) resistance in bacteria and fungi. FEMS Microbiol Rev. 38(4):633–59.

Viti C., Mini A., Ranalli G. et al. (2006). Response of Microbial Communities to Different Doses of Chromate in Soil Microcosms. App. Soil Ecol. 34, 125–39.

Vocciante M., Caretta A., Bua L. et al. (2016). Enhancements in ElectroKinetic Remediation Technology: Environmental assessment in comparison with other configurations and consolidated solutions. Chem. Eng. J. 289, 123–134.

Wang G, Huang L, Zhang Y. (2008). Cathodic reduction of hexavalent chromium [Cr(VI)] coupled with electricity generation in microbial fuel cells. Biotechnol Lett; 30(11):1959–66.

Wang H, Luo H, Fallgren PH, Jin S, Ren ZJ. (2015). Bioelectrochemical system platform for sustainable environmental remediation and energy generation. Biotechnol. Adv. 33(3–4):317–34

Wang H, Ren ZJ. (2014). Bioelectrochemical metal recovery from wastewater: A review. Vol. 66, Water Research. p. 219–32.

Wang H., Ren Z.J. (2013). A comprehensive review of microbial electrochemical systems as a platform technology, Biotechnol. Adv. 31(8): 1796−1807.

Wang P.C., Mori T., Toda K., Ohtake H. (1990). Membrane-associated chromate reductase activity from Enterobacter cloacae, J. Bacteriol. 172(3):1670–1672

Wang PC, Mori T, Komori K, Sasatsu M, Toda K, Ohtake H. (1989). Isolation and characterization of an Enterobacter cloacae strain that reduces hexavalent chromium under anaerobic conditions. Appl Environ Microbiol; 55(7):1665–9.

Wang Q, Huang L, Pan Y, Quan X, Li Puma G. (2017). Impact of Fe(III) as an effective electron-shuttle mediator for enhanced Cr(VI) reduction in microbial fuel cells: Reduction of diffusional resistances and cathode overpotentials. J Hazard Mater. Elsevier B.V.;321:896–906.

Wang X., Cai Z., Zhou Q., Zhang Z., Chen C. (2012). Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells, Biotechnol. Bioeng. 109(2): 426−433.

Wang Y., Pan Z., Lang J., Xu J., Zheng Y. (2007). Bioleaching of chromium from tannery sludge by indigenous Acidithiobacillus thiooxidans. Journal of Hazardous Materials 147, 319–324.

Wang, C., Deng, H., Zhao, F., (2016). The Remediation of Chromium (VI)-Contaminated Soils Using Microbial Fuel Cells. Soil Sediment Contam. 25, 1–12. https://doi.org/10.1080/15320383.2016.1085833.

Wang, H., Luo, H., Fallgren, P.H., Jin, S., Ren, Z.J., (2015). Bioelectrochemical system platform for sustainable environmental remediation and energy generation. Biotechnol. Adv. 33, 317–334. https://doi.org/10.1016/j.biotechadv.2015.04.003

Wang, H., Zhang, S., Wang, J., Song, Q., Zhang, W., He, Q., Song, J., Ma, F., (2018). Comparison of performance and microbial communities in a bioelectrochemical system for simultaneous denitrification and chromium removal: Effects of pH. Process Biochem. 73, 154–161. https://doi.org/10.1016/j.procbio.2018.08.007

Wang, Y., Qian, P.Y., (2009). Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One 4. https://doi.org/10.1371/journal.pone.0007401.

Wei X., Guo S., Wu B. et al. (2016). Effects of reducing agent and approaching anodes on chromium removal in electrokinetic soil remediation. Front. Env. Sci. Eng. 10(2), 253–261.

WHO World Health Organization (2004). Chromium in drinkingwater. Guidelines for drinking-water quality, 2nd ed.; World Health Organization: Geneva, 2004; Vol. 2 (health criteria and other supporting information).

WHO World Health Organization (2013). Inorganic Chromium(VI) Compounds. International Programme on Chemical Safety (IPCS), Concise International Chemical Assessment Document 78.

WHO (2000). WHO Regional Office for Europe, Copenhagen, Denmark, 2000. http://www.euro.who.int/__data/assets/pdf_file/0017/123074/AQG2ndEd_6_4Chromium.

Wick LY, Mattle PA, Wattiau P, Harms H (2004). Electrokinetic transport of PAH-degrading bacteria in model aquifers and soil. Enviro Sci Technol 38(17):4596–4602.

Wieczorek S., Weigand H., Schmid M., Marb C. (2005). Electrokinetic remediation of an electroplating site: design and scale-up for an in-situ application in the unsaturated zone. Engineering Geology 77 (2005) 203–215.

Wielinga B., Mizuba M.M., Hansel C.M., Fendorf S. (2001). Iron Promoted Reduction of Chromate by Dissimilatory Iron-Reducing Bacteria. Environmental Science and Technology 2001, 35, 522-527.

Wilkin R.T., AcreeS.D., Ross R.R., Puls R-W-. Lee T.R., Woods L.L. (2014), Fifteen-year assessment of a permeable reactive barrier for treatment of chromate and trichloroethylene in groundwater, Science of the Total Environment, 468-469, 186-194.

Williams KH, Nevin KP, Franks A, Englert A, Long PE, Lovley DR. (2010). Electrode-based approach for monitoring in situ microbial activity during subsurface bioremediation. Environ Sci Technol; 44(1):47–54.

Wu J, Zhang J, Xiao C. (2016a). Focus on factors affecting pH, flow of Cr and transformation between Cr(VI) and Cr(III) in the soil with different electrolytes. Electrochim Acta. Pergamon; 211:652–62.

Wu X, Tong F, Yong X, Zhou J, Zhang L, Jia H, et al. (2016b). Effect of NaX zeolite-modified graphite felts on hexavalent chromium removal in biocathode microbial fuel cells. J Hazard Mater. Elsevier B.V.; 2016;308:303–11.

Wu X, Zhu X, Song T, Zhang L, Jia H, Wei P. (2015). Effect of acclimatization on hexavalent chromium reduction in a biocathode microbial fuel cell. Bioresour Technol. Elsevier Ltd; 2015;180:185–91.

Wu Y, Qi H, Gao Y, Wang L. (2018a). Heavy Metal Sensor Research Based on Microbial Fuel Cell. Int J Environ Monit Anal. 2018a;6(2):53–64.

Wu, X., Ren, X., Owens, G., Brunetti, G., Zhou, J., (2018b). A Facultative Electroactive Chromium (VI) -Reducing Bacterium Aerobically Isolated From a Biocathode Microbial Fuel Cell. Front. Microbiol. 9, 1–10. https://doi.org/10.3389/fmicb.2018.02883

Wu, Y., Jing, X., Gao, C., Huang, Q., Cai, P., (2018c). Recent advances in microbial electrochemical system for soil bioremediation. Chemosphere 211, 156–163. https://doi.org/10.1016/j.chemosphere.2018.07.089

Xafenias, N., Anunobi, M.O., Mapelli, V., (2015a). Electrochemical startup increases 1,3-propanediol titers in mixed-culture glycerol fermentations. Process Biochem. 50, 1499–1508. https://doi.org/10.1016/j.procbio.2015.06.020

Xafenias, N., Mapelli, V., (2014). Performance and bacterial enrichment of bioelectrochemical systems during methane and acetate production. Int. J. Hydrogen Energy 39, 21864–21875. https://doi.org/10.1016/j.ijhydene.2014.05.038

Xafenias, N., Zhang, Y., Banks, C.J., (2013). Enhanced performance of hexavalent chromium reducing cathodes in the presence of Shewanella oneidensis MR-1 and lactate. Environ. Sci. Technol. https://doi.org/10.1021/es304606u

Xafenias, N., Zhang, Y., Banks, C.J., Banks, N.X., (2015b). Evaluating hexavalent chromium reduction and electricity production in microbial fuel cells with alkaline cathodes. Int. J. Environ. Sci. Technol. 12, 2435–2446. https://doi.org/10.1007/s13762-014-0651-7.

Xie D, Yu H, Li C, Ren Y, Wei C, Feng C. (2014). Competitive microbial reduction of perchlorate and nitrate with a cathode directly serving as the electron donor. Electrochim Acta. Pergamon;133:217–23.

Xie Y.; Cwiertny D.M. (2010). Use of Dithionite to Extend the Reactive Lifetime of Nanoscale Zero-Valent Iron Treatment Systems. Environ. Sci. Technol. 44, 8649–8655.

Xu X., Li H., Li X. et al. (2004). Reduction of hexavalent chromium by ascorbic acid in aqueous solutions. Chemosphere. 57, 609-613.

Xue, G., Lai, S., Li, X., Zhang, W., You, J., Chen, H., Qian, Y., Gao, P., Liu, Z., Liu, Y., (2018). Efficient bioconversion of organic wastes to high optical activity of L-lactic acid stimulated by cathode in mixed microbial consortium. Water Res. 131, 1–10. https://doi.org/10.1016/j.watres.2017.12.024.

Yan D.Y.S., Lo I.M.C. (2012a). Pyrophosphate coupling with chelant-enhanced soil flushing of field contaminated soils for heavy metal extraction. J. Hazard. Mater. 199–200, 51–57.

Yan Y., Xue F., Muhammad F. et al. (2018). Application of iron-loaded activated carbon electrodes for electrokinetic remediation of chromium-contaminated soil in a three-dimensional electrode system. Scientific Reports. 8(1), 1–11.

Yan, Z., Song, N., Cai, H., Tay, J.-H., Jiang, H. (2012b). Enhanced degradation of phenanthrene and pyrene in freshwater sediments by combined employment of sediment microbial fuel cell and amorphous ferric hydroxide. J. Hazard. Mater. 199-200: 217–225.

Yang J., He M., Wang G. (2009). Removal of toxic chromate using free and immobilized Cr(VI)-reducing bacterial cells of Intrasporangium sp Q5-1, World J. Microbiol. Biotechnol. 25:1579-1587.

Yang Y., Ding Y., Hu Y., Cao B., Rice S. A., Kjelleberg S., and Song H. (2015). "Enhancing Bidirectional Electron Transfer of Shewanella oneidensis by a Synthetic Flavin Pathway," ACS Synth. Biol., vol. 4, no. 7, pp. 815–823.

Yaqiao S., Lei D. (2016). The influence mechanism of ash-flushing water from power plant on groundwater environment. Proceeding of 4th International Conference on Bioinformatics and Biomedical Engineering, IWBBIO 2016, Granada, Spain, (40772160), 4–7.

Yeon RE, Kim M, Lee SJ. (2011). Characterization of microbial fuel cells enriched using Cr(VI)-containing sludge. J Microbiol Biotechnol; 21(2):187–91.

Yoon I.H., Kim K.W. (2018). Effect of pH and Dissolved Oxygen on the Cr(VI) Removal by Zero-Valent Iron. (unpublished; manuscript in preparation).

Yun, H., Liang, B., Kong, D., Wang, A., (2018). Improving biocathode community multifunctionality by polarity inversion for simultaneous bioelectroreduction processes in domestic wastewater. Chemosphere 194, 553–561. https://doi.org/10.1016/j.chemosphere.2017.12.030

Zaybak Z., Pisciotta J. M., Tokash J. C., and Logan B. E. (2013). "Enhanced start-up of anaerobic facultatively autotrophic biocathodes in bioelectrochemical systems," J. Biotechnol., vol. 168, no. 4.

Zayed A.M., Terry N. (2003). Chromium in the environment: factors affecting biological remediation. Plant and Soil 249: 139-156, 2003.

Zhang JL, Cao ZP, Zhang HW, Zhao LM, Sun XD, Mei F (2013). Degradation characteristics of 2,4-dichlorophenoxyacetic acid in electro-biological system. J Hazard Mater 262:137–142.

Zhang P., Jin C., Zhao Z. et al. (2010a). 2D Crossed Electric Field for Electrokinetic Remediation of Chromium Contaminated Soil. J. Hazard. Mater. 177(1–3), 1126–33.

Zhang T., Gannon S.M., Nevin K.P., Franks A.E., Lovley D.R. (2010b). Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor, Environ. Microbiol. 12(4): 1011−1020.

Zhang X, Feng H, Shan D, Shentu J, Wang M, Yin J, Shen D, Huang B, Ding Y (2014). The effect of electricity on 2-fluoroaniline removal in a bioelectrochemically assisted microbial system (BEAMS). Electrochim Acta 135:439–446.

Zhang, D., Li, Z., Zhang, C., Zhou, X., Xiao, Z., Awata, T., and Katayama, A. (2017). Phenol-degrading anode biofilm with high coulombic efficiency in graphite electrodes microbial fuel cell. J Biosci Bioeng 123: 364–369.

Zhao HP, Van Ginkel S, Tang Y, Kang DW, Rittmann B, Krajmalnik-Brown R. (2011). Interactions between perchlorate and nitrate reductions in the biofilm of a hydrogen-based membrane biofilm reactor. Environ Sci Technol; 45(23):10155–62.

Zhao S, Liu P, Niu Y, Chen Z, Khan A, Zhang P, et al. (2018). A Novel Early Warning System Based on a Sediment Microbial Fuel Cell for In Situ and Real Time Hexavalent Chromium Detection in Industrial Wastewater. Sensors (Basel). Multidisciplinary Digital Publishing Institute (MDPI); 18(2).

Zhou H, Liu B, Wang Q, Sun J, Xie G, Ren N, Ren ZJ, Xing D (2017a). Pulse electromagnetic fields enhance extracellular electron transfer in magnetic bioelectrochemical systems. Biotechnol Biofuels 10(1):238.

Zhou T, Han H, Liu P, Xiong J, Tian F, Li X. (2017b). Microbial fuels cell-based biosensor for toxicity detection: A review. Vol. 17, Sensors (Switzerland). Multidisciplinary Digital Publishing Institute; p. 2230.

Zhou, D.M., Deng, C.F., Cang, L., Alshawabkeh, A.N., (2005). Electrokinetic remediation of a Cu-Zn contaminated red soil by controlling the voltage and conditioning catholyte pH. Chemosphere 61, 519–527. https://doi.org/10.1016/j.chemosphere.2005.02.055.

ATTACHMENT A – REVIEW TABLE

Table 4.1: Summary of key studies regarding Cr(VI) reduction in BESs.

BES Type[1]	Anode material	Anodic inoculum/ mediator[2]	Cathode material	Cathodic inoculum/ mediator	Cathode Potential [V vs. SHE]	Initial Cr(VI) [mg/L]	Initial pH	Test period [h]	Cr^{VI} removal [%]	Rate [$mgCr^{VI}/L/h$] (Specific rate [$mgCr^{VI}/g_{SSV}/h$])[3]	Refs
2CMFC-PEM, H(b)	graphite plate	anaerobic sludge; ED: acetate	graphite plate	Cr(VI) enriched denitrifying and anaerobic mixed culture	NA	22	7.2-7.6	552	100	0.14 (0.18)	(Tandukar et al., 2009a)
						31				(0.22)	
						40				(0.36)	
						63				0.45 (0.46)	
2CMFC, PEM, H(b)	graphite plate	MFC effluent; ED: acetate	graphite plate and granules	Anaerobic mixed culture from contaminated soil	NA	13	≈7	7	100	3.8 (2)	(Huang et al., 2010)
					NA	39				5.3 (2.4)	
2CMFC, CEM, TR(b)	graphite fiber	effluent and acclimated anode from MFC	graphite fiber (C/A=3)	MFC anaerobic effluent	0.013	20	5	3	98.5	6.57	(Huang et al., 2011b)
					0.03		8		61	4.07	
					NA	40	7	3.5	75	8.57	
					-0.05	50		3	NA	(5.2)	
					NA	20		5	100	4.08 (12.4)	
			graphite fiber (C/A=10)					4		5.12 (15.5)	
			graphite fiber (C/A=20)					3		6.8 (20.6)	
			graphite fiber (C/A=3)					5	90.25	3.61 ± 0.1 (11.3 ± 2.2)	
			graphite felt (C/A=3)						76.75	3.07 ± 0.12 (9.5 ± 1)	
			graphite granules (C/A=3)						86.5	3.46 ± 0.09 (5.6 ± 0.6)	
2CMFC, CEM, TR(b)	graphite brush	anaerobic wastewater ED: acetate	graphite granules	WWTP primary clarifier effluent	NA	20	7	24	70	0.6	(Huang et al., 2011a)
2CBES (poised cathode), CEM, TR(b)	graphite brush		graphite granules		-0.45				55	0.43	
					-0.3				100	0.83	
					-0.15				100	0.83	
					0.2				43	0.36	

BES Type[(1)]	Anode material	Anodic inoculum/ mediator[(2)]	Cathode material	Cathodic inoculum/ mediator	Cathode Potential [V vs. SHE]	Initial Cr(VI) [mg/L]	Initial pH	Test period [h]	Cr^{VI} removal [%]	Rate [$mgCr^{VI}/L/h$] (Specific rate [$mgCr^{VI}/g_{SSV}/h$])[(3)]	Refs
2CMFC, CEM, H(sb)	reticulated vitreous carbon	Shewanella oneidensis MR-1; ED: lactate	reticulated vitreous carbon	Shewanella oneidensis MR1	NA	2.5 (3 cycles)	7	72 (each cycle)	60-100	0.02-0.04	(Hsu et al., 2012)
				Shewanella putrefaciens					92-100	0.03-0.04	
				Shewanella amazonensis					72-100	0.025-0.04	
				Shewanella sp ANA3					32-100	0.01-0.04	
				Shewanella loihica					44-100	0.015-0.04	
				Shewanella sp MR-4					20-100	0.007-0.04	
2CMFC, PEM, H(b)	graphite felt	S.oneidensis MR-1; ED: lactate	graphite felt	Shewanella oneidensis MR-1	NA	10 (6 cycles)	7	300	90	0.1 – 1.1	(Xafenias et al., 2013a)
2CBES (poised cathode), PEM, H(b)	graphite felt	Shewanella oneidensis MR-1, ED: lactate	graphite felt	Shewanella oneidensis MR1					10	0.5	
				Shewanella oneidensis MR1; MED: riboflavin					13	0.65	
				Shewanella oneidensis MR1; ED: lactate	-0.3	20	7	4	45	2.25	
SCMFC (b)	carbon brush	municipal wastewater; ED: acetate	carbon cloth with Pt	municipal wastewater; ED: acetate	2.93	1	6.5	120	89	0.01	(Li et al., 2014)
					3.03	3			95.7	0.02	
					3.13	10			98.8	0.08	
2CMFC, CEM, TR(b)	graphite brush	MFC anodic effluent; ED: acetate	graphite felt	WWTP primary clarifier effluent acclimatated to Cr(VI)	-0.074	5	5.8	4	100	1.21	(Huang et al., 2015)
					NA	5 (with Cu(II), Cd(II))			39	0.49	
				WWTP primary clarifier effluent, acclimated to Cr(VI), Cu(II) and Cd(II)	NA	5 (Cu(II), Cd(II))			100	1.24	

BES Type[(1)]	Anode material	Anodic inoculum/ mediator[(2)]	Cathode material	Cathodic inoculum/ mediator	Cathode Potential [V vs. SHE]	Initial Cr(VI) [mg/L]	Initial pH	Test period [h]	Cr^{VI} removal [%]	Rate [mgCr^{VI}/L/h] (Specific rate [mgCr^{VI}/g_{SSV}/h])[(3)]	Refs
2CMFC, PEM, H(b)	graphite felt	Shewanella oneidensis	graphite felt	Shewanella oneidensis	NA	10 (8 cycles)	8	840	90-100	0.9-1.95	(Xafenias et al., 2015b)
2CMFC, PEM, C(b)	graphite felt	anaerobic sludge; ED: glucose	graphite felt	mixed culture from MFC anode (ex situ)	NA	20	7	24	79.3	0.66	(Wu et al., 2015)
				anaerobic digester sludge enriched in presence of Cr(VI) (in situ)					20.2	0.17	
SCMEC, Cyl(c)	carbon rod	anaerobic sludge	graphite felt	anaerobic sludge	NA	100	7	16 (HRT)	43.12 - 96.68	2.69-6.04	(Chen et al., 2016)
						100 (200 NO_3^-, 100 p-FNB)			41.38	2.59	
						100 (200 NO_3^-, 150 p-FNB)	7		49.14	3.07	
						100 (200 NO_3^-, 200 p-FNB)			55.21	3.45	
						100 (200 NO_3^-, 300 p-FNB)			58.93	3.68	
2CMFC-PEM, C(b)	graphite felt	anaerobic sludge; ED: glucose	graphite felt	acclimated MFC anode	NA	20	7	5	28.3	1.13 ± 0.01	(Wu et al., 2016b)
			graphite felt/NaX		NA				69	2.76 ± 0.09	
			graphite felt/NaX-HNO_3		NA			3	100	10.39 ± 0.28	
2CMFC-PEM, H(b)	graphite felt	sewage sludge; ED: glucose	graphite felt	acclimated MFC	NA	40	7	48	58.3	0.49	(Song et al., 2016)
			graphene modified graphite felt						100	0.83	
2CMFC, CEM, Cyl(b)	graphite felt		graphite felt		-0.04	20	5.8	5	74 – 83	2.96 – 3.32	

BES Type[(1)]	Anode material	Anodic inoculum/ mediator[(2)]	Cathode material	Cathodic inoculum/ mediator	Cathode Potential [V vs. SHE]	Initial Cr(VI) [mg/L]	Initial pH	Test period [h]	Cr^{VI} removal [%]	Rate [mgCr^{VI}/L/h] (Specific rate [mgCr^{VI}/g_{SSV}/h])[(3)]	Refs
		MFC anodic effluent; ED: acetate		Stenotrophomonas sp., S. maltophilia, Serratia marcescens, Achromobacter xylosoxidans	-0.05	20 (20 mg/L Cd(II))			63 – 71	2.52 – 2.84	(Huang et al., 2018)
SCMEC, Cyl(c)	graphite rod	activated sludge	carbon felt	activated sludge	NA	30 (20 mg/L NO_3^-)	6	20 h HRT	58.96	0.88	(Wang et al., 2018b)
							7		72.65	1.09	
							8		65.08	0.98	
2CMFC-CEM, H(b)	graphite felt	anaerobic sludge	graphite felt	Bacillus Cereus	NA	27	7	25	100	2.56	(Wu et al., 2018a)

Notes: (1) 2CMFC: Double chamber Microbial Fuel Cell, 2CBES-poised cathode: potentiostatically controlled bioelectrochemical systems; SCMFC: Single-Chamber Microbial Fuel Cell, SCMEC: Single-Chamber Microbial Electrolysis Cell; 3CMDC: three-chamber Microbial Desalinization Cell, 3CMFC: three-chamber Microbial Fuel Cell (anode and double cathode); PEM: Proton Exchange Membrane, CEM: Cation Exchange Membrane, BPM: Bipolar membrane. H = H-type reactor; Cyl = Cylindrical reactor; C = cubic chambers reactor; TR = tubular reactor, (b) = batch mode operation, (sb) = semi-batch mode operation, i.e. continuous flow anode chamber and batch cathodic chamber; (c) continuous flow operation (2) ED: Electron Donor, MED: mediator, for chemical or biological activity; WWTP (Wastewater Treatment Plant); PPy (polypyrrole); AQS (9,10-anthraquinone-2-sulfonic acid sodium salt); AQDS (anthraquinone-2-sulfonate). (3) Specific rate normalized by the mass of suspended volatile solids (SSV) in the cathode compartment. NA: no available information.

ATTACHMENT B – IN-HOUSE POTENTIOSTAT

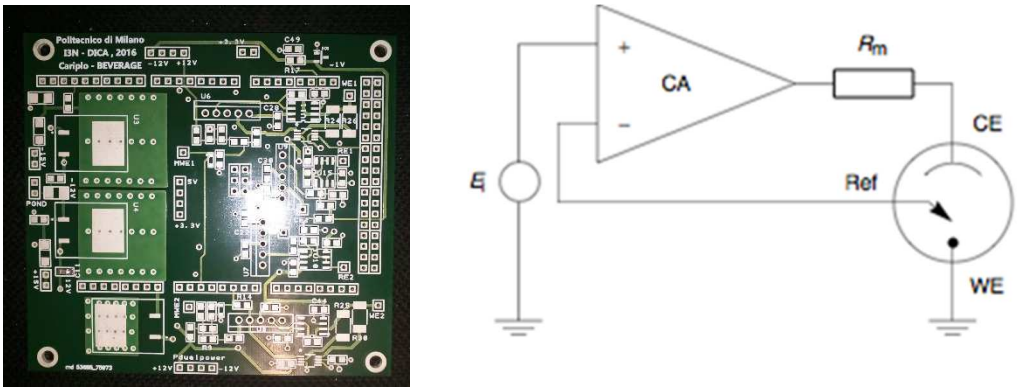
Such a device allows to set the electric potential of the working electrode in respect of a reference and to measure the current at the working electrode and the voltage at the counter electrode, and it is necessary for any research activity on BESs. The construction of a cost-effective instrument suitable for research purposes offered the chance to investigate in detail the operating methods of the system and the precautions in the tests set-up to prevent incorrect operation of the instruments (e.g. shielding to electrical interferences, etc.). Tests of BES with M3Cs could be performed.

A potentiostat is the electronic hardware required to control a three electrode cell and run most electroanalytical experiments. The system functions by maintaining the potential of the WE at a constant level with respect to the RE by adjusting the CE current. This equipment is fundamental to study reaction mechanisms related to BESs. Modern potentiostats are designed to interface and operate through a dedicated software package. Some peculiarities are:

- Electric potential range (measured and applied).
- Accuracy in potential (measured and applied).
- Range of scan rate: how slow or fast a potential window can be scanned. This is most important for experiments that require high scan rates, like in BES.
- Electric current range (measured and applied): the maximum range over which current can be sampled. Applying large currents is important for experiments that pass a great deal of current like a large bulk electrolysis. Measuring small currents is important for experiments that pass small currents like in BES.
- Current resolution: determines the operational range of a specific experiment.
- Accuracy in current (measured and applied).
- Number of working channels: how many WE can the instrument control. A polypotentiostat may be important for controlling biological experiments with more WE.
- Interface.

The in-house system was programmed starting from a card designed by Professor Giorgio Ferrari of the DEIB (Dipartimento di Elettronica, Informazione e

Bioingegneria) - Politecnico di Milano (Figure A.1a); built and realized by the staff of the labs and the PhD candidate. The collaboration established between the two departments and their respective laboratories is a positive result; direct collaboration in the creation and programming of the instrument, designed for the experimental tests covered by the thesis, allows flexibility in the development and modulation of the system, also for future instrumental modifications/implementations. The card, which has a microcontroller to allow the management of the instrument from a computer with a USB cable, is powered with a ± 15V input voltage and has 3 outputs: the reference electrode (RE), the working electrode (WE) and the counter-electrode (CE) (Figure A.1b). The project layout is represented in Figure A.2. Current and voltage signs adapt to the IUPAC convention; the current is positive when oxidation occurs.



a) b)

Figure A.1. Electronic board (a) and "schematic" of a 3-electrode potentiostat (b).

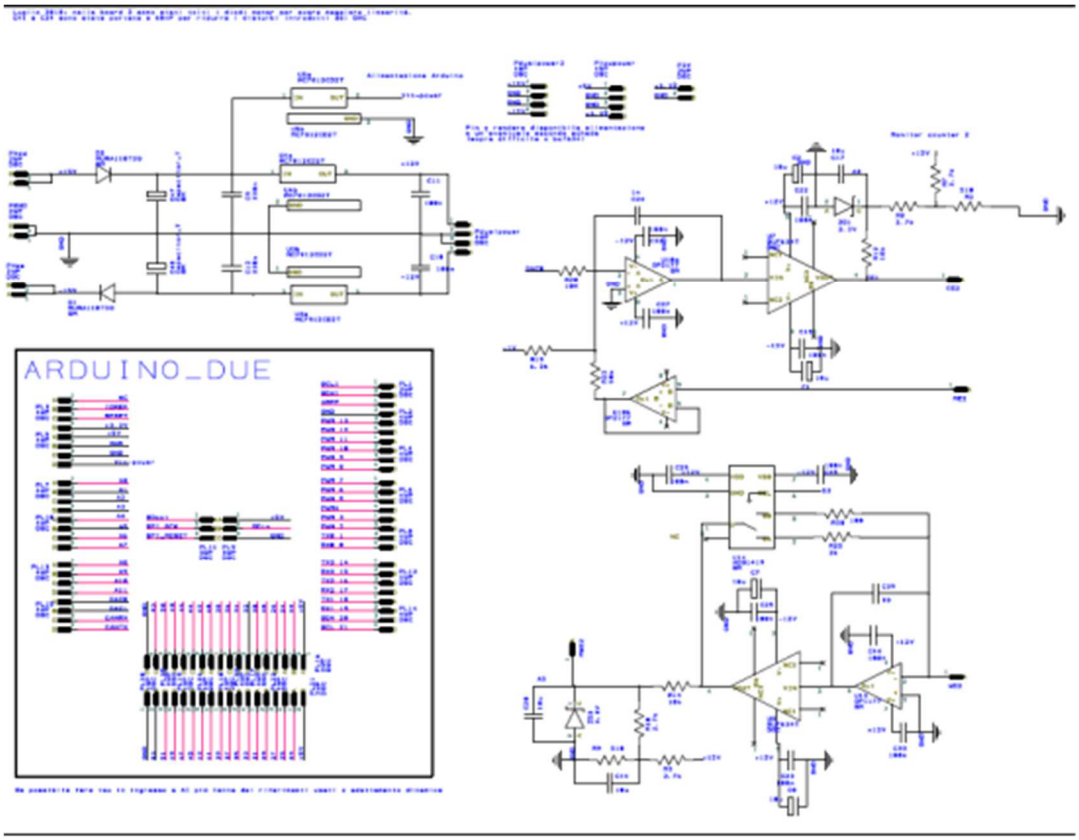


Figure A.2. Layout.

The input voltage is chosen between RE and WE between -5V and + 5V with an accuracy of about 10 mV; the current is read in the WE and the voltage in the CE. The CE voltage has a maximum value of about ± 10V. The current measurement has two selectable ranges: the maximum range is about 100 mA, the minimum 5 mA. The minimum measurable current is of the order of 1 μA. The system is completely bidirectional: the currents can be incoming or outgoing and the voltages both positive or negative.

The card has been designed for the simultaneous management of two separate channels. The assembly of potentiostat was structured in different phases:

1. Welding of the different components on the board (Figure A.3a).
2. Implementation of the program using Arduino and programming of the user interface in Matlab (Figure A.4) for managing the instrument from a PC.
3. Calibration of the channels (Figure A.3b) for reading of the voltage and current.
4. Use and validation in dedicated experimental tests.

a) b)

Figure A.3. Capacitors, resistors, operational, pins for connection to the Arduino board 2 (a); the different components were welded using a tin wire with Pb. The board with mounted and welded connectors is calibrated (b).

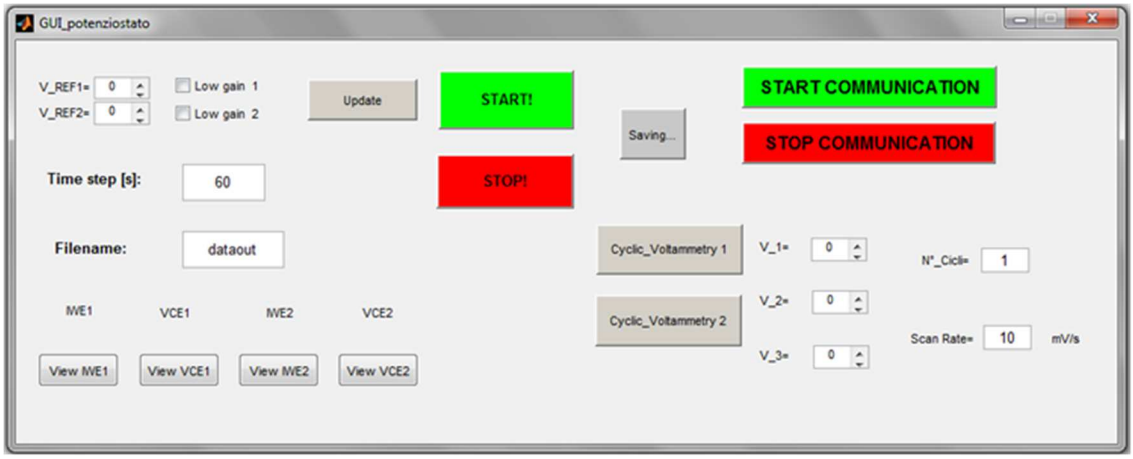
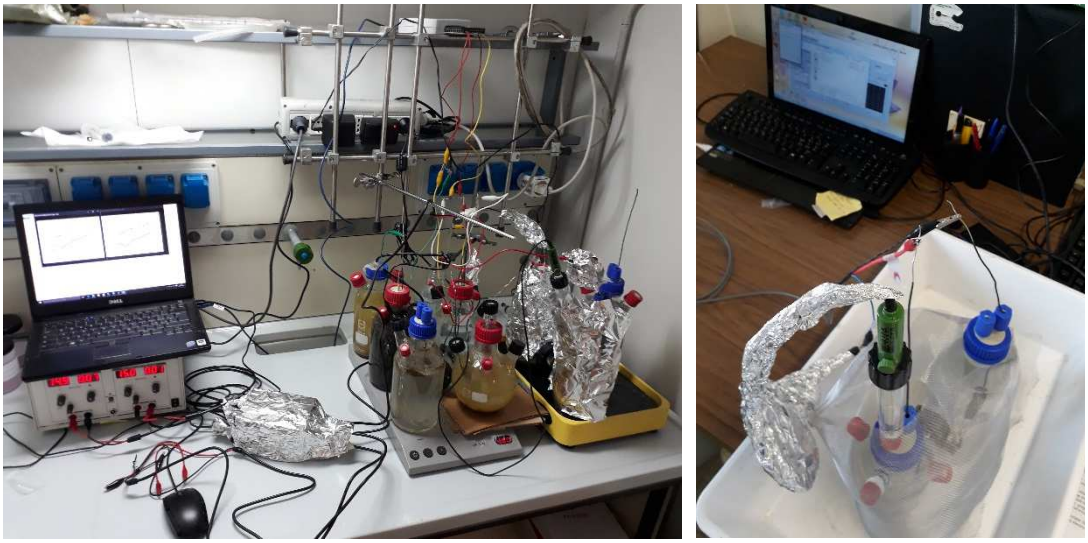


Figure A.4. Matlab graphic interface for users to use the potentiostat; Graphical User Interface.

It is possible to fix the potential to the WE, record the voltage data to the counter-electrode and the current data to the WE (on both channels, processing 4 graphics in real time) and manage the saving of data in txt format according to the chosen sampling time through the Matlab's graphic interface. It is also possible to establish the profit with which you intend to work. It is also possible to perform CV tests on both channels: the interface allows to select the potential interval in which to scan, to set the number of cycles and the scanning speed; the program processes a voltage-current graph. A Matlab code was elaborated to program the user interface and continuously update.

It was necessary to calibrate both channels for the voltage and current reading for a correct use of the instrument. By setting an input voltage value, it is converted into a digital code through Matlab by interpolating the collected calibration points; a function is obtained that allows to convert the digital signal output of the instrument into the equivalent analog signal. Different tests were carried out with the use of the potentiostat in operating conditions similar to the experimental tests to be conducted (Figure A.5).



a) b)

Figure A.5. Preliminary tests for setting and operation control.

The observations from these tests allowed to optimize the functioning of the instrument related to aspects such as the attenuation of background noise in the signals due to interference from electronic equipment for setting up the BES. Devices such as the Faraday Cage have allowed the collection of data less affected by errors. These aspects are very significant for the conduct of the CV tests and with low currents.
For example the slowing down of the potentiostat control loop determines response times in the order of 5-10 ms, enough to conduct CV with low scan rate up to 1 mV/s; the potentiostat control loop controls the voltage of the counter in order to filter out disturbances introduced by the digital-analog converters.

FIGURE INDEX

TABLE INDEX