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FACULTY OF ENGINEERING AND THE ENVIRONMENT

Cr(VI) removal in bioelectrochemical systems with electrodes as electron donors

by

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Abstract

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Hexavalent chromium (Cr(VI)) is a highly toxic and soluble substance present in a wide range of industrial effluents. An effective treatment method is the biochemical Cr(VI) reduction to the less toxic trivalent chromium (Cr(III)), and such a transformation has recently been demonstrated in bioelectrochemical systems. However, depending on the pH of the catholyte, a biocatalyst might be required in the cathode and also the process can be very much inhibited by Cr(III) products which tend to form on the electrode surface and deactivate it.

Herein is demonstrated how an electrophilic bacterium, that is *Shewanella oneidensis* MR-1, can be used as a bacterial catalyst in Cr(VI) reducing cathodes of bioelectrochemical systems. Starting with potentiostatically controlled experiments (-500 mV vs. Ag/AgCl for 4 h), the abiotic cathode's (AC) performance was shown to be affected by the Cr(VI)-reduction products that are deactivating the cathode and are severely inhibiting further Cr(VI) reduction. The presence of metal chelators like lactate delayed this deactivating effect and enhanced the system's performance to a large extent; in the presence of 30 mM lactate, the AC delivered approximately 3 times more electrons to Cr(VI). In addition, approximately 15 times more electrons were delivered when 5 μM of the electron shuttle riboflavin was also added in the AC. However, the presence of riboflavin did not have any effect in the absence of lactate. The MR-1 biocathode also exhibited an enhanced current production and Cr(VI) reduction, though the pre-treatment conditions were found to be important. When pre-treated in an aerated chamber with a poised electrode at +300 mV vs. Ag/AgCl, the MR-1 biocathode mediated 70% more electrons than the AC with 30 mM lactate, and only 39% more electrons when the electrode was initially poised at -500 mV vs. Ag/AgCl. Cr(VI) reduction was also enhanced, with a decrease in concentration over the 4 h operating period of 9 mg L^{-1} Cr(VI) in the aerobically pretreated MR-1 chamber, compared to only 1 and 3 mg L^{-1} in the AC without lactate and in the AC with 30 mM lactate respectively. On the other hand, when pre-treated anaerobically in the presence or absence of Cr(VI), the performance of the MR-1 biocathode was not much different than that of the AC with 30 mM lactate.

The positive effect of lactate was further demonstrated in microbial fuel cell (MFC) cathodes, where maximum power densities produced were up to 44 times the power densities reported elsewhere for abiotic cathodes (8.8 mW m^{-2} vs. 0.2 mW m^{-2} at pH 7) and at similar levels to the power densities of biotic cathodes at pH 6 and 7. Considerable Cr(VI) reduction was also observed at alkaline pH abiotic cathodes and maximum power densities were 31 times the ones reported elsewhere for biotic cathodes at pH 8 (21.4 mW m^{-2} vs. 0.7 mW m^{-2}).

In MFCs, the presence of MR-1 enhanced the performance of pH 7 cathodes; in the presence of 30 mM lactate, the MR-1 biocathode bioelectrochemically reduced 3 times the amount of Cr(VI) reduced by the AC with the same amount of lactate. Compared to the results in the absence of an electrode, the MFC results suggested that different Cr(VI) reduction pathways could be utilised by MR-1 when the electron donor is in the poised electrode form rather than in the lactate form. In conclusion, effective and continuous Cr(VI) reduction with associated current production were achieved when MR-1 and lactate were both present in the biocathodes.

KEYWORDS: Chromium remediation, bioelectrochemical systems, microbial fuel cells, biocathodes, *Shewanella*, flavins, lactate, metal chelation

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DECLARATION OF AUTHORSHIP

I, Nikolaos Xafenias, declare that the thesis entitled "Cr(VI) removal in bioelectrochemical systems with electrodes as electron donors" and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
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  XAFENIAS, N., ZHANG, Y. & BANKS, C. 2013. Enhanced performance of hexavalent chromium reducing cathodes in the presence of Shewanella oneidensis MR-1 and lactate. Environmental Science & Technology, 47, 4512-4520. (http://dx.doi.org/10.1021/es304606u)
  XAFENIAS, N., ZHANG, Y. & BANKS, C. Enhanced performance of hexavalent chromium reducing cathodes in the presence of Shewanella oneidensis MR-1 and lactate. European International Society for Microbial Electrochemical Technologies Meeting, 2012 Ghent, Belgium.
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Signed: …… ……..

Date:……23/01/2014……..

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DEFINITIONS AND ABBREVIATIONS

AEM: Anion Exchange Membrane

Ag/AgCl: Silver/silver chloride reference electrode

BES: Bioelectrochemical System

CE: Coulombic Efficiency or Counter Electrode

CEM: Cation Exchange Membrane

COD: Chemical Oxygen Demand

Cr_{tot}: Total Chromium

Cr(II): Divalent Chromium

Cr(III): Trivalent Chromium

Cr(VI): Hexavalent Chromium

CV: Cyclic Voltammetry

E_{cell}: Cell Potential

E^0: Standard Cell Potential

FAD: Flavin Adenine Dinucleotide

FMN: Flavin Mononucleotide

I: Electrical Current

MEC: Microbial Electrolysis Cell

MFC: Microbial Fuel Cell

MM: Minimal Medium

NAD^+: Nicotinamide Adenine Dinucleotide

NADH: The reduced form of Nicotinamide Adenine Dinucleotide

$NADP^+$: Nicotinamide Adenine Dinucleotide Phosphate

NADPH: The reduced form of Nicotinamide Adenine Dinucleotide Phosphate

OCV: Open Circuit Voltage

pH: the negative logarithm of the H^+ cations' activity($-\log\{H^+\}$)

PEM: Proton Exchange Membrane

PSA: Projected Surface Area

RE: Reference Electrode

R_{ext}: External Resistance

RF: Riboflavin

R_{int}: Internal Resistance

Redox: Reduction-Oxidation

SHE: Standard Hydrogen Electrode

TASA: Total Apparent Surface Area

TSS: Total Suspended Solids

TVSS: Total Volatile Suspended Solids

VFA: Volatile Fatty Acid

WE: Working Electrode

Chapter 1 Research overview

1.1. Introduction

Being the product of a wide range of anthropogenic processes and an abundant inorganic groundwater pollutant found at hazardous waste sites, carcinogenic hexavalent chromium (Cr(VI)) has received a lot of attention during the last decades. Various methods to detoxify this pollutant have been developed, including a range of conventional physicochemical processes. Although effective, these treatments usually require an excess of chemicals and produce secondary toxic wastes, significantly increasing the related costs and reducing their sustainability.

Biological treatment on the other hand, has been proposed as an alternative to lower the costs and improve sustainability. By utilising inorganic or organic energy sources, a wide range of microorganisms are capable to reduce Cr(VI) and remove it from the polluted wastewater. Amongst them, there are certain species which have the ability to respire using conductive solid surfaces as electron donors or acceptors (e.g. *Shewanella* sp.). With such respiration mechanisms being abundant in nature, these species could be exploited in bioelectrochemical systems (BESs) known as microbial fuel cells (MFCs) for removing poisonous metals like Cr(VI) from polluted wastewater.

MFCs have received a lot of research attention, especially during the last 15 years, representing a clean and renewable energy source which converts chemical energy available in wastewater, to electrical energy. Even if power generation is still extremely limited and a range of technical challenges must be overcome before becoming practical for renewable energy production, on-going research is conducted in a wide range of possible applications; from wastewater treatment, BOD monitoring and groundwater remediation, to powering implantable medical devices and even to robotic and space applications.

The majority of MFC configurations used so far has focused on the oxidation of the organic electron donors in the anode with oxygen as an electron acceptor in the cathode. On the other hand, the design of a relatively limited number of MFCs has been made for the concurrent treatment of reducible pollutants in the cathode, including hexavalent chromium. Amongst them, an even more limited number of studies have considered the benefits of microbially catalysed cathodes, a configuration which could eventually reduce high costs and increase the low operational sustainability related to the use of specialised chemical catalysts. With a lot

more ground to be explored, this study attempts to look deeper into the mechanisms involved in Cr(VI) reducing MFCs, in order to improve their configuration and maximise the benefits associated with this process. The major outcomes of this project are a more integrated view of the BES technology potential for the biological treatment of Cr(VI) and a deeper insight into the electron transfer and Cr(VI) removal mechanisms.

1.2. Statement of research objectives

The **aim** of this research project can be summarised as follows:

To contribute to the understanding of the processes taking place in BESs which treat Cr(VI) containing wastewater and to exploit their potential, so as to develop a sustainable configuration for bioelectrochemical Cr(VI) remediation.

Considering the aim above, the following **objectives** have been set:

1. to investigate how the performance of abiotic Cr(VI) reducing cathodes can be improved
2. to confirm whether *Shewanella* is capable of both Cr(VI) reduction and current generation in BES, to identify the critical parameters affecting its performance in Cr(VI) reducing BES, and to compare its performance with that of mixed populations
3. to investigate the dominant electron transfer mechanisms in Cr(VI) treating biocathodes with *Shewanella* as the biocatalyst, enhance Cr(VI) removal and promote current generation

1.3. Research outline

This thesis consists of five chapters that describe the theoretical and experimental work that was conducted. The research outline of the thesis is graphically presented in Figure 1-1, where the connection between the experiments and the objectives is also shown.

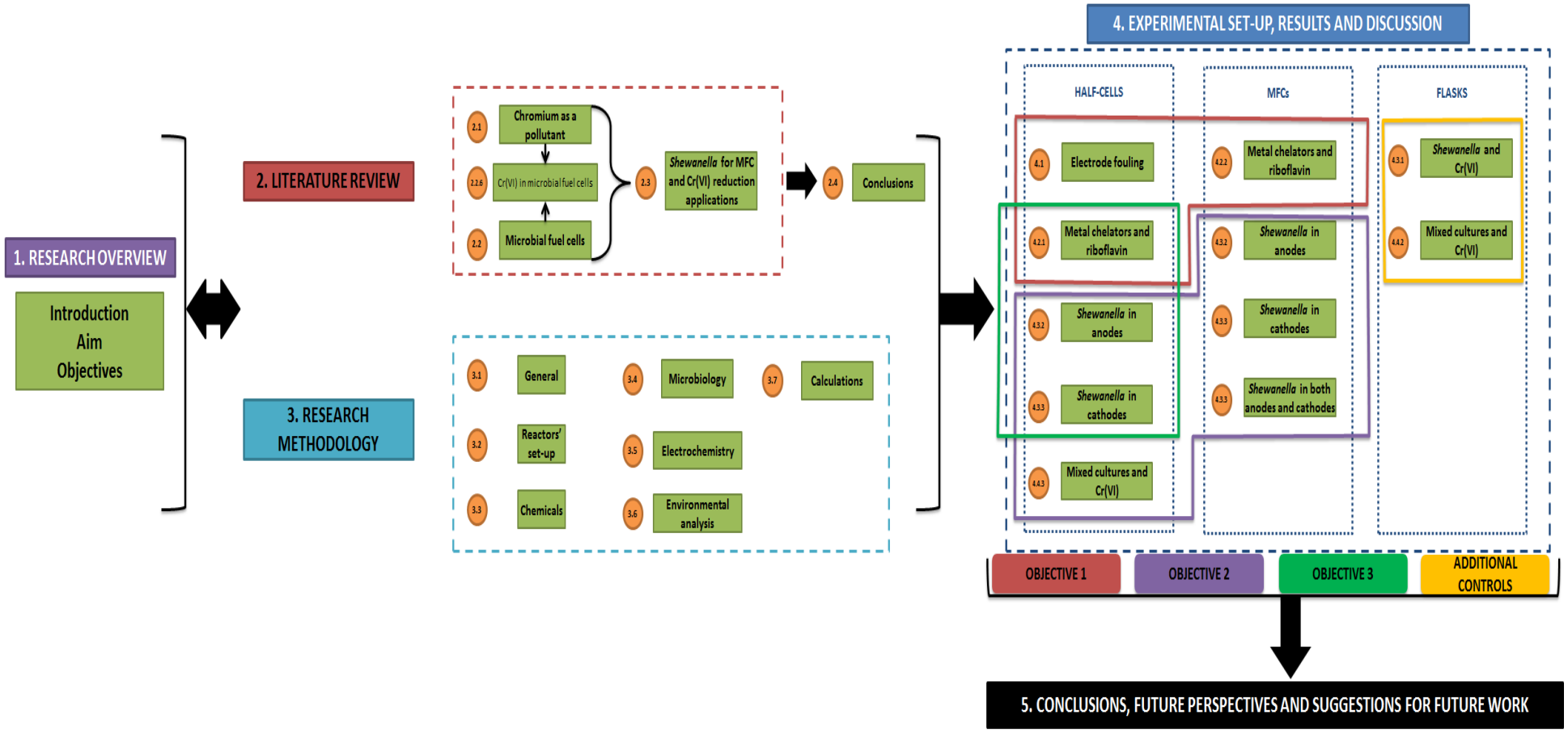

Figure 1-1: Research outline of the thesis.

Chapter 2 Literature review

2.1. Chromium as a pollutant

2.1.1. Toxicity and regulations

A great variety of chemical compounds can be found in water, not all of them of the same concentration or significance. Even with respect to the same chemical element, different aquatic conditions like the reduction/oxidation (redox) potential and pH may reveal a variety of different properties of varying importance. Amongst other inorganic contaminants, chromium can be found in water as a result of anthropogenic processes like electroplating, leather tanning, textile and pigment manufacturing and wood preserving (USEPA, 2000). In the U.S.A., chromium is considered to be the second most abundant pollutant found at hazardous waste sites (Blowes, 2002), mainly as a result of the disposal of waste produced by the above mentioned processes.

The main oxidation states that chromium can be found in nature are Cr(III) and Cr(VI), with chromium being significantly more toxic and mobile when in the hexavalent state (Palmer and Puls, 1994). Genetic studies on the effect of Cr(VI) in animal cells *in vivo* and animal and human cells *in vitro*, revealed various effects "including DNA damage, gene mutation, sister chromatid exchange, chromosomal aberrations, cell transformation and dominant lethal mutation" (WHO, 1997). For the above reasons, Cr(VI) has been characterised by the World Health Organisation as "carcinogenic to humans (Group 1)" (WHO, 1997); on the other hand, Cr(III) compounds have been categorised as "not classifiable as to their carcinogenicity to humans (Group 3)" (WHO, 1997). Furthermore, Cr(III) is considered as a micronutrient and safe for human health at the concentrations found in ambient water environments (Brandhuber et al., 2004).

As a result of the above, the World Health Organisation has set a provisional guideline value of 50 μg L^{-1} for total chromium in drinking water (WHO, 2011). The same value has been adopted by the Council of the European Union (EU, 1998), while the U.S. Environmental Protection Agency's maximum contaminant level has been set to 100 μg-Cr_{total} L^{-1} without setting separate standards for Cr(VI) and Cr(III) (CEPA, 1999). On the other hand, California's Environmental Protection Agency has more stringent standards for total and hexavalent chromium with the maximum level set to 50 μg-Cr_{total} L^{-1} and an issued Public Health Goal of 2.5 μg-Cr_{total} L^{-1} (CEPA,

1999). However, newer findings suggest the adoption of the even lower Public Health Goal of 0.06 μg-Cr(VI) L^{-1} (CEPA, 2009).

2.1.2. The chemistry of chromium

Figure 2-1 shows the Eh-pH diagram for aqueous chromium species in the water-chromium system, where Cr(III) and Cr(VI) are the only stable valent states of chromium; the exact speciation will vary with temperature, Cr concentration and ionic strength (Palmer and Wittbrodt, 1991). Depending on the pH, the following equilibria exist in aqueous solutions, with $H_2CrO_4^{2-}$ being the dominant Cr(VI) species at pH<1, $Cr_2O_7^{2-}$ in equilibrium with $HCrO_4^-$ at 2≤pH≤6 and CrO_4^{2-} formation at pH>6 (Saha and Orvig, 2010):

$$H_2CrO_4 = HCrO_4^- + H^+ \qquad \text{(Reaction 2.1)}$$

$$Cr_2O_7^{2-} + H_2O = 2HCrO_4^- \qquad \text{(Reaction 2.2)}$$

$$HCrO_4^- = CrO_4^{2-} + H^+ \qquad \text{(Reaction 2.3)}$$

Following Cr(VI) reduction, Cr(III) will start to precipitate at pH above 6.5 as $Cr(OH)_3$ (Molokwane and Nkhalambayausi-Chirwa, 2009), as also seen in Figure 2-2. However, positively charged ions $Cr(OH)^{2+}$ and $Cr(OH)_2^+$ will also form at aquatic pH 6-8 and will remain in a soluble state. The positive charge of the produced species and also the low conductivity of the hydroxides might cause a decline in the efficiency of electrochemical treatments, as is discussed in Chapter 4.

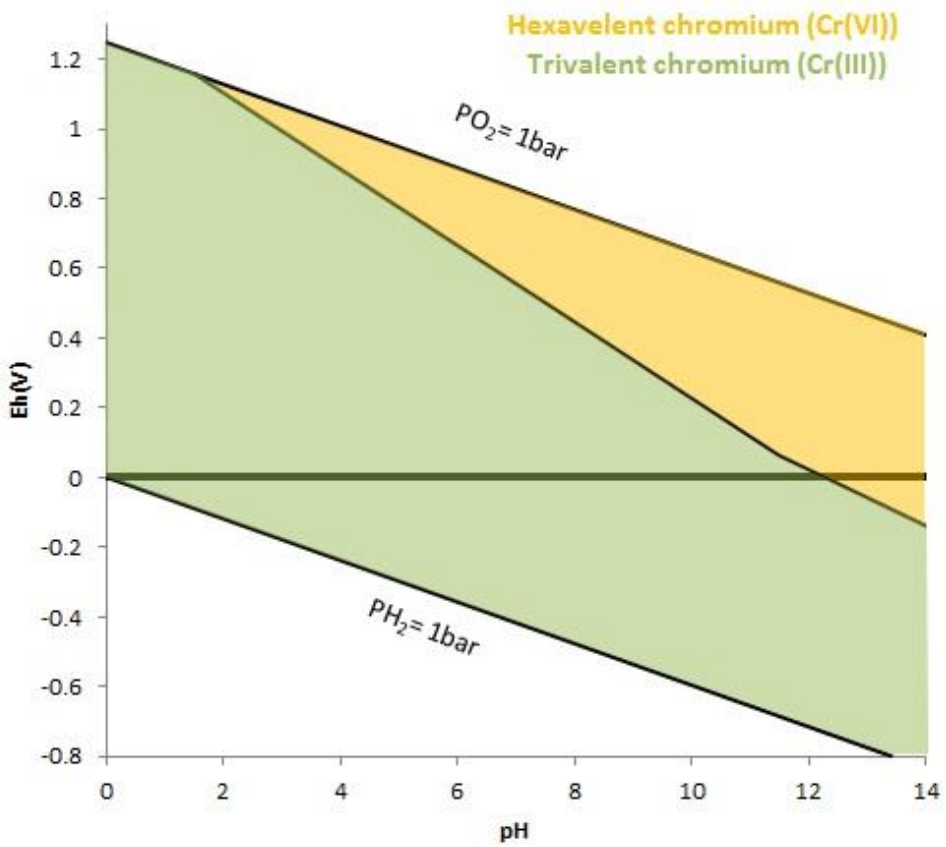

Figure 2-1: The Eh-pH diagram for aqueous chromium species in a chromium-water system (Adapted from Palmer and Wittbrodt (1991)).

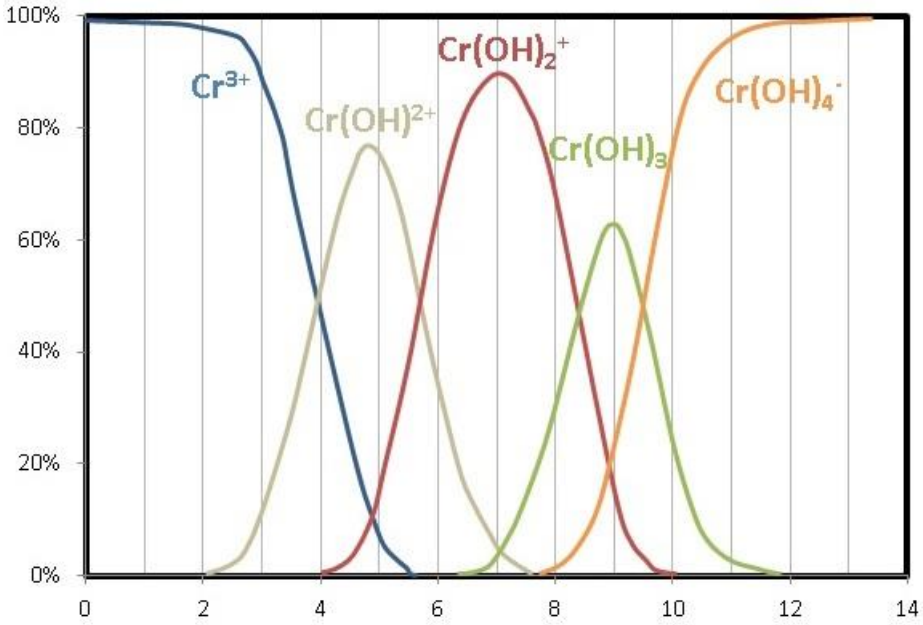

Figure 2-2: Cr(III) speciation changes with pH (Adapted from Sun et al. (2010)).

Given the relatively high redox potential of the Cr(VI)/Cr(III) couple, only a few naturally occurring oxidants may oxidise Cr(III) back to Cr(VI) (Stanin, 2005) and bacteria do not seem to prefer this pathway (Schmieman et al., 1998). However, chromium can be oxidised by some disinfectants used for potable water treatment such as free chlorine, chloramines and hydrogen peroxide (Brandhuber et al., 2004), and this should be seriously considered during water treatment close to Cr(VI)-contaminated sites. According to the reports made by Stanin (2005), only dissolved oxygen and MnO_2 are known to oxidise Cr(III) back to Cr(VI) in the natural environment, with MnO_2 being the most common. On the other hand, studies on the oxidation of Cr(III) by dissolved oxygen at pH as high as 12.5 for 24 days, resulted in very little or no oxidation to Cr(VI) (Palmer and Puls, 1994). Especially with the lack of mediator species, this certain pathway of Cr(III) oxidation has been reported to be negligible (Stanin, 2005). In aqueous environments with slightly acidic to basic pH, this reaction can be relatively slow, requiring several months and allowing faster reactions such as sorption or precipitation to occur first (Schmieman et al., 1998, Krishna and Philip, 2005, Stanin, 2005, Molokwane and Nkhalambayausi-Chirwa, 2009). Consequently, because of the limited routes of Cr(III) oxidation and the low toxicity of Cr(III), the reduction of Cr(VI) to Cr(III) seems to be a feasible route for Cr(VI) remediation and has been applied within a range of treatment processes.

2.1.3. Cr(VI) wastewater treatment

2.1.3.1. Physicochemical treatment

Cr(VI) is produced by a variety of industrial processes and at a range of effluent concentrations, usually between 0.1 to 200 mg-Cr(VI) L^{-1} (Owlad et al., 2009). For the removal of chromium from industrial effluents, various physicochemical methods have been applied, with the main ones being:

- **Chemical reduction** (e.g. zero-valent iron (Franco et al., 2009), ferrous sulphate (Ludwig et al., 2007))
- **Electrochemical reduction** (Akbal and Camcl, 2011)
- **Adsorption** (e.g. activated carbon (Mohan and Pittman Jr, 2006), dried biomass (Gavrilescu, 2004), chitosan (Udaybhaskar et al., 1990))
- **Filtration** (Hafiane et al., 2000)
- **Ion exchange** (Mustafa et al., 2009)

Even effective for heavy metals removal, physicochemical methods usually come with high costs due to the energy consumption and chemicals addition, particularly when the dissolved metals are in the range of 1–100 mg L^{-1} (Zahoor and Rehman, 2009). Physicochemical techniques will also generate secondary wastes (Dermou et al., 2005, Molokwane and Nkhalambayausi-Chirwa, 2009) and in many of these applications chromium keeps its toxic hexavalent state (Kurniawan et al., 2006); thus, such techniques are rarely the last step of Cr(VI) remediation and usually further treatment is required.

2.1.3.2. Biological treatment

On the other hand, the usage of bacteria for Cr(VI) reduction does not require high energy inputs nor does it produce significant chemical by-products (Molokwane and Nkhalambayausi-Chirwa, 2009). For the above reason and also due to the relatively low costs that microbial remediation of Cr polluted sites has to offer (Fruchter, 2002), microbial Cr(VI) reduction has been extensively studied, with most studies focusing on Gram negative species of the genera *Pseudomonas, Shewanella, Desulfovibrio* and *Escherichia* (Viamajala et al., 2008). Table 2-1 summarises some of the species found in the literature with the ability to reduce Cr(VI). In general, the vast majority of Cr(VI) reducers consists of heterotrophic bacteria which are also more tolerant to Cr(VI) exposures than autotrophic ones are (Vaiopoulou and Gikas, 2012).

Biological Cr(VI) reduction is generally a complex process with various possible pathways and unstable redox intermediates (Daulton et al., 2007). It can take place both directly through enzymatic reactions within the cell or the cell wall and indirectly through reduction by reduced species produced by microbial respiration (Stewart et al., 2007). Amongst all these processes, enzymatic reduction of Cr(VI) to the less toxic Cr(III) is believed to be the preferred pathway selected by bacteria living in Cr(VI) contaminated environments, as part of their protection mechanism against toxic substances (Chirwa and Wang, 1997b).

Under aerobic conditions, Cr(VI) reduction takes place as a two or three step process, with NADH, NADPH and endogenous electron reserves acting as electron donors under both aerobic and anaerobic conditions (Cheung and Gu, 2007). In the absence of oxygen, both soluble and membrane-associated enzymes including cytochromes b and c were found to mediate Cr(VI) reduction (Cheung and Gu, 2007).

Table 2-1: Microorganisms able to reduce Cr(VI)

Organism	e^- donor	Redox conditions	pH	Temperature (°C)	Reference
Achromobacter eurydice	acetate, glucose	anaerobic	-	-	Derived from Wang and Shen (1995)
Acinetobacter haemolyticus	tryptone, yeast extract	aerobic	7.2	30	Pei et al. (2009)
Aeromonas dechromatica	various sugars	anaerobic	-	-	Derived from Wang and Shen (1995)
Agrobacterium radiobacter	various sugars	aerobic	-	-	Derived from Wang and Shen (1995)
***Bacillus* sp.**	glucose	aerobic	9.0	37	Liu et al. (2006)
***Cellulomonas* sp.**	enzymatic and indirect reduction via Fe(III) reduction	anaerobic	7.2	-	Viamajala et al. (2008)
Desulfovibrio vulgaris	yeast extract, lactate	anaerobic	7.4	37	Goulhen et al. (2006)
Enterobacter cloacae	sucrose	anaerobic	7.0	30	Rege et al. (1997)
Escherichia coli	glucose	aerobic	7.0	37	Bae et al. (2000)
Micrococcus roseus	acetate, glucose	anaerobic	-	-	Derived from Wang and Shen (1995)
Pseudomonas aeruginosa	glucose, benzoate, mPlate Count Broth	aerobic	7.0	37	Oh and Choi (1997)
Pseudomonas ambigua G-1	nutrient broth	aerobic	7.0	30	Horitsu et al. (1987)
Pseudomonas dechromaticans	peptone, glucose	anaerobic	-	-	Derived from Wang and Shen (1995)
Pseudomonas chromatophila	various sugars, lactate, acetate, succinate, fumarate, butyrate	anaerobic	-	-	Derived from Wang and Shen (1995)
Pseudomonas fluorescens LB 300	glucose	aerobic	7.0	30	Chirwa and Wang (1997a)
Pseudomonas putida PRS 2000	NADH, NADPH	aerobic	7.0	30	Ishibasi et al. (1990)

Organism	e^- donor	Redox conditions	pH	Temperature (^oC)	Reference
Shewanella alga	lactate	anaerobic	7.0	-	Guha (2004)
Shewanella oneidensis	lactate	-	-	-	Alam et al. (2006)
Sphaerotilus natans	glucose	aerobic	7.0	-	Caravelli et al. (2008)
Staphylococcus capitis	acetate	-	7.0	37	Zahoor and Rehman (2009)
Streptomyces griseus	glucose, peptone, yeast, beef and malt extract	-	6.0	28	Laxman and More (2002)

2.1.3.3. Microbial Fuel Cell (MFC) treatment

In general, the MFC is considered to be a sustainable technology developed for power production from wastewater (Rabaey and Verstraete, 2005) which also allows the concurrent treatment of different types of wastewater (Clauwaert et al., 2007a). In order to overcome some major drawbacks of conventional metal removal techniques, and especially the ones related to physicochemical treatment (e.g. high energy and chemical requirements, secondary waste production) and to demonstrate an energetic benefit from the treatment process, the use of MFC technology for Cr(VI) removal has recently been proposed (Li et al., 2008, Wang et al., 2008, Li et al., 2009, Tandukar et al., 2009, Huang et al., 2010, Huang et al., 2011a, Huang et al., 2011b, Liu et al., 2011, Pandit et al., 2011, Hsu et al., 2012, Zhang et al., 2012).

By using an appropriate type of wastewater in the anode, Cr(VI) polluted wastewater can be treated in the cathode with simultaneous power production. Moreover, due to the higher standard redox potential of Cr(VI) (at acidic pH) when compared to other electron acceptors applied in the cathode of common MFCs such as oxygen and hexacyanoferrate (1.33 V, compared to 1.23 V and 0.36 V respectively vs. SHE), Cr(VI) was considered as a theoretically more favourable electron acceptor at acidic pH (Li et al., 2008, Tandukar et al., 2009). This fact allowed several studies to regard Cr(VI) not only as a toxic pollutant that needs to be sufficiently treated, but also as an efficient oxidant for electric power production. Further discussion on the use of Cr(VI) in MFCs is given in Section 2.2.6.

2.2. Microbial Fuel Cells

2.2.1. General

A microbial fuel cell is a certain type of biofuel cell, “a system in which microorganisms function as catalysts to convert chemical energy into electrical energy” (Rabaey, 2010). Microbial fuel cells belong to the broad family of bioelectrochemical systems which share the common characteristic of exploiting the bacterial metabolic pathways to accelerate oxidation/reduction reactions on electrode surfaces. In general, bioelectrochemical systems are receiving a lot of research attention recently (Pant et al., 2010), as they provide the potential for various applications including:

- ***Power production***: MFCs can be used to generate power, by utilising COD loading rates in the range 0.1-10 Kg COD m^{-3} of reactor d^{-1} and are expected to provide a power density output between 0.01 and 1.25 KW m^{-3} (Rabaey and Verstraete, 2005).

Substantial efforts are currently being made to produce power for real applications (e.g. robotics and electronics applications (Ieropoulos et al., 2012b, Ieropoulos et al., 2013)); however, with the power production yet remaining low, there are still economic and efficiency challenges that need to be overcome, before the MFC technology can become competitive for power production (Du et al., 2007, Fornero et al., 2010, McCarty et al., 2011).

- ***Wastewater treatment***: an increasing number of real wastewaters have been studied, including both municipal and industrial types (Fornero et al., 2010, Velasquez-Orta et al., 2011, Ieropoulos et al., 2012a). With the estimated energy content available in raw wastewater exceeding the electricity requirements of a municipal wastewater treatment plant by a factor of 9.3, and with an achieved COD removal from municipal wastewater of approximately 80%, the MFC technology may appear attractive for wastewater treatment (Liu et al., 2004, Shizas and Bagley, 2004, Logan and Regan, 2006).

- ***Bioremediation***: modified MFCs could also be used for bioremediation of contaminated sites; however, such systems are generally not designed for electricity production (Logan and Regan, 2006). As an example, subsurface snorkels have been suggested for the direct bioremediation of organic pollutants without the use of an external resistor, and therefore without any power production (Lovley, 2011).

- ***Sensors for monitoring and control***: MFCs could be used as sensors for the analysis of pollution and for in situ monitoring and control, especially in water environments that are lacking the ease of access (Logan and Regan, 2006, Du et al., 2007). As an example, a Biological Oxygen Demand analyser has been developed and is on the market by Korbi Inc, South Korea.

- ***Desalination:*** with appropriate reactor modifications, MFCs are being tested for the desalination of water, where the electrical potential created is desalinating the water, by driving the ions through ion selective membranes (Kim and Logan, 2013).

- ***Hydrogen, methane and commodity chemicals production***: with anodic current produced at potentials higher than approximately -300 mV vs. SHE, bioelectrochemical systems can be modified to produce H_2 and CH_4 gases in the cathode by applying an additional theoretical potential of 0.11 V (Logan and Regan, 2006, Du et al., 2007). In a similar configuration, such reactors can produce organic commodity chemicals (e.g.

acetate) by utilising cathodophilic microorganisms with the ability to combine CO_2 and electricity into complex organic molecules (Lovley and Nevin, 2013).

2.2.2. General configuration

A conventional MFC generally consists of two chambers, a biological anode chamber and an abiotic cathode chamber separated by a CEM (He and Angenent, 2006). An oxidisable substrate able to provide electrons to the microorganisms and consequently to the anode's electrode is used in the anode chamber, whilst oxygen or another substrate with a higher redox potential is used in the cathode. Electrons generated by oxidation of the substrate in the anode travel through an external circuit to reach the cathode, whilst protons need to pass through the CEM to keep an ionic balance in both chambers and sustain the produced current. The working principle of a MFC with an abiotic oxygen-reducing cathode is presented in Figure 2-3.

The cost of the materials used for construction determines to a large degree the successful application of MFCs in large scale (Logan and Regan, 2006). Materials used as electrodes in MFC applications need to be highly conductive and non-corrosive, with the ability to provide an adequate surface area and strength for microbial attachment and growth (Logan, 2008). Also, CEMs need to have a low internal resistance, be resistant to fouling and prevent crossover of electron acceptors to the anode and of electron donors to the cathode (Logan, 2008). Materials used in the cathode sometimes also include catalysts such as Pt or artificial redox mediators such as ferricyanide, but these eventually decrease the sustainability and cost effectiveness of the system (Logan, 2008).

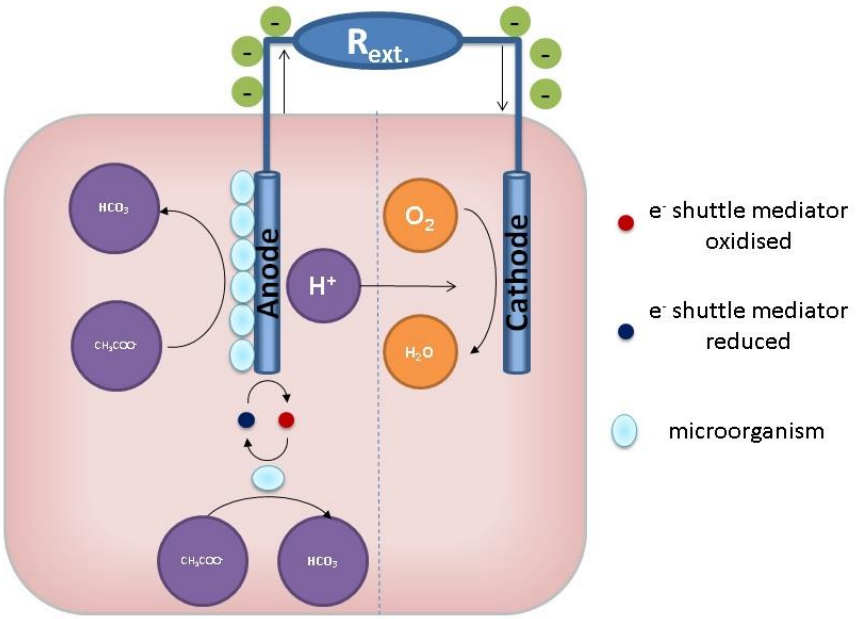

Figure 2-3: The working principle of a MFC with an abiotic oxygen-reducing cathode.

2.2.3. Voltage losses

In a MFC, the electromotive force is defined as the potential difference between the cathode and the anode (Logan et al., 2006):

$$E_{emf} = E_{cat} - E_{an} \qquad \text{(Eq. 2.1)},$$

where E_{cat} and E_{an} are calculated according to the Nernst equation (see Eq. 2.3 in Section 2.2.6.1):

Considering the internal losses of the system, the finally measured cell potential will be given by Eq. 2.2 (Logan et al., 2006):

$$E_{cell} = E_{emf} - (\sum \eta_a + |\sum \eta_c| + IR_\Omega) \qquad \text{(Eq. 2.2)}$$

$\sum \eta_a, \sum \eta_c$: the total overpotentials of the anode and the cathode respectively

IR_Ω: the sum of all ohmic losses

Potential losses are an important aspect of MFC configuration and they need to be minimised, as they reduce the efficiency of the system. Potential losses can be (Logan et al., 2006): *i) concentration* losses, *ii) activation* losses (dominant in the low current density range

(Clauwaert et al., 2008)), *iii) bacterial/metabolic* losses and *iv) ohmic* losses. Losses of potential in an MFC can be described by the following Figure 2-4. The reduction of the overpotentials (concentration, activation and bacterial/metabolic losses) and the ohmic losses in a MFC is critical and will affect the power output of the system. Losses in a MFC can be minimised (Logan et al., 2006), however they will never reach zero levels. Increasing the mixing in an MFC is a way to decrease the concentration losses, however excessive mixing might affect the biofilm formation. Decreasing the oxidised/reduced species ratio at the anode and the reduced/oxidised species ratio at the cathode will also decrease the concentration losses. However, as will be also shown in Chapter 4, there might be cases where, for example, the presence of an oxidised species (e.g. oxygen) in the anode, can enhance current production.

There are various ways by which the activation and the bacterial/metabolic losses can be minimised, and these can include increasing the electrode's specific area and improving the electrode catalysis. Increasing the operating temperature will also have an effect on the system's losses, though one has to balance the benefits of increasing the temperature with the perhaps unsustainable operation at high temperatures. In addition, excessive temperatures will harm the bacterial "catalyst" and can therefore be meaningless. Establishing an enriched biofilm on the cathode is also crucial for maximising performance; again, there is a sensitive balance between minimising the bacterial/metabolic losses and maintaining the growth of a healthy biofilm on the anode. Keeping the anode's potential low enough so as to increase the voltage gained by the MFC and at the same time keeping the potential difference between the anode and the substrate high enough to keep the biofilm satisfied and avoid other bacterial energy producing pathways which do not involve extracellular electron transfer, is also important. In some cases, minimising the crossover of the substrate and the mix-up of anodic and cathodic reagents will also help to minimise the system's losses (Clauwaert et al., 2008). Finally, ohmic losses can be minimised by reducing the electrodes' spacing and the membrane's resistivity, by increasing the solution's conductivity to the maximum tolerated by the bacteria, and by replacing loose or corroded contacts (Logan et al., 2006).

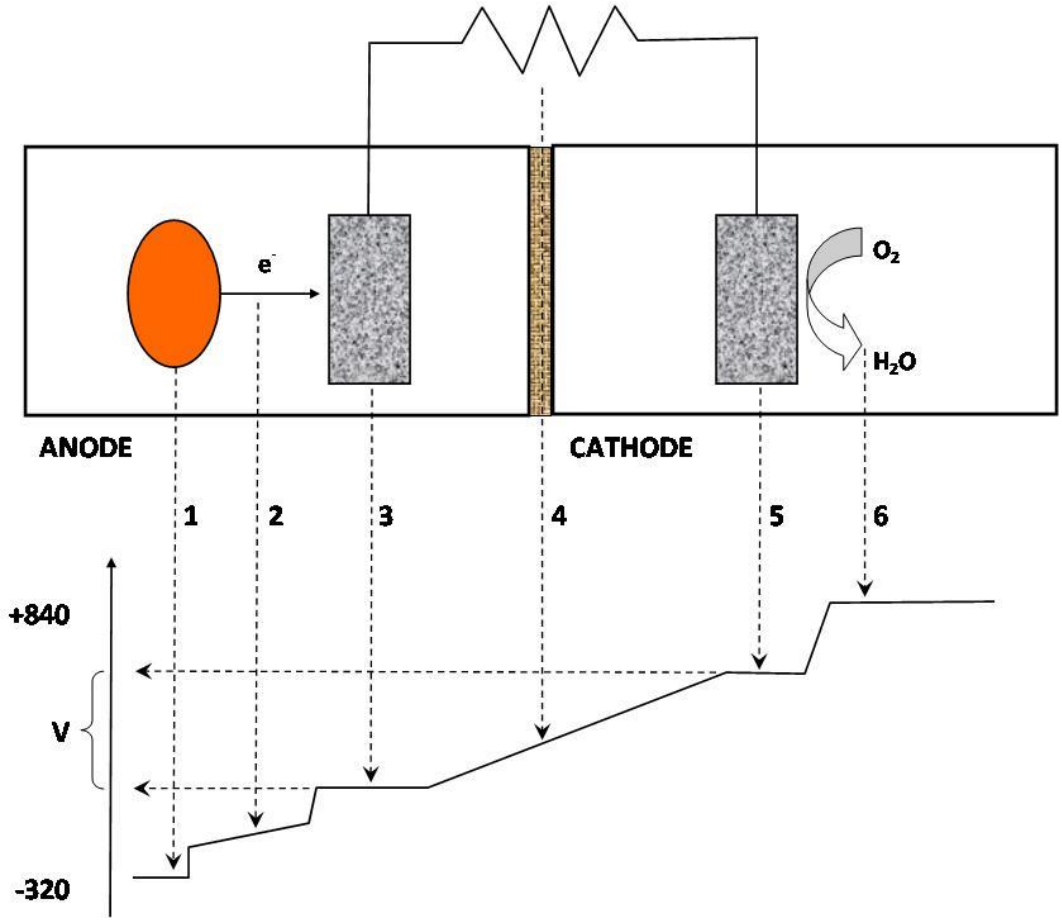

Figure 2-4: Potential losses in a MFC due to the: 1) bacterial electron transfer, 2) electrolyte resistance, 3) anode, 4) MFC resistance (useful potential difference) and membrane resistance, 5) cathode, 6) electron acceptor reduction (Adapted from Rabaey and Verstraete (2005)).

2.2.4. Processes and characteristics of the anode

In a MFC anode, the removal of organic matter is the complex outcome of diverse microbial metabolisms, mainly of electrode-involving respiration, fermentation and methanogenesis (Zhuang et al., 2010). While both respiration and fermentation may take place either separately or simultaneously (Rinaldi et al., 2008), electrode-reducing microorganisms*, which

*Found in the literature also as "electricigens", which are "microorganisms which conserve energy to support growth by oxidizing organic compounds to carbon dioxide with direct quantitative electron

can only utilise a limited range of simple organic electron donors, need to rely on fermentation microorganisms when a more complex substrate is provided (Chaudhuri and Lovley, 2003). When it comes to electrical performance, this tight collaboration of different microbial consortia may be regarded as the reason why mixed culture MFCs are typically more efficient than the pure culture counterparts (Rinaldi et al., 2008). However, fermentation can become antagonistic to electricity production, as under oxygen limiting conditions, many microorganisms might prefer fermentation from electrode respiration, depending on the electrode potential (Hamelers et al., 2010).

In general, the way microbes gain energy through their respiratory pathways (e.g. the tricarboxylic acid cycle) is considered to be the driving force of power generation in a MFC. ATP production by proton movement through the ATPase enzyme involves a series of processes, including substrate oxidation and electron extraction, electron transfer to the respiratory enzymes by NADH and a final electron deposition to an electron acceptor like oxygen (Figure 2-5). In the case where electrons exit the respiratory chain at some reduction potential less (e.g. cytochrome c) than that of the final electron acceptor (electrode), then the bacteria will obtain less ATP but the remaining potential can be used for electricity generation in a MFC (Logan and Regan, 2006).

When the anode's electrode potential becomes too low, fermentation of complex matter becomes advantageous, providing higher amounts of energy for the microorganisms (Logan et al., 2006). From a thermodynamic point of view and when a more complex substrate such as glucose (-429 mV vs. SHE) is used as the electron donor, microorganisms will eventually prefer to produce electric current from glucose than ferment glucose to acetate only when the electrode's potential will be higher than the acetate's potential (-289 mV vs. SHE) (Hamelers et al., 2010). Experimentally, it has been shown that in anodes operating with mixed populations, the direct oxidation of glucose was not the key process taking place (Freguia et al., 2008b). As a result, when working with more complex substrates like glucose, one must bear in mind that only the energetic potential of acetate will be transferred to the electrode, reducing the voltage and the energy efficiency of the system (Hamelers et al., 2010).

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transfer to electrodes" LOVLEY, D. R. 2006. Bug juice: harvesting electricity with microorganisms. *Nat Rev Micro,* 4**,** 497-508.

A wide range of microorganisms is capable of respiring with the aid of an electrode (Clauwaert et al., 2008), with Gram-negative bacteria being more efficient to perform extracellular electron transfer and thus produce electric current than Gram-positive ones (Read et al., 2010). Since it is the bacterial anaerobic metabolism that needs to be promoted in order to effectively convert the organic matter to electricity (Lovley, 2006), anaerobic sludge has been used extensively and has been shown to be a suitable biocatalyst for that purpose (Li et al., 2008). In addition, anaerobic sludge is readily available at wastewater treatment plants and it contains a very broad range of bacterial communities, including electrochemically active ones (Kim et al., 2005). However, one must bear in mind that methanogens will eventually compete with the electrochemically active bacteria for the substrate, thus causing a decrease in the MFC performance (Kim et al., 2005).

Regarding the electron transfer from the substrate to the electrode, four primary mechanisms have been observed (Lovley, 2006):

i) *mediatorless and indirect* electron transfer caused by the abiotic oxidation of microbial fermentation products on the anode surface,

ii) *mediated by artificial electron mediators,*

iii) *mediated by electron mediators produced by the microorganisms, and*

iv) *direct electron transfer* (c-cytochromes and nanowires)

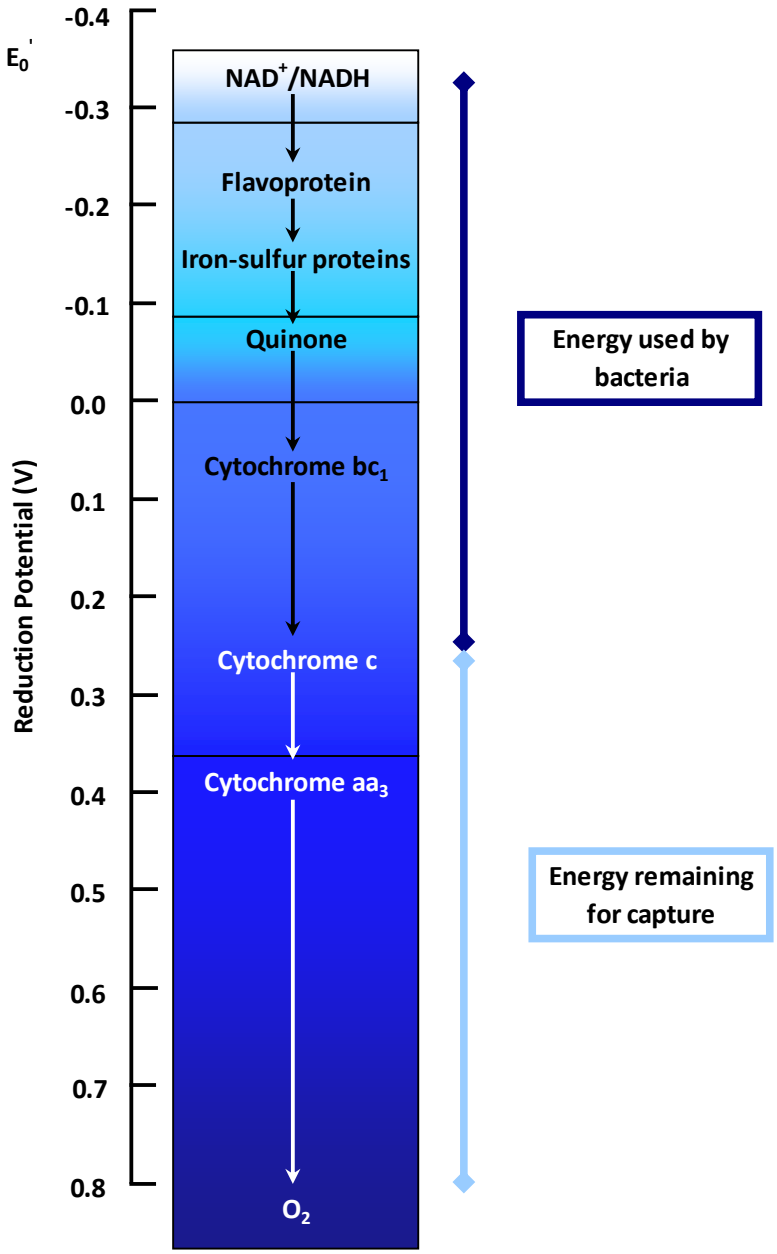

Figure 2-5: Respiratory chain of *Paracoccus denitrificans*, showing the dependence of the voltage produced in a MFC with the location where electrons exit the chain of respiratory enzymes (Adapted from Logan and Regan (2006)).

Among all, the first mechanism is relatively ineffective; fermentative products react very slowly with the electrodes and finally accumulation of oxidation products occurs in the electrode chambers, causing an efficiency decrease (Lovley, 2006). Regarding the second mechanism, electron shuttles are carrying the process out by entering the cell, accepting the electrons from within the cell and transferring them onto the electrode (Lovley, 2006). Although electrochemical mediators are sometimes essential in MFCs (Roller et al., 1984, Bennetto,

1990, Kim et al., 2002), their behaviour is generally unstable and not effective for long-term performance (Chaudhuri and Lovley, 2003). Furthermore, their use can be expensive and even toxic to the microorganisms (Liu and Logan, 2004) and eventually the MFC performance would be very sensitive to the mediator's structure, electrochemistry and biochemistry (Sund et al., 2007).

Various species have been observed to be producing their own electron shuttles (third mechanism), including *Shewanella oneidensis* MR-1 (flavins) (von Canstein et al., 2008), *Geothrix ferementans* (an electron shuttle with "characteristics similar to those of a water-soluble quinone") (Nevin and Lovley, 2002, Bond and Lovley, 2005), *Klebsiella Pmeumoniae* (2,6-di-tert-butyl-p-benzoquinon) (Deng et al., 2010), *Escherichia coli* (Zhang et al., 2008b), *Pseudomonas chlororaphis* (phenazine-1-carboxamide) (Hernandez et al., 2004) and *Pseudomonas aeruginosa* (pyocyanin and phenazine-1-carboxamide) (Rabaey et al., 2005a). Even effective in closed systems, the microbial synthesis of an electron shuttle is energetically expensive (Lovley, 2006) and can lead to reductions in the biomass yield (Mahadevan et al., 2006). As a result of the mediators' loss, decreases in the MFC efficiency have been observed even after a one-time medium replacement (Bond and Lovley, 2005, Deng et al., 2010).

A very important mechanism of electron transfer in MFCs seems to be the direct contact between the electrode and the microorganisms. Especially regarding practical applications, exoelectrogens able to conduct direct electron transfer with an electrode are of great interest (Peng et al., 2010a). Cytochromes localised in the outer cell membrane have been considered responsible for electron transfer between the bacterial cells of *Shewanella* sp. and various terminal electron acceptors, including the electrodes' surfaces (Kim et al., 2002, Bretschger et al., 2007, Meitl et al., 2009, Bouhenni et al., 2010, Bücking et al., 2010, Peng et al., 2010b). In addition, the formation of electrically conductive pili-like structures known as nanowires has also been observed on both *Shewanella* (Gorby et al., 2006) and Geobacter (Reguera et al., 2005) cells. Nanowires are essential for the formation of thick electrically conductive biofilms on the electrodes' surfaces, because of their ability for long-range electron transfer (Gorby et al., 2006, Lovley, 2006, Clauwaert et al., 2008). Specifically regarding the electron transfer mechanisms preferred by *Shewanella*, these are described in more detail in Section 2.3.2.3.

2.2.5. Processes and characteristics of the cathode

2.2.5.1. Conventional cathodes

Considering all the cathodic mechanisms, oxygen is the most common electron acceptor in the cathode chamber (Logan et al., 2006). However, for power producing applications, a solid soluble substrate such as potassium ferricyanide might be more convenient (Bennetto, 1990). When water remediation is the main concern, the reducing environment of the cathode also offers the potential for the reduction of a plethora of oxidised pollutants such as perchlorate, nitrate, bromate, chlorinated solvents, heavy metals and even radionuclides (Tandukar et al., 2009). Consequently, cathodes can operate in either an aerobic or an anaerobic environment, depending on the nature of the study and the application.

Even though the performance of MFCs has been greatly improved during the last years, the performance of the cathode still remains one of the main limitations (Chen et al., 2008). Due to oxygen's poor reduction kinetics under MFC conditions (De Schamphelaire et al., 2010) and also because of high overpotentials associated with oxygen and proton reduction on unmodified carbon and graphite based cathodes, the use of a catalyst is generally required (Clauwaert, 2009). A range of catalysts such as platinum and also artificial electron redox mediators including ferricyanide, neutral red, thionin, methyl viologen, 2,6-dichlorophenolindophenol, 2-hydroxy-1,4-naphthoquinone, phenazines, phenothiazines, phenoxoazines and iron chelates have been used (Lovley, 2006, Chen et al., 2008).

Regardless of platinum's efficiency, its cost and possible poisoning by components of the substrate or by by-products originating from the anode chamber can considerably limit its application (Erable et al., 2010). Also ferricyanide, a common catalyst which acts mainly as a terminal electron acceptor, requires continuous replacement and thus cannot be considered as a sustainable chemical (He and Angenent, 2006). In conclusion, despite the performance improvement artificial electron mediators can achieve, their use not only increases the cost to considerably high levels (Lovley, 2006, Chen et al., 2008) but could also have toxic effects on humans and the environment (Lovley, 2006).

2.2.5.2. Biocathodes

Biocatalysts on the other hand, i.e. microorganisms acting as catalysts in a cathode, could help overcome problems such as the ones described above (Mao et al., 2010). With biocathodes reducing significantly the capital costs of BESs, the use of microbes in the cathode of MFCs has

become a rapidly emerging research area and future innovations within this field are expected to arise from their development (Rozendal et al., 2008a). Also, the better compatibility of the biocatalysts with the operating conditions, when compared to that of metal-based catalysts, may be more encouraging for some future MFC applications (Rismani-Yazdi et al., 2008). The main advantages of a biocathode when compared to an abiotic cathode have been summarised by He and Angenent (2006), and include:

- lower cost of construction and operation
- improved sustainability
- removal of unwanted compounds and production of useful products

Selected studies with biocathodes have been summarised and are presented in Table 2-2.

While the exact electron transfer mechanisms in biocathodes are still uncertain, the mechanisms are expected to be similar to the ones in the anodes (Clauwaert, 2010). In order to study the electron transfer mechanism in oxygen reducing biocathodes, Freguia et al. (2010), used pure cultures of two electrochemically active bacteria, namely *Acinetobacter calcoaceticus* and *Shewanella putrefaciens* and compared their cyclic voltammograms with the ones produced for the abiotic cathodes containing pyrroloquinoline quinone (PQQ) (comparison with *A. calcoaceticus*) and hemin (comparison with *S. putrefaciens*). As the authors state, their results suggested the involvement of free PQQ in the catalysis mechanism of *A. calcoaceticus*, with PQQ accepting the electrons from the cathode and transferring them to c-cytochromes, which finally reduce dioxygen to water. For *S. putrefaciens*, the authors attributed its behaviour to a mechanism where membrane-attached compounds like cytochromes or cytochrome-containing nanowires (which could also be released to the surrounding medium) are generating a catalytic current similar to that of free hemin and where hemin could also be released by cell lysis, catalysing current production.

According to Clauwaert (2010), two types of biocathodes exist: ***i)*** biocathodes in which living microorganisms consume part of the electrical energy with simultaneous ATP production, and ***ii)*** biocathodes where no electrical energy is converted into ATP. Chemolithoautotrophs[*] are believed to play the major role in the biocathodes where electrical energy is consumed for ATP

[*] Microorganisms utilizing inorganic carbon to sustain growth

production (Clauwaert, 2010), however the optimum trophic environment for the biocathodes still remains vague.

A study conducted by Rozendal et al. (2008b), demonstrated a trophic flexibility of their Microbial Electrolysis Cells' (MECs) cathodic inoculum. After a start-up period where the bioanode was receiving an organic carbon source (acetate) and H_2, and was producing CO_2 and protons, the same chamber ended up operating as the cathode after a polarity reversal, producing H_2 under autotrophic conditions or even without any inorganic carbon source present. Similar flexibility was also observed in the study by Cheng et al. (2012), who found that the same electroactive biofilm could alternately catalyse both acetate oxidation and nitrate reduction, depending on the electrode potential.

In another study, *Geobacter lovleyi* was used for fumarate and tetrachlorethene reduction (Strycharz et al., 2008). For this purpose, an adaptation period was applied where *G. lovleyi* was utilizing both the electrode and acetate (and lactate in an intermediate step) for tetrachlorethene reduction. By poising an electrode at -300 mV, *G. lovleyi* was able to reduce fumarate and tetrachlorethene without the presence of an organic electron donor. This is in accordance with an earlier study by Gregory et al. (2004), who found that *Geobacter sulfurreducens* was capable of cathodic current production with fumarate as the final electron acceptor. Acetate was also used as a carbon source during start-up and fumarate fermentation was also reported in this study.

After 9 months of continuous operation and with oxygen as the electron acceptor, Freguia et al. (2008a), reported an increase in the cathode's performance (compared to the non-catalysed cathode), when they connected the acetate-containing anodic effluent with the cathode. The potential development of two bacterial communities in the cathode was then reported by the authors: ***i)*** cathodophilic bacteria which catalyse the electron transfer from the cathode to oxygen and ***ii)*** heterotrophic aerobic bacteria which take advantage of acetate's presence in the aerobic cathode. Despite the improved performance, the researchers drew attention to the fact that unnecessary BOD loading to the cathode should be avoided due to the excessive growth of heterotrophs, which eventually compete with the cathodic biofilm for the electron acceptor.

An increase in performance was also demonstrated when controlled quantities of anodic effluent were spiked in a mixed inoculum cathodic chamber (Rabaey et al., 2008). In this case, the authors attributed this performance improvement to lithoheterotrophic growth but also to

the enhanced proton transfer from the anode to the cathode (better pH control). However, as the authors also conclude, more research is to be done to clarify the cathode's main metabolic pathways and how these contribute to the overall performance.

Table 2-2: Studies with biocathodes in Bioelectrochemical Systems (MFCs, unless otherwise indicated)

Cathodic inoculum	Terminal electron acceptor	Source
Geobacter metallireducens*, *Geobacter sulfurreducens	Nitrate, fumarate*	Gregory et al. (2004)
Seawater biofilm	Oxygen	Bergel et al. (2005)
Geobacter sulfurreducens	Uranium(VI)*	Gregory and Lovley (2005)
A microorganism enriched from anaerobic sludge taken from a sewage treatment plant	Nitrate†	Park et al. (2005)
Leptothrix discophora	Oxygen	Rhoads et al. (2005)
A mixture of aerobic sludge, anaerobic sludge and sediment	Nitrate	Clauwaert et al. (2007a)
Originating from different types of sludge and sediment	Oxygen	Clauwaert et al. (2007b)
Originating from an anoxic tank of a lab-scale aerobic/anoxic/oxic wastewater treatment process	Oxygen, nitrate	Chen et al. (2008)
A mixture of cathodophilic and heterotrophic bacteria originating from the anode's effluent (sequential anode-cathode configuration)	Oxygen	Freguia et al. (2008a)
A mixed culture of denitrifying bacteria originating from a lab-scale reactor	Nitrate	Lefebvre et al. (2008)
A mixture of environmental samples obtained from rusted metal poles at a freshwater section, pond sediment and mixed liquor from a domestic activated sludge plant	Oxygen	Rabaey et al. (2008)
A mixed culture of electrochemically active microorganisms	H^+ (water)‡	Rozendal et al. (2008b)
Geobacter lovleyi	Tetrachloroethene, fumarate*	Strycharz et al. (2008)
A mixed denitrifying sludge treating domestic wastewater	Nitrate	Virdis et al. (2008)

* Poised electrode- constant potential

† Poised electrode- constant current

‡ Microbial Electrolysis Cell

Cathodic inoculum	Terminal electron acceptor	Source
Aerobic activated sludge collected from the aeration tank of a wastewater treatment plant	Oxygen	Zhang et al. (2008a)
A mixture of aerobic and anaerobic sludge from a phototrophic anode chamber	Bicarbonate	Cao et al. (2009)
A mixture of denitrifying and anaerobic mixed cultures enriched in the presence of Cr(VI)	Chromium(VI)	Tandukar et al. (2009)
Aerobic activated sludge from a secondary sedimentation tank	Oxygen	You et al. (2009)
Originating from brackish sediments and enriched in a medium containing trichloroethene and H_2	Trichloroethene*	Aulenta et al. (2010)
Effluent from the biocathode of a denitrifying MFC, activated sludge, a chlorate reducing enrichment from lake sediment and a pure culture of *Dechloromonas* sp. PC1	Perchlorate	Butler et al. (2010)
Seawater biofilm	Oxygen	Erable et al. (2010)
Inoculum from a Cr(VI) contaminated soil	Chromium(VI)	Huang et al. (2010)
Effluent from a previous biocathode setup (Rozendal et al., 2008b)	H^+ (water)†	Jeremiasse et al. (2010)
Aerobic sludge treating dye wastewater in a sequencing batch reactor	Azo dye	Li et al. (2010b)
Ferro-manganese oxidizing bacteria	Oxygen	Mao et al. (2010)
Water from previously operating Sediment MFCs with biocathodes	Oxygen (sediment cathode)	De Schamphelaire et al. (2010)
A microbial consortium previously operating in a MFC performing nitrogen removal	Nitrate, oxygen	Virdis et al. (2010)
Activated sludge contacted with 2,4-dichlorophenol	2,4-dichlorophenol*	Zhang et al. (2010)
Primary clarifier effluent	Chromium(VI)	Huang et al. (2011a)
A mixed bacterial culture obtained from an MFC utilizing Cr(VI) as an electron acceptor	Chromium(VI)	Huang et al. (2011b)

* Poised electrode- constant potential

† Microbial Electrolysis Cell

2.2.6. Cr(VI) in MFCs

2.2.6.1. Reduction potential in MFC cathodes

The higher standard redox potential of Cr(VI) when compared to other potential electron acceptors such as oxygen and hexacyanoferrate (1.33 V, 1.23 V and 0.36 V respectively vs. SHE), makes Cr(VI) a theoretically more favourable electricity generator especially at low pH ranges (Li et al., 2008, Tandukar et al., 2009). As an experimental proof, a study conducted by Wang et al. (2008), indicated a higher CE for dichromate at pH 2 (62%) than for oxygen (21%, without Pt catalyst) and hexacyanoferrate (44%), with correspondingly higher power densities. However, as pH increases and depending on other factors such as solubility and incomplete oxygen reduction, oxygen might become a more preferred electron acceptor as it is less dependent on the presence of H^+. In order to receive 6 moles of e^-, the complete O_2 reduction requires only 6 moles of H^+ (Reaction 2.4), compared to the 14 moles of H^+ that dichromate requires at acidic pH (Reaction 2.5) and the 16 moles of H^+ that chromate requires at neutral-alkaline pH (Reaction 2.6). This could be a limitation for Cr(VI) reduction especially at neutral-alkaline pH and when O_2 is also present in the cathode, consuming the available H^+.

$$\frac{3}{2}O_2 + 6H^+ + 6e^- = 3H_2O \quad \text{(Reaction 2.4)}$$

$$Cr_2O_7{}^{2-} + 14H^+ + 6e^- = 2Cr^{3+} + 7H_2O \quad \text{(Reaction 2.5) (Li et al., 2008)}$$

$$2CrO_4{}^{2-} + 16H^+ + 6e^- = 2Cr^{3+} + 8H_2O \quad \text{(Reaction 2.6) (Stewart et al., 2007)}$$

In either case, the Nernst equation is the one to express the equilibrium potential at open circuit conditions (Clauwaert, 2009):

$$E_{cat} = E^0_{cat} + 2.303 \times \frac{RT}{nF} \times \log\left(\frac{c_O}{c_R}\right) \text{ (Eq. 2.3),}$$

$\boldsymbol{E_{cat}{}^0}$: the standard cathode potential at pH 0

$\boldsymbol{R}$: the molar gas constant= 8.31447 J mol^{-1} $^oK^{-1}$

$\boldsymbol{T}$: temperature, ^oK

$\boldsymbol{n}$: the amount of electrons exchanged

$\boldsymbol{F}$: Faraday's constant= 96,485.3 C mol^{-1}

$\boldsymbol{c_O}$: the concentration of the oxidised species on the electrode's surface

c_R: the concentration of the reduced species on the electrode's surface

At acidic pH where the dichromate ion is dominant, E^0 will be equal to 1.33 V (Wang et al., 2008), whilst at neutral-alkaline pH where the chromate ion is dominant, E^0 will be equal to 1.51 V (Stewart et al., 2007) and E_{cat} will be equal to (at 25 ^oC and assuming that the concentrations on the electrode surface are equal to the ones in the bulk solution):

$$E_{cat} = 1.33 - 4.28 \times 10^{-3} \times ln \frac{[Cr^{3+}]^2}{[Cr_2O_7{}^{2-}] \times [H^+]^{14}} \quad \text{acidic pH}$$

$$E_{cat} = 1.51 - 4.28 \times 10^{-3} \times ln \frac{[Cr^{3+}]^2}{[CrO_4{}^{2-}]^2 \times [H^+]^{16}} \quad \text{near neutral-alkaline pH}$$

At low pH values where H^+ are abundant, cathodic Cr(VI) reduction proceeds relatively fast and without the need for a catalyst (Li et al., 2008, Wang et al., 2008). In the neutral pH region, however, the reaction kinetics are slower due to low H^+ availability (Reaction 2.6) but also because of the overpotentials imposed by Cr(III) hydroxides (Figure 2-2) which form on the electrode surface and deactivate it (Clark and McCreery, 2002). Therefore, in the neutral-alkaline pH region the presence of a suitable bacterial catalyst and probably that of a metal chelator will be beneficial for the process, as it will increase the potential of the cathode.

2.2.6.2. Studies with abiotic Cr(VI) reducing cathodes

Studies reported using Cr(VI) as the electron acceptor in the abiotic cathodes of MFCs are summarised in Table 2-3 and Table 2-4. Li et al. (2008) were the first ones to report Cr(VI) reduction in the cathode. By using two rectangular chambers separated by a 45 cm^2 Nafion™ 117 CEM, they managed to achieve a power density of up to 800 mW m^{-2} total apparent surface area (TASA) when the cathode's pH was equal to 2 and with an external resistance of 100 Ω. Sludge originating from the anaerobic digester of a wastewater treatment plant was inoculated in the anode and acetate was utilised as the electron donor. This study is the only one so far reporting the use of real Cr(VI) containing electroplating wastewater in the cathode of a MFC. The maximum power density obtained has been one of the highest reported by now (Table 2-3) but this might also be due to the relatively higher surface area of the membrane used, which can potentially enhance power generation (Oh and Logan, 2006). Because Cr(VI) reduction is highly pH dependent (Reaction 2.5), a higher membrane surface area will enhance power generation by increasing the proton and electron transfer rates from the anode to the cathode; however, such an approach would highly increase the cost of such an application.

Using an "H-type" MFC, Wang et al. (2008), observed the best performance at pH 2 (within the pH range 2-6) as the above mentioned study, whilst higher Cr(VI) concentration also affected positively the potential, as indicated by Eq. 2.1. However, even if abiotic cathodes might be suitable for acidic wastewaters containing high concentrations of Cr(VI), there are two main issues that need to be addressed (Tandukar et al., 2009); ***i***) in low pH, Cr(III) ions remain soluble (Figure 2-2) and thus an alkali must be added to achieve Cr precipitation, therefore increasing the costs of the process; ***ii***) pH in the cathode lower than that of the anode might cause back migration of the protons, affecting the anode's performance and stability. Protons back migration was indeed observed by Wang et al. (2008), where the pH in the anolyte decreased, in all the experiments where pH in the catholyte was ranging from 2 to 5.

Li et al. (2009) utilised natural rutile as the cathodic catalyst coated on the cathode's electrode (polished graphite) in a Cr(VI) containing chamber. In the anode, acetate was utilised as the electron donor for the anaerobic sludge used. As the researchers noticed, the cathode's potential was higher by 250 mV in the presence of light than in the dark, with a corresponding increase of the Cr(VI) reduction efficiency. According to the authors, this was due to a potential "*visible light-induced photocatalysis*" which reduced the cathodic activation losses.

Liu et al. (2011) examined the effect of hydrogen peroxide in an abiotic cathode containing Cr(VI). According to the authors and also to the study conducted by Fu et al. (2010), hydrogen peroxide can be formed in an aerobic cathode MFC by the incomplete reduction of oxygen. This can then reduce Cr(VI), mainly in acidic pH (2.0-3.0) where the redox potential for H_2O_2 is lower than for Cr(VI) (+560 mV and +1,080 mV vs. SHE respectively at pH 2). When the cathode was flushed with air or when H_2O_2 was directly added to the cathode, current and Cr(VI) reduction rates increased compared to the cathode flushed with N_2. In their aerobic and anaerobic cathodes, 26 mW m^{-2} TASA and 3.9 mW m^{-2} TASA were respectively produced, however the authors do not provide information about their membrane's surface area, for comparison with other studies. Also efficiency did not improve much at pH higher than 4.0, indicating that at less acidic pH a catalyst might be required to drive Cr(VI) reduction.

Pandit et al. (2011) tested the performance of AEMs in MFCs with Cr(VI) cathodes. Even if their study lacks any kind of information on Cr(VI) reduction, the authors report a power density of 46 mW m^{-2} at pH 7.0 (13.5 cm^2 membrane, 4 cm electrode distance, 184 mg-Cr(VI) L^{-1}), with cell potential decreasing for every pH increase, as predicted by the Nernst equation (Eq. 2.3). However, the authors used LB medium in the anode and thus redox mediators present in this medium (Masuda et al., 2010) could have artificially enhanced power production, making

comparison with other studies more difficult. Also, under open circuit conditions, a rapid initial decrease in the catholyte pH from 7.0 to less than 5.0 and from 5.0 to less than 4.5 occurred in this study, which was not observed when the cathode was at pH 3.0 and cannot be explained with respect to the catholyte's chemistry. This brings to doubt the initial conditions of these experiments. Finally, this study lacks any kind of information with respect to Cr(VI) migration in the anode as $Cr_2O_7^{2-}$ or CrO_4^{2-} (their study only lasted 62-65 h), affecting the performance of the anode.

Zhang et al. (2012) utilised a dual-chamber MFC to treat synthetic wastewater containing two different electron acceptors present in vanadium containing wastewaters, i.e. V(V) and Cr(VI). By adding 250 mg L^{-1} of both these electron acceptors, the authors observed an increased power density (422 mW m^{-2}) when compared to adding 500 mg L^{-1} of vanadium(V) alone (270 mW m^{-2}); according to the authors, this was due to the higher redox potential of Cr(VI) compared to that of V(V). An even higher power density was observed when adding 500 mg L^{-1} of Cr(VI) alone (448 mW m^{-2}). This is one of the highest power densities observed in MFC studies until now and was produced using carbon fiber felt electrodes with dimensions of 40 mm $\times$ 40 mm $\times$ 3 mm. However, once again the authors do not provide information on the membrane surface area used, for comparison.

2.2.6.3. Studies with Cr(VI) reducing biocathodes

Up until the present study, studies with Cr(VI) reducing biocathodes were conducted by three main research groups. Following a proof-of-concept study conducted by Dr Tandukar, Dr Huang then focused on improving the processes by studying various materials, operating conditions and MFC configurations. Later on, Dr Hsu made an attempt to study the performance of various *Shewanella* strains in Cr(VI) reducing biocathodes. What follows in this paragraph is a critical review of these studies.

Tandukar et al. (2009) were the first ones who used a bacterial population as a catalyst for Cr(VI) reduction in the cathode. The cathodic inoculum in this study was developed after mixing a denitrifying mixed culture and a methanogenic mixed culture grown with acetate, with a mixing ratio of 80:20 (information given personally by Dr Tandukar). Using 5.4 cm × 2.5 cm × 0.6 cm graphite plate electrodes in both the anode and the cathode and a 1,000 Ω resistor, the authors reported maximum current densities ranging from 46.6 to 123.4 mA m^{-2} for 22 to 63 mg L^{-1} initial Cr(VI) concentrations, respectively. Corresponding power densities for

this study were from 7.9 to 55.5 mW m^{-2} and Coulombic efficiencies in the cathode were from 85 to 139%.

As the authors reported, Cr(VI) reduction in the cathode was carried out under largely autotrophic conditions, with *Trichococcus pasteurii* and *Pseudomonas aeruginosa* being the dominant species. However, the strictly autotrophic operating conditions in the cathode can be questioned, as substrate can still leak from the anode when using a CEM (Logan et al., 2006, Kim et al., 2007) and also cell lysis can release organic carbon. *P. aeruginosa* has been shown to be capable for both Cr(VI) reduction and anodic current production in MFC anodes (Oh and Choi, 1997, Li et al., 2006, Kong et al., 2009, Xu et al., 2009), however an organic carbon source was utilised in both cases. Direct biosorption of Cr(VI) by functional groups of the cell surface has also been observed (Kang et al., 2007) but eventually this pathway would not contribute to current generation in a MFC. Nevertheless, *P. aeruginosa* can grow even in tap water under substrate limiting conditions (Kooij et al., 1982) and this can be an advantage for its survival in MFC biocathodes. On the other hand, *Trichococcus* sp. are known to be fermentatives (Janssen et al., 1995, Liu et al., 2002), therefore their survival in a strictly autotrophic environment for a long time period could be argued and therefore long-term studies are needed on this aspect. Even if lithoautotrophic respiration is the case for this study, limited growth of bacterial cells without an external organic source or continuous supply of new biomass might eventually reduce the efficiency of the system. Again, longer term experiments and in-depth study of the electron transfer mechanisms are needed to show which species, under what conditions and how they are contributing to Cr(VI) electroreduction.

In a similar study to that of Tandukar et al. (2009), Huang et al. (2010), also used a batch-fed dual chamber MFC with a Cr(VI) reducing biocathode, which they inoculated with a mixed microbial consortium found in a Cr(VI) contaminated site. Considering that the cathode was the limiting part of the MFC and in order to increase the specific area of its surface, they used granular graphite with a higher surface area (average of 620 m^2 m^{-3}) than the anode's graphite plate electrode. According to the authors, this strategy, along with the use of a pre-acclimated biological anode, led to the acceleration of the biofilm start-up on the cathode and shortened the acclimation period of the biocathode. Also the authors used a solution with a higher buffer capacity than Tandukar et al. (2009). As a result, the reported maximum power and current generated were higher (Table 2-4) compared to the previous study, along with the increase of the specific Cr(VI) reduction rate.

The effect of conductivity on the performance of the MFC was also tested by Huang et al. (2010). According to the authors, the use of a 100 mM phosphate buffer solution with a 10.6 mS cm^{-1} conductivity raised power production by 58%, the CE by 7% and decreased the internal resistance (calculated from the polarisation graphs) from 485 Ω to 258 Ω when compared to the use of a 10 mM phosphate buffer solution with a 1.5 mS cm^{-1} conductivity. In addition, Cr(VI) reduction rate increased by 25%, indicating according to the authors, an improvement on ion transport around the biofilm and the bulk phase.

According to the same study, a strategy of improving the MFC performance would be the screening and utilisation of bacteria, which extracellularly transform Cr(VI) to soluble Cr(III), in order to avoid $Cr(OH)_3$ precipitation in the cathode. However, this approach would probably require a slightly acidic pH (Figure 2-2), affecting the optimum operating conditions for the bacteria in the cathode. Also, other operational issues like back migration of H^+ towards the anode would then need to be considered, thus affecting the anodophilic population, and also an inevitable use of chemicals after the MFC step for a final Cr(III) precipitation. However, as the authors state, the development of a Cr(VI) reducing biocathode is still in its infancy and more extensive research is needed for its improvement, including studying the bacterial metabolisms along with their electron transfer mechanisms.

Huang et al. (2011a) tried a potentiostatic start-up approach; they applied electrode potentials varying from +200 mV to -450 mV vs. SHE and studied the effect on the establishment and performance of a Cr(VI) reducing biocathode. After the acclimatisation period, all biocathodes were connected to their anodes via 200 Ω external resistances. The biocathode poised at -300 mV vs. SHE during start-up gave the maximum power and Cr(VI) reduction rates (6.4 W m^{-3}, 20.2 mg L^{-1} d^{-1}) and these results were similar to the biocathode poised at -150 mV vs. SHE (5.9 W m^{-3}, 19.8 mg L^{-1} d^{-1}). In comparison, control reactors without potentiostatic start-up had a maximum Cr(VI) reduction rate of 14.0 mg L^{-1} d^{-1} and a maximum power production of 4.0 W m^{-3}. In addition, precipitates and pili-like structures were observed by the authors on the bacterial surfaces when the cathodes were poised at -150 and -300 mV vs. SHE, whereas no such pili structures were observed in the control biocathodes.

Huang et al. (2011b) conducted a study with three different materials in Cr(VI) reducing biocathodes (graphite granules, felt and fibres). According to the authors, graphite fibres produced the higher power and specific Cr(VI) reduction rates (4.5 W m^{-3} and 11.3 mg-Cr(VI) g^{-1} VSS h^{-1}). However, for this comparison the authors did not take into consideration the specific surface areas of the different materials (for example, the pores of graphite felt were not taken

into consideration for this comparison; information given personally by Prof Liping Huang) and performances were compared per volume and not per surface area as would be appropriate when comparing materials with different specific surface areas. Also other parameters contributing to these high reported values are the short distance between the electrodes (1.75 cm) and the high membrane surface area (80.4 cm^2), in addition to the small cathodic working volume used (85 mL). All these parameters contribute to the minimisation of the system's internal resistance, however maximising the membrane's surface area and minimising the working volume will finally result in non-economical and even impractical systems. The authors concluded that the cathode to anode (C/A) surface area ratio also affects the performance of Cr(VI) biocathodes. They conducted a study using graphite fibers with three C/A ratios (3, 10, 20) and concluded that higher C/A ratio resulted in higher power produced and also higher Cr(VI) reduction rate: 6.8 W m^{-3} and 4.08 mg L^{-1} h^{-1} for C/A ratio of 3, 13 W m^{-3} and 5.12 mg L^{-1} h^{-1} for ratio 10; 15 W m^{-3} and 6.8 mg L^{-1} h^{-1} for ratio 20. Nevertheless, the authors did not specify in their paper whether they altered the C/A ratio by altering the anode's, the cathode's or both the electrode's surface area. This information is vital for the correct evaluation of their results on the basis of the cathodic electrode surface area. Finally, the authors report a complete 20 mg-Cr(VI) L^{-1} reduction in 3, 4 and 5 h for C/A of 20, 10 and 3 respectively, however they did not use the same external resistances in all three reactors. In fact, they used smaller $R_{ext.}$ for higher C/A ratios (80, 100 and 200 Ω for C/A 20, 10 and 3 respectively) and this was most probably done to maximise power production by all three systems ($R_{int.}$ were 82, 96 and 231 Ω for C/A 20, 10 and 3 respectively). In the same study, kinetic experiments demonstrated pseudo-first order kinetics for Cr(VI) initial concentrations ranging from 20 to 40 mg L^{-1}, however an inhibitory effect was observed at 50 mg L^{-1}. Also, a decrease in the cathodic pH from 8.0 to 5.0 resulted in an increase of the biocathode potential from 30 mV to 130 mV vs. Ag/AgCl, a concurrent increase of the cell potential and also a higher Cr(VI) reduction rate. Finally, the authors reported an increase in the Cr(VI) reduction rate with an increase in temperature from 22 to 35 and then to 50 ^{o}C, however the high temperature of 50 ^{o}C had a negative effect on power production, which was attributed to the reduced electrochemical activity of the anode.

Hsu et al. (2012) studied six different *Shewanella* strains for their performance in Cr(VI) reducing biocathodes. The authors used a simple dual-chamber configuration with reticulated vitreous carbon electrodes (10 cm × 5 cm × 0.5 cm, 80 pores per inch) connected via a 10 Ohms resistor and *Shewanella* as the biocatalyst in both the anode and the cathode. An

acclimatisation step was initially applied in the cathode, where *Shewanella* was using the electrode as an electron donor to bio-catalyse fumarate reduction. After fumarate was depleted, three Cr(VI) spikes were conducted at intervals of 3 days each to increase Cr(VI) concentration in the cathode to 2.5 mg L^{-1}. Despite the fact that the inoculum lacked any organics in solution before the start, the cathodic efficiency of the fumarate reducing cathode was poor for all strains (2–10%). This was attributed by the authors to an extra electron pool probably stored in the extracellular biofilm matrix; a similar behaviour had also been observed by Freguia et al. (2010). In addition, maximum power production during fumarate reduction ranged from 10 nW cm^{-2} for MR-1 to 59 nW cm^{-2} for W3-18-1 (normalisation surface area is not specified). During Cr(VI) reduction there was no clear enhancement in performance using *Shewanella*, as sterile cathodes had similar or even better performance than the *Shewanella* inoculated cathodes. For instance, total Cr(VI) reduced during three spikes was approximately 6.4 mg L^{-1} for the sterile cathode, 6.0 mg L^{-1} for the MR-1, PV-4 and ANA-3 cathodes and 4.8 mg L^{-1} for the MR-4 cathode. An extra electron pool (probably organic carbon in dead cells and also succinate produced during fumarate reduction) and also Cr(VI) adsorption on the electrode surface were considered responsible by the authors for the 67% cathodic efficiency observed for the MR-1 biocathodes; similar results were also obtained from the sterile cathode. However, Cr(VI) adsorption could be questioned on these conclusions, as the negatively charged CrO_4^{2-} anion would eventually have low affinity for the negatively charged cathode.

The higher current and power densities reported by Huang et al. (2010), Huang et al. (2011a) and Huang et al. (2011b), when compared to those of Hsu et al. (2012) and Tandukar et al. (2009), need to be further evaluated (no power or current values could be derived from Hsu et al. (2012)). As will be seen in Chapter 4, electrochemical Cr(VI) reduction is strongly affected by Cr(VI) reduction products, which accumulate on the electrode's surface and deactivate it. Therefore, in order to compare the performance of different conditions in the cathode, current and power normalisation should be done according to the electrode's surface area and not according to the cathode's working volume as was done by Huang et al. (2010), Huang et al. (2011a) and Huang et al. (2011b). In this case, the maximum performance reported in W m^{-3} for the biocathode in Huang et al. (2010) would only be 3.9 mW m^{-2} (620 m^2 m^{-3}) with a 40 mg L^{-1} $Cr(VI)_0$, in Huang et al. (2011a) would be 10.3 mW m^{-2} (620 m^2 m^{-3}) with a 20 mg L^{-1} $Cr(VI)_0$ and in Huang et al. (2011b) would be 1.6 mW m^{-2} (9,600 m^2 m^{-3}) with a 20 mg L^{-1} $Cr(VI)_0$. As can be seen, maximum power densities obtained by normalising to the cathode surface area can

be comparable to the ones reported by Tandukar et al. (2009). This finally could bring under discussion the conclusion made by Huang et al. (2010), who claimed an enhanced performance, that is, enhanced power production and Cr(VI) reduction of their system, compared to the system described in Tandukar et al. (2009).

Table 2-3: Studies with Cr(VI) as the electron acceptor (abiotic cathodes)

	Li et al. (2008)	Wang et al. (2008)	Li et al. (2009)	Liu et al. (2011)	Pandit et al. (2011)	Zhang et al. (2012)
Wastewater type	Real electroplating wastewater	Synthetic	Synthetic	Synthetic	Synthetic	Synthetic
Cathodic catalyst/mediator	N/A	-	Rutile	H_2O_2	-	-
Anodic catalyst	Anaerobic sludge	N/A	Anaerobic sludge collected from lake sediments	Mixed culture/ *S. decolorationis*/ *K. pneumoniae*	*S. putrefaciens*	Sludge and effluent of MFCs
Cathode (dimensions)	Graphite paper (2×5×0.02 cm^3)	Graphite plate (4.5×2.5×1 cm^3)	Rutile-coated polished graphite (5×7×0.5 cm^3)	Carbon felt (4.5×4.5 cm^2)	Graphite block (31.5 cm^2)	Carbon fiber felt (4×4×0.3 cm^3)
Anode (dimensions)	Carbon felt (2×5×0.2 cm^3)	Graphite plate (4.5×2.5×1 cm^3)	Unpolished graphite plate (5×7×0.5 cm^3)	Carbon felt, (4.5×4.5 cm^2)	Stainless steel block (31.5 cm^2)	Carbon fiber felt (4×4×0.3 cm^3)
Membrane (dimensions)	Nafion™ 117 PEM (45 cm^2)	Nafion™ 117 PEM (4.9 cm^2)	Nafion™ 117 PEM (4.9 cm^2)	ESC-7000 CEM (N/A)	Ralex™ AEM (13.5 cm^2)	Nafion™ 117 PEM (N/A)
Electron donor	Acetate	Acetate	Acetate	Glucose	LB medium	Glucose
Maximum power density observed*	800 mW m^{-2}	46 mW m^{-2}	N/A	26 mW m^{-2}	49 mW m^{-2}	448 mW m^{-2}
Current density for maximum power*	2,000 mA m^{-2}	114 mA m^{-2}	N/A	100 mA m^{-2}	170 mA m^{-2}	1,104 mA m^{-2}
Cathodic pH reported	1.0-6.0	2.0-6.0	2.0	2.0-7.0	3.0, 5.0, 7.0, 7.5	2
Maximum Coulombic efficiency (%)	12†	59†	N/A	8†, 70‡	N/A	26†
Maximum Cr(VI) removal from solution (%)	99	100	97	100	N/A	75
Maximum Cr_{total} removal from solution(%)	66	N/A	N/A	N/A	N/A	45

*Values given by the authors were normalised to the total apparent surface area of the cathode

†Calculated based on COD removal

‡Calculated based on Cr(VI) removal

Table 2-4: Studies with Cr(VI) as the electron acceptor (biocathodes)

	Tandukar et al. (2009)	Huang et al. (2010)	Huang et al. (2011a)	Huang et al. (2011b)	Hsu et al. (2012)
Wastewater type	Synthetic	Synthetic	Synthetic	Synthetic	Synthetic
Cathodic catalyst/mediator	Denitrifying and anaerobic mixed cultures enriched in the presence of Cr(VI)	Inoculum from a Cr(VI) contaminated soil	Primary sludge	Mixed culture obtained from a MFC	6 *Shewanella* strains
Anodic catalyst	Mixed anaerobic sludge	Primary sludge	Mixed culture obtained from a MFC	Mixed culture obtained from a MFC	*Shewanella* MR-1
Cathode (dimensions)	Graphite plate (5.4×2.5×0.6 cm^3)	Graphite granules (1.5-4.0 mm diameter, 0.35 porosity, 620 m^2 m^{-3} of reactor)	Graphite granules (620 m^2 m^{-3} of reactor)	Graphite fibres, felt and granules * (N/A)	Reticulated vitreous carbon (80ppi, 10×5×0.5 cm^3)
Anode (dimensions)	Graphite plate (5.4×2.5×0.6 cm^3)	Graphite plate (4.5×2.5×1 cm^3)	Graphite brush (N/A)	Graphite fibres (N/A)	Reticulated vitreous carbon (80ppi, 10×5×0.5 cm^3)
Membrane (dimensions)	Nafion™ 117 PEM (N/A)	Nafion™ 117 PEM (4.9 cm^2)	CMI-7000 (80.4 cm^2)	CMI-7000 (80.4 cm^2)	CMI-7000 (N/A)
Electron donor	Acetate	Acetate	Acetate	Acetate	Lactate
Maximum power density observed†	56 mW m^{-2}	3.9 mW m^{-2} (2.4 W m^{-3})	10.3 mW m^{-2} (6.4 W m^{-3+})	1.6 mW m^{-2} (15 W m^{-3})	N/A (59 nW cm^{-2} during fumarate reduction; normalisation area is not specified)
Current density for maximum power†	100 mA m^{-2}	11.1 mA m^{-2} (6.9 A m^{-3})	33.9 mA m^{-2} (21 A m^{-3})	5 mA m^{-2} (48 A m^{-3})	N/A
Cathodic pH reported	7.0	5.7-7.0	7.0	5.0-8.0	7.0
Maximum Coulombic efficiency (%)	>100‡	100‡	N/A	100	100§
Maximum Cr(VI) removal from solution (%)	100	99	100	100	100
Maximum Cr_{total} removal from solution(%)	N/A (authors report 25% adsorption of Cr_{total} on the electrode)	55	100	80	N/A

* Specific surface area per m^3 of reactor; graphite fibres: 9600 m^2 m^{-3}, graphite felt: 2000 m^2 m^{-3}, graphite granules: 800 m^2 m^{-3}

† Values given by the authors were normalised to the total apparent surface area of the cathode

‡ Calculated based on COD removal

§ Calculated based on Cr(VI) removal

2.3. *Shewanella* for MFC and Cr(VI) reduction applications

2.3.1. General

Shewanellae are γ-Proteobacteria, gram-negative rods 2–3 μm in length and 0.4–0.7 μm in diameter (Hau and Gralnick, 2007). They are organoheterotrophic metal reducers with the ability to adapt easily in environments where the electron donor is abundant but the electron acceptor can vary and be limited and variable within short distances (Pinchuk et al., 2010). They are the most diverse respiratory organisms described so far (Hau and Gralnick, 2007, Gao et al., 2010), with an electron transport system that includes (at least) 39 c-type cytochromes (Tiedje, 2002). They are facultative anaerobes and have been isolated from a range of salt concentrations, barometric pressures and temperatures (Hau and Gralnick, 2007).

In particular, *S. oneidensis* MR-1 bacteria are able to utilise lactate, acetate, pyruvate and amino acids as carbon sources, with the tricarboxylic cycle being the major metabolic pathway under both aerobic and oxygen-limited conditions (Tang et al., 2007a). Under aerobic conditions, acetate is metabolised but at lower rates than lactate, after lactate has dropped to low concentrations (Tang et al., 2007b). Under anaerobic conditions, acetate is not utilised by *Shewanella* (Lanthier et al., 2008), and partial oxidation of lactate to acetate can cause acetate accumulation. In general, *Shewanella* grow with oxygen, fumarate, nitrate and metal oxides as electron acceptors (Gao et al., 2010), however growth has also been observed when electrode surfaces were serving as the sole electron acceptors (Lanthier et al., 2008). On the other hand, it has been evidenced that acetate can inhibit the growth of MR-1 at concentrations over 10 mM (Tang et al., 2007b).

2.3.2. *Shewanella* in MFC and electron transfer studies

2.3.2.1. *Shewanella* in MFC anodes

2.3.2.1.1. General

Shewanella sp., along with *Geobacter* sp., have been up to now the most extensively studied microorganisms in MFC anodes. This section is presenting some important studies on *Shewanella* sp. operating anodes and is divided according to the redox environment of the anodes (anaerobic/aerobic), which however might differ from the redox environment of the initial cultivation. Various operation parameters have been applied in these studies and the key conclusions are summarised in this section.

2.3.2.1.2. Anaerobic anodes

Kim et al. (1999b), were the first to report the electrochemical activity of anaerobically grown cells of *S. putrefaciens* IR-1 and Kim et al. (1999c), first talked about a lactate biosensor-type MFC utilising IR-1. In this first study by Kim et al. (1999b), c-type cytochromes localised on the outer membrane of anaerobically grown IR-1 were considered responsible for this catalytic effect of IR-1. No electrochemical activity was found in these early studies when *Shewanella* was grown aerobically, however this issue is further debated in Section 2.3.2.1.3.

Planktonic cells of *S. oneidensis* MR-1 have been found in higher concentration than electrode-attached ones (Lanthier et al., 2008) and have also been considered primarily responsible for current generation under anaerobic conditions (Biffinger et al., 2009). However, extensive biofilm formation has been observed under aerobic conditions with either lactate or glucose as the carbon source (Biffinger et al., 2009).

The pH is also very important in *Shewanella* operating MFCs. Biffinger et al. (2008b), showed that when under acidity stress (pH 5), MR-1 produced 70% more electron shuttle mediators than *Shewanella oneidensis* DSP10 did and outperformed DSP10 in terms of power and current generation. However, in the same study DSP10 exhibited higher current generation than MR-1 did at pH 6 and 7.

Other crucial parameters in *Shewanella* operated MFCs are the electrode's operating potential and also the fuel's concentration. Cho and Ellington (2007), suggested that increasing the applied potential or lactate concentration might be a good strategy for the adaptation of aerobically grown MR-1 to anaerobic environments and the enhancement of their electrochemical activity. In their study, they showed that increasing the poised electrode's potential from 0 to 500 mV vs. Ag/AgCl or lactate's concentration from 5 mM to 500 mM reduced the lag period for current production from 90 h to 5 h and from 50 h to 5 h respectively. However, a further increase to over 750 mV vs. Ag/AgCl had a negative effect in this study.

On the other hand, Peng et al. (2010b) reported weaker anodic electrochemical activity of MR-1 (grown aerobically, incubated anaerobically) when poised at 0 V than when poised at -240 mV vs. SCE (saturate calomel electrode; +244 mV vs. SHE). According to the authors, this was due to the expression of weakly electroactive OmcA/MtrC cytochromes at 0 mV vs. SCE, hindering the contact between the electrochemically active flavins and the electrode, thus

producing less oxidative current than when poised at lower potentials where OmcA/MtrC were not expressed.

2.3.2.1.3. Aerobic anodes

After the first studies conducted by Kim et al. (1999b), which showed no electrochemical activity of aerobically grown cells of IR-1, these results were again confirmed by Kim et al. (1999a), who studied the effect of nitrate and oxygen exposure to anaerobically grown IR-1. As the authors noticed, the electrochemical activity was inhibited after oxygen exposure (total inhibition after 3 h) but not after nitrate addition. However, this kind of inhibition was not irreversible and was better restored by poising the electrode with a positive potential at +200 mV vs. Ag/AgCl. Finally, no electrochemical activity was attributed in this study to aerobically grown cells or cells grown with nitrate as the sole electron acceptor. Oxygen was considered to inhibit the expression of electroactive components on the surface of IR-1 and this was also observed by Kim et al. (2002) for aerobically grown cells of MR-1.

Regardless these earlier studies, more recent studies have shown that *Shewanella* species exposed or grown under oxygen rich conditions can effectively operate in MFC anodes. One of these studies was conducted by Ringeisen et al. (2007). In this study, power was produced by aerobically grown *S. oneidensis* DSP10 under both aerobic and anaerobic conditions, however under aerobic conditions DSP10 had 50% the Coulombic efficiency and produced 60% the power produced under anaerobic conditions. AQDS addition in this study increased power production in both cases, however power in aerobic MFCs was still 33% less than in anaerobic ones.

An interesting trend was observed by Biffinger et al. (2008a), when they utilised glucose as the carbon source in MFCs inoculated with DSP10 under both aerobic and anaerobic conditions. According to the authors, maximum power and short circuit current under aerobic conditions were almost three times that under anaerobic conditions and this was attributed to the ineffective utilisation of glucose by DSP10 under anaerobic conditions. In the same study, fructose and ascorbic acid also generated current under aerobic conditions, possibly due to the higher levels of proteins' expression under aerobic conditions by *S. oneidensis*, even if all these nutrients are considered as unusable for anaerobic growth on Fe(III) (Biffinger et al., 2008a).

In a later study, Biffinger et al. (2009), compared aerobically and anaerobically operated MFCs inoculated with MR-1 and lactate or glucose as the carbon source. With lactate as the e^- donor, the authors noticed an eight-fold more current generated by air-exposed MR-1 than by

anaerobically operated MR-1. Also increased current and Coulombic efficiency were observed as the biofilm was forming on the surface of air-exposed anodes but not on anaerobically operated ones. Positive current responses for every lactate addition were reported, however repetitive glucose additions under anaerobic conditions did not have the same current producing effect in this study.

After potentiostatically controlling anaerobically grown MR-1 at +400 mV vs. SHE for 21 days, Rosenbaum et al. (2010) started aerating the anode and recorded the current response. Their findings demonstrated an almost three times rise of current soon after aeration and this enhanced current production continued for several days after the medium tank was switched back to a CO_2/N_2 environment. This enhanced performance was attributed by the authors to ***i)*** the complete oxidation of lactate under aerobic conditions and ***ii)*** the higher biomass density of *S. oneidensis* produced under aerobic conditions. However, the CE remained low (22%), due to the non-electricity producing metabolic pathways when oxygen is provided (Rosenbaum et al., 2010).

Li et al. (2010a), also noticed an enhanced current generation by *Shewanella decolorationis* NTOU1 under aerobic conditions, during the 6 days of a potentiostatically controlled operation (+400 mV vs. Ag/AgCl). In this study, higher metabolic rates and NADH production induced by oxygen were positively correlated by the authors to the higher current production. Finally, the authors found higher concentrations of free flavin produced under aerobic conditions and also that spiked free flavin had little effect on current generation under anaerobic conditions.

2.3.2.2. Shewanella in MFC cathodes

Despite extensive studies with *Shewanella* sp. in MFC anodes, studies in the cathodes remain very limited. These are presented in this section, with the exception of the study conducted by Hsu et al. (2012) which has already been presented earlier in Section 2.2.6.3. Freguia et al. (2010) were the first ones who studied *S. putrefaciens* as a catalyst in an oxygen reducing biocathode. In a half-cell cathode poised at -200 mV vs. SHE, *S. putrefaciens* produced a catalytic current attributed in this paper to catalytic oxygen reduction. Current increased slowly within the first 20 h and this slow rate was attributed by the authors to the possible storage of organic carbon within the cells that could act as an alternative electron donor. After 24 h at -200 mV vs. SHE, cyclic voltammetry revealed the same shape and magnitude for both harvested cells and the supernatant, with the catalytic current having an onset at a potential similar to that of cytochromes OmcA and MtrC (Freguia et al., 2010). According to the authors,

this indicated that compounds, which are present on the outer membrane surface and are responsible for the catalytic current may also be released to the surrounding medium.

In the study of Biffinger et al. (2011), it was found that redox mediators other than riboflavin were produced by *Shewanella japonica*, which enhanced the abiotic reduction of oxygen. In this study, these biologically produced redox mediators were considered responsible for a positive shift (0.13 V) of the oxygen reduction wave observed in the cyclic voltammograms, which translates into a decrease of the cathode overpotentials. On the other hand, in the study of Liu et al. (2012), it was claimed that it is flavins produced by anode-respiring *Shewanella* cells that are adsorbing on the electrode surface and enhancing cathodic oxygen reduction. Finally, in the most recent study, conducted by Varia et al. (2013), cells of *Shewanella putrefaciens* CN32 were found capable of catalysing the reduction of Au^{3+} and Fe^{3+} ions in acidic media (pH 2). No catalytic effect was found for Co^{2+} electroreduction and the exact catalysing mechanism was not clarified in this study. However, the authors give as possible explanations the presence of c-cytochromes, redox mediators and also biosorption/desorption mechanisms acting via the lipopolysaccharide bacterial membrane.

2.3.2.3. The electron transfer mechanisms

2.3.2.3.1. General

The electron transfer mechanisms of *Shewanella* have been extensively studied, revealing a broad respiratory network consisting of soluble flavins, c-cytochromes and nanowires. Studies on the electron transfer mechanisms included studying the efficiency of mutants to produce current, form biofilms and reduce solid metal oxides, the conductivity of nanowires, the importance of flavins in distant electron shuttling etc.. As a summary, the Mtr respiratory pathway plays a central role in respiration, with ΔOmcA ΔMtrC mutants exhibiting a reduced ability to attach to and reduce solid surfaces, to reduce soluble flavins and to produce electrically conductive nanowires. Flavin production and utilisation is essential in extracellular electron transfer that *Shewanella* have advanced to reach electron acceptors when the latter are not abundant. Key studies on the electron transfer mechanisms are presented below.

2.3.2.3.2. Direct electron transfer

The importance of cytochromes has mainly been investigated in the literature by studying the performance of mutants lacking the genes for their expression. A study conducted by Bretschger et al. (2007), demonstrated that mutants lacking the genes for MtrA, MtrB and

OmcA MtrC expression all exhibited limited ferric and electrode reduction capability when compared to the wild strain, with the ΔmtrA and ΔmtrB mutants exhibiting the lower performance. Also mutants deficient in type IV prepilin peptidase or type II secretion (ΔpilD, ΔgspG and ΔgspD), all demonstrated reduced efficiency in both Fe(III) and Mn(IV) reduction, electrode colonisation and current production. However, the fact that the ΔomcA ΔmtrC mutant exhibited higher Mn(IV) reduction even compared to the wild strain, made the authors support that this mutant was not limited in the ability to transfer electrons to solid surfaces in general, but there may be different mechanisms employed for metal oxide reduction than for current generation.

Bouhenni et al. (2010), also found reduced ability of the ΔomcA ΔmtrC and ΔgspG MR-1 mutant to produce current and reduce Fe(III) oxides. In addition, they found that the deficiency of ΔpilD mutant in current and Fe(III) oxide reduction was not due to the loss of type IV pili or flagella (Δflg and ΔpilM-Q mutants produced twice as current as the wild type strain and could reduce Fe(III) oxides equally to the wild strain, despite the same levels of riboflavin produced by the mutants and the wild type strain) but rather due to type II secretion dysfunction (absence of c-cytochromes). Their findings led to the conclusions that the mechanisms for electron transfer to metal oxides and anodes are the same for MR-1, however they did not test Mn(IV) oxides as Bretschger et al. (2007) did.

A later study by Carmona-Martinez et al. (2011), who tested ΔomcA ΔmtrC, ΔpilM-Q, ΔmshH-Q, ΔpilM-Q ΔmshH-Q and Δflg mutants of *S. oneidensis* MR-1, found that the catalytic current associated with the direct electron transfer mechanisms was not totally inhibited in either of these mutants. On the contrary, cyclic voltammetry signals associated with both direct and mediated electron transfer mechanisms were observed for all mutants and the wild strain, supporting the hypothesis that when a redox protein cannot be used, bypass molecules can replace it. Also in this study, the contribution of mediated electron transfer was increased when pili, outer surface cytochromes or flagella could not be utilised as direct electron transfer pathways, highlighting the flexibility of *Shewanella* respiration pathways.

Another way of direct electron transfer is through conductive appendages called nanowires. The study of Gorby et al. (2006) first reported their production by MR-1 cells previously exposed to a limited electron acceptor environment. In this study, mutants lacking OmcA MtrC or a functional type II secretion system exhibited decreased electrochemical activity in MFC anodes and this was believed to be due to the poor electrical conductance of their nanowires. This was later proved by El-Naggar et al. (2010), who measured the electrical conductivity of

these nanowires produced by MR-1 and found them conductive along the micrometre-length scales but non-conductive in ΔMtrC ΔOmcA mutants. Similar pili-like structures had previously been observed on the surface of *Geobacter sulfurreducens* by Reguera et al. (2005), who showed that these structures are there to aid the cells reach and reduce solid Fe(III)-oxides.

2.3.2.3.3. Mediated electron transfer

An early study conducted by Newman and Kolter (2000), indicated that when mutants of *Shewanella putrefaciens* lacking a gene involved in the biosynthesis of menaquinone were streaked on plates with AQDS as the sole e^- acceptor, no AQDS reduction was observed, however when the mutants were streaked in the vicinity of the wild-type strain, they were able to grow and reduce AQDS. This fact made the authors conclude that a soluble substance, probably a quinone produced by the wild-type strain, could be directly involved in extracellular electron transfer. Nevertheless, a later study by Myers and Myers (2004), found that the substance produced by the wild-type strain could actually restore the ability of the mutants to synthesise menaquinone rather than act as a redox mediator itself.

In a later study, riboflavin and riboflavin-5'-phosphate were considered responsible for current production in biofilm reactors inoculated with anaerobically grown *Shewanella oneidensis* MR-1 and *Shewanella* sp. MR-4 (Marsili et al., 2008). In this study, removal of riboflavin from the biofilms caused a 70% reduction in current production, however current recovered up to 96 h after medium replacement, along with the production of electron shuttle mediators by the attached cells. The authors also found that flavins have the ability to be adsorbed on the electrode surface and on other surfaces that *Shewanella* sp. can use as electron acceptors like Fe(III) and Mn(IV) oxides.

In a parallel study, von Canstein et al. (2008), identified flavin mononucleotide (FMN) as the dominant flavin produced by *Shewanella* strains, including MR-1, while riboflavin and flavin adenine dinucleotide (FAD) were also detected. Flavins were excreted equally efficiently in the presence or absence of oxygen and this was attributed by the authors to *Shewanella*'s ability for quick adaptation to changes in the oxic-anoxic environments where they normally live. FMN production was mainly related to growth, in contrast to riboflavin that reached its maximum levels in the stationery phase. Also FAD was the dominant intracellular flavin in this study and it was released by *Shewanella* only after cell lysis. To conclude, this study supported the fact that flavins are produced nomatter if Fe(III) is in soluble or insoluble form but are only required for insoluble Fe(III) reduction.

In another study by Baron et al. (2009), the addition of soluble flavins not only enhanced current generation by MR-1 but also allowed the catalytic effect to occur at lower applied potentials. Current production by ΔomcA and ΔmtrC mutants was not enhanced by riboflavin addition in this study, underperforming the wild-type, regardless of their maintained ability to attach to electrodes.

Coursolle et al. (2010), also supported the idea of electron shuttling by MR-1 excreted flavins, which were found to stimulate the reduction of insoluble iron more than AQDS does. The Mtr respiration pathway was considered by the authors to be responsible for at least 90% of the flavins reduction activity and flavin-assisted electrode reduction rates were found considerably higher than the rates achieved by outer membrane cytochromes alone. Both MtrC and OmcA were believed in this study to have a crucial role in solid surfaces' reduction, with mutants lacking OmcA exhibiting a reduced ability to attach and consequently reduce solid surfaces, despite their unaffected ability to reduce soluble substrates like flavins. On the other hand, ΔmtrC mutants were found defective in reducing both soluble (flavins) and insoluble (electrodes) electron acceptors, despite maintaining their ability to attach onto solid surfaces.

In a later study, Velasquez-Orta et al. (2010), added FMN and riboflavin in MFC anodes inoculated with MR-1 and found that current increased at least three times when compared to MFCs without added flavins. FMN and riboflavin were equally effective as electron shuttles, and when FMN was added, its concentration remained stable for as long as lactate was present. On the other hand, when riboflavin was added, its concentration decreased even in the presence of lactate. This, according to the authors, could be either due to the utilisation by the cells or due to the adsorption on the electrode surface, as described previously by Marsili et al. (2008).

2.3.3. *Shewanella oneidensis* MR-1 in Cr(VI) reduction studies

2.3.3.1. General

Cr(VI) reduction by MR-1 has been interpreted to be cell associated, with both intracellular and extracellular compounds participating in the process. Type c-cytochromes responsible for current generation in MFCs have also been considered to be responsible for part of the Cr(VI) reductase activity in MR-1, whilst biosorption does not contribute to Cr(VI) reduction. Cr(VI) can inhibit the growth of MR-1, however inhibition varies depending on Cr(VI) concentrations and culture conditions. Finally, Cr(VI) can inhibit the reduction of other electron acceptors like nitrate and vice versa. Key studies presented below support these statements.

2.3.3.2. Mechanism of Cr(VI) reduction

According to Viamajala et al. (2003), the Cr(VI) reduction mechanism by MR-1 appears to be enzymatic- and cell-associated, able to be modelled using an enzyme kinetic analysis. Using cultures grown with either nitrate or fumarate as the e^- acceptor, the authors developed a model describing anaerobic Cr(VI) reduction by two parallel mechanisms. The first mechanism involves the rapid Cr(VI) reduction by an enzyme which rapidly deactivates during Cr(VI) reduction, and the second mechanism involves a 10 times slower but more stable reduction by another enzyme. When the authors compared fumarate-grown with nitrate-grown cultures, faster reduction rates were obtained by the latter for both the deactivating (8.54×10^{-10} vs. 6.89×10^{-10} mmol Cr(VI) h^{-1} $cell^{-1}$) and the stable enzymes (6.89×10^{-11} vs. 5.23×10^{-11} mmol Cr(VI) h^{-1} $cell^{-1}$). The model equation given by the authors to describe Cr(VI) reduction in a batch system is as follows:

$$[Cr(VI)] = [Cr(VI)]_0 - \left[r_{s0}t + \frac{r_{d0}}{k'_d}\left(1 - e^{-k'_d t}\right)\right]$$

where:

[Cr(VI)]: Cr(VI) concentration, mM

$[Cr(VI)]_0$: initial Cr(VI) concentration, mM

r_{s0}: the initial rate of reaction caused by the stable enzyme, mM Cr(VI) h^{-1}

t: time, h

r_{d0}: the initial rate of reaction caused by the deactivating enzyme, mM Cr(VI) h^{-1}

k'_d: a factor similar to a first-order constant, h^{-1}

As the authors supported, biosorption is not the case for MR-1, where the negatively charged bacterial cells are unable to bond with the CrO_4^{2-} and $Cr_2O_7^{2-}$ ions present at neutral and acidic pH values. However, under strongly acidic conditions Cr(VI) uptake can be possible by "acid adsorption", where the abundance of protons in the liquid can cause anion exchange (Srinath et al., 2002).

Regarding the MR-1 Cr(VI) reductase activity, Myers et al. (2000) suggested that this was localised in the cytoplasmic membrane, it was not associated with the presence of Cr(VI) during growth, and it was not irreversibly inhibited by the presence of oxygen. In comparison, fumarate and ferric reductase activities were found in distinct locations in their study, i.e. the periplasm and the outer membrane, respectively. Following these findings and also due to

Cr(VI) reduction inhibition by electron transport inhibitors, the authors suggested that an energy generation pathway utilizing Cr(VI) as the electron acceptor might be present.

In a later study, Daulton et al. (2007), reported accumulated Cr(II) near the cytoplasmic membrane of MR-1, even before the formation of insoluble Cr(III) precipitates on the cells' surface. According to the authors, this indicated that the final reduction step was intracellularly localised and that Cr(II), as the final reduction product, is further oxidised in the presence of available oxidisers (e.g. oxygen) to the more stable Cr(III) organic complexes and/or hydroxides after being expelled from the cell and bonded to the negatively charged extracellular biopolymers available.

In a more recent study, Gao et al. (2010) studied Cr(VI) reduction by 13 different mutants of MR-1, amongst which some lacking cytochromes OmcA, MtrD and MtrC. During their study, the authors noticed that mutants lacking OmcA and MtrD cytochromes exhibited worse performance with regards to Cr(VI) reduction than MtrC lacking mutants, suggesting, according to the researchers, that MtrC was not the major terminal Cr(VI) reductase. As it has been shown previously, cytochromes MtrC and OmcA are outer membrane decaheme cytochromes important for the energy generation by MR-1 (Bretschger et al., 2007) and also for electron transfer to MFC anodes, however they might not be the only Fe(III) and Mn(IV) terminal reductases (Gorby et al., 2006). Other cytochromes characterised by the authors as critical for anaerobic Cr(VI) reduction included cytochromes PetC, CcoO and CcoP.

On the other hand, by studying cytochromes MtrC and OmcA alone, Belchik et al. (2011) suggested that these are the MR-1 terminal Cr(VI) reductases. By creating mutants lacking one or both of these cytochromes, Cr(VI) was reduced by up to 68.9% less when compared to the wild type ($[Cr(VI)]_0$= 200 μM= 10.4 mg L^{-1}). Also, since ΔMtrC and ΔOmcA mutants still reduced Cr(VI), and Cr(III) precipitates were found inside their cells, the authors acknowledged that an amount of Cr(VI) must be intracellularly reduced by MR-1. Cr(III) present in the cytoplasm was attributed by the authors to be responsible for the toxic effects observed, as this was believed to bind to *Shewanella*'s cellular components and interfere with its cellular functions. Finally, the Michaelis-Menten K_m values for MtrC and OmcA measured by the authors (34.1 and 41.7 μM respectively) led to the conclusion that MtrC and OmcA-mediated Cr(VI) reduction should work optimally at μM-level Cr(VI) concentrations.

2.3.3.3. Inhibition effects

2.3.3.3.1. Inhibition of growth

In an early study by Middleton et al. (2003), growth of aerobic cultures was inhibited when exposed to Cr(VI) concentrations of 7.8-20.8 mg L^{-1} (150-400 μM) but not when exposed to 3.1 mg L^{-1} (60 μM). In the same study, the authors found that anaerobic cultures were able to reduce approximately 4.5 times more Cr(VI) within the same time period when they were spiked with sequential low Cr(VI) concentrations, compared to when spiked with single but higher Cr(VI) concentrations.

Later on, Brown et al. (2006), found that MR-1 grown aerobically in the presence of 0.3 mg L^{-1} or 6.5 mg L^{-1} Cr(VI), showed slightly decreased growth rates when compared to growth in the absence of Cr(VI). However, similar maximum cell density was obtained after 48 h in their study. Also MR-1 was unable to grow in the presence of 104 mg L^{-1} Cr(VI) during this 48 h period, whilst growth in the presence of 13 mg L^{-1}, 26 mg L^{-1} and 52 mg L^{-1} Cr(VI) revealed extended lag periods (20 h-40 h), followed by biphasic growth. Finally, after studying the genes involved in Cr(VI) reduction, Brown et al. (2006), concluded to "a combination of different regulatory networks that involve genes with annotated functions in oxidative stress protection, detoxification, protein stress protection, iron and sulfur acquisition and SOS-controlled DNA repair mechanisms".

In another study, Tang et al. (2006) studied MR-1 growth during Cr(VI) reduction, whilst continuously monitoring and controlling temperature, pH and dissolved oxygen levels. As they report, growth was not inhibited by the addition of 5.2 mg L^{-1} Cr(VI) at neutral pH and temperature of 30-35 ^{o}C, however it was inhibited when the same concentration was added into cultures growing at temperatures <25 ^{o}C or when pH was less than 6.5 or greater than 8.5. Finally, growth was also inhibited when MR-1 was exposed to dissolved oxygen levels >80% and that was attributed in this study to the toxicity of the produced free radicals of oxygen.

2.3.3.3.2. Competition with other electron acceptors

In a study conducted by Middleton et al. (2003), Cr(VI) supressed nitrate reduction and nitrite production when added at 2.3 mg L^{-1} but not when added at 0.3 mg L^{-1}. A complete stop of nitrate reduction occurred when Cr(VI) was added at 9.9 mg L^{-1}; according to the authors, a higher electron flow towards Cr(VI) was observed when initial Cr(VI) concentrations were also higher. Also, even if no growth was observed in this study when Cr(VI) was available as the sole

e^- acceptor, the transformation capacity (mg-Cr(VI) reduced per mg of protein) in this case was higher than when nitrate or oxygen were also present (1.4 and 9.8 times respectively).

Cr(VI) reduction inhibition caused by nitrate in nitrate-grown MR-1 cultures had been reported earlier by Viamajala et al. (2002), however this was not the case for fumarate-grown cultures. On the other hand, fumarate did not exhibit any kind of Cr(VI) reduction inhibition in neither fumarate or nitrate-grown cultures as reported by the authors. Finally, nitrite inhibited Cr(VI) reduction in both fumarate and nitrate-grown MR-1 cultures in this study, and inhibition was slightly higher in the nitrate reducing cultures.

2.4. Conclusions from the literature review

As discussed earlier, there are limited studies on Cr(VI) reducing cathodes and therefore there is great potential for research on the topic. However, successful attempts on Cr(VI) reducing biocathodes have only been made by two research groups until now (those of Dr Tandukar and Dr Huang); this is mainly because there has not been a clear demonstration of the important mechanisms and/or weaknesses involved in these systems. The proof-of-concept has been shown, however studying the mechanisms behind the process will help identify the key elements and make the process reproducible by others in the field.

Under these terms, studying a pure culture can be very useful because one has to deal with a more defined system (compared to the mixed populations used in other studies) from which safer conclusions can be drawn regarding the different pathways involved. *Shewanella* sp. is a very good candidate for this study as it is a well-known metal reducer that has been extensively studied in MFC anodes. Therefore, if studied appropriately, a connection with other MFC studies can be made in terms of the biocatalysts' behaviour and the electron transfer mechanisms involved.

On the other hand, the biocatalyst is a vital but not the only important component in Cr(VI) reducing MFCs and the literature still needs to address numerous challenges in the process as a whole in order to improve its performance. For example, no particular study has been conducted on how the performance of cathodes can be improved without the use of bacterial catalysts at non-acidic pH ranges; until now studies have either used abiotic MFC cathodes only as control reactors for biotic cathodes at non-acidic pH, or they have focused on acidic pH cathodes. Another question not yet adequately addressed is whether AEMs are suitable for Cr(VI) reducing MFCs. If questions like these are more appropriately studied, then the MFC technology will be able to move a step further ahead.

Considering the above, the following main **hypotheses** complement the literature and are being studied (amongst others) in the present PhD study:

- the presence of metal chelators can enhance Cr(VI) electroreduction and prevent fouling of the electrode with Cr(VI) reduction by-products
- electrophilic metal-reducers like *Shewanella* sp. can be utilised as biocatalysts in both the anode and cathode of a Cr(VI) reducing MFC
- bacterial electron shuttle mediating is a major electron transfer mechanism in Cr(VI) reducing biocathodes with *Shewanella* sp. as biocatalysts and affects both anodic and cathodic currents

Chapter 3 Research methodology

3.1. General

This chapter introduces the research methodology generally followed throughout this study, including common procedures and features of the experiments conducted (e.g. reactors' configuration, electrode preparation, data monitoring and calculations). In general, the present work consists of flask experiments and BES experiments in either MFCs or potentiostatically poised reactors. Mixed populations of microorganisms have been used in this study, however pure cultures of *Shewanella oneidensis* MR-1 were mainly used and have a central role in it. More detailed information on each experimental setup can be found in Chapter 4.

3.2. Reactors set-up

As shown in Figure 3-1 and Figure 3-2, common dual-chamber MFCs with a single chamber working volume of 200 mL were made by joining two 250 mL borosilicate bottles, each specially modified with a sidearm tube. The joint was made using underwater epoxy resin and the membrane was fixed in the middle of the joint, occupying 4 cm^2 for electrical connection between the two chambers. Ventilation and gas sparging tubes were inserted through the top lid and each chamber had a sampling port at an appropriate height. Rubber stoppers were used to keep the reactors airtight and to run the electrodes' connecting copper wires. Where applicable, an electrical circuit was closed with a suitable external resistance connected to the electrodes present in each compartment. When working with pure cultures, except otherwise stated, the MFCs were autoclaved (121 ^{o}C, 15 min) prior to use, with all the components fixed. When reactors were flushed with oxygen-free N_2 or air, gases were supplied by a compressed cylinder and an aquarium pump (160 mL min^{-1}) respectively. If pure cultures were present, the gases were first passed through 0.2 μm filters. All bioelectrochemical experiments were conducted at room temperature (22 ± 4 ^{o}C) with reactors covered with aluminium foil to prevent light exposure. Unless otherwise stated, all WE and MFC chambers were stirred at 250 rpm using a magnetic stirrer.

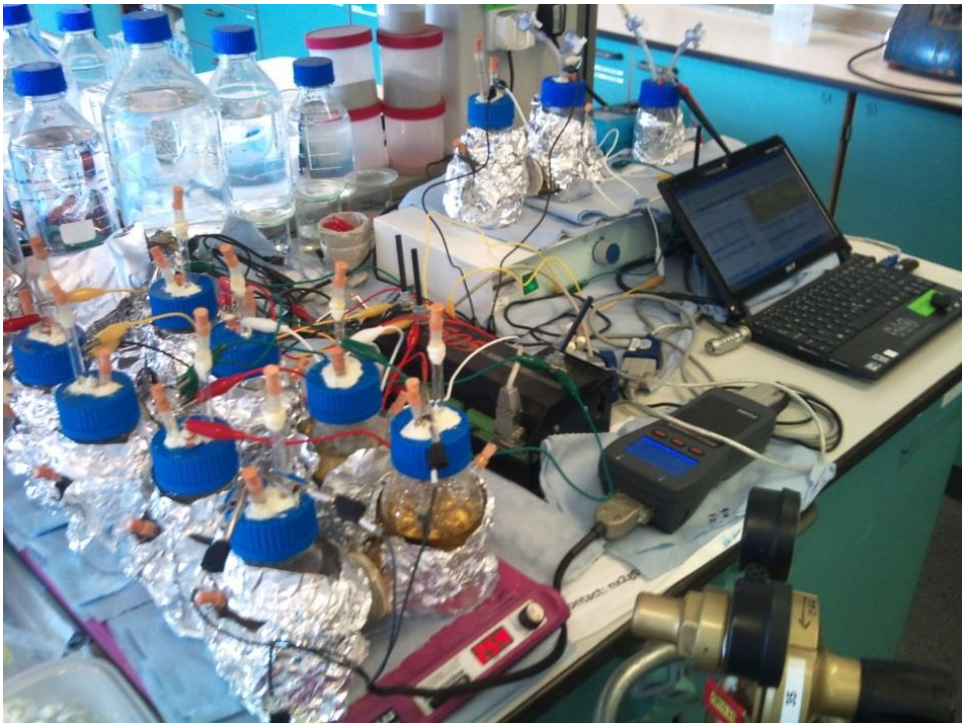

Figure 3-1: General experimental setup with dual-chamber MFCs connected to a potentiostat or a datalogger and a PC for data recording and experimental control.

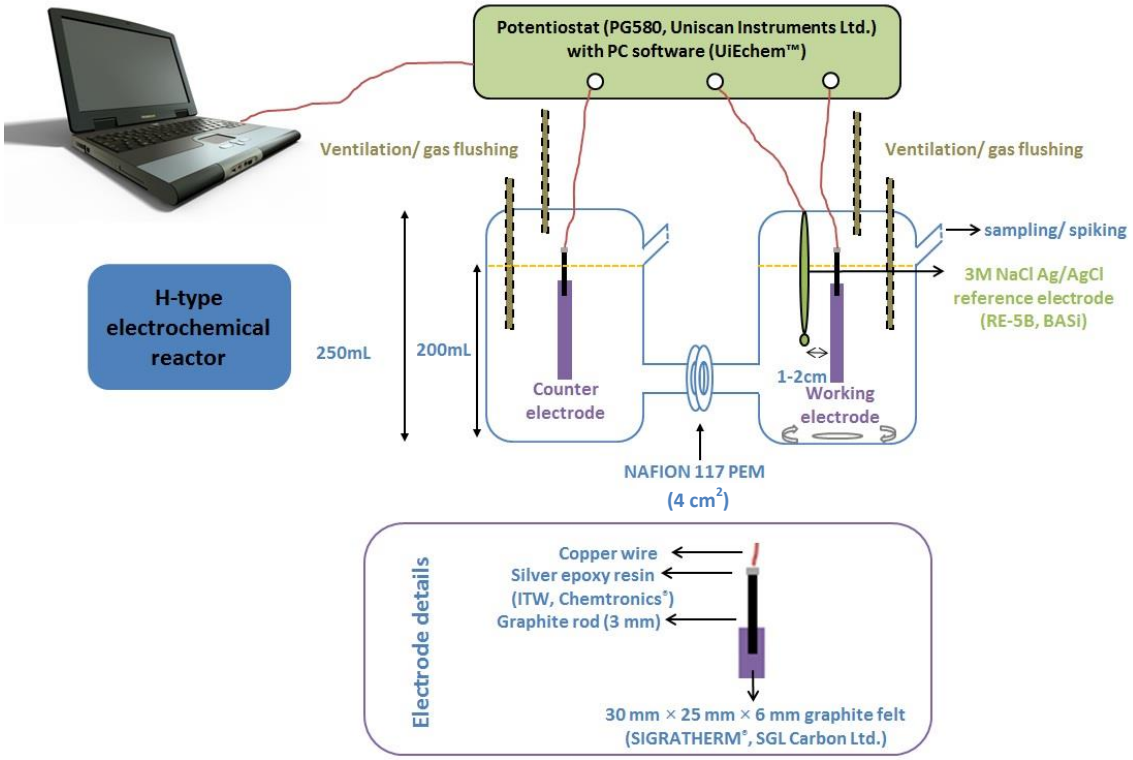

Figure 3-2: H-type reactor and data recording assembly.

Except otherwise stated, working (WE) and counter (CE) electrodes (anodes and cathodes in MFCs) were made of 30 mm × 25 mm × 6 mm (20 cm^2 exposed total apparent surface area; TASA) graphite felt (SIGRATHERM®, SGL Carbon Ltd., 430-580 g m^{-2}). Graphite felt was joined with 3 mm ∅ graphite rods to avoid direct contact of the solution with the copper wire used to externally connect the two chambers. To join the felt with the rod, a hole was drilled in the felt and the two materials were fixed together using underwater epoxy resin (Bondloc Ltd., UK) on the free surface of both materials (Figure 3-3). To connect the felt-rod electrode with the 1 mm copper wire, a hole was drilled in the graphite rod and the two materials were electrically connected using silver conductive epoxy resin (ITW Chemtronics®). An extra underwater epoxy layer was then applied on the rod-wire exposed surface. Prior to fixation, graphite felt was cleaned with 1 M NaOH followed by 1 M HCl and stored in deionised water before use, as described by Chaudhuri and Lovley (2003). During storage in deionised water, the water was frequently replenished until the pH of the graphite felt pores was neutral. Most electrodes had a final resistance of 2-3 Ω and electrodes with a higher resistance were discarded. To avoid interference with contamination caused by deposited Cr(III) oxides and hydroxides formed after Cr(VI) reduction, new electrodes were used for each experiment.

Where reference electrodes (RE) were used for monitoring or controlling an electrode's potential, these were Ag/AgCl electrodes (3 M NaCl, RE-5B, BASi) placed within 1 cm from the WE. When not in use, REs were stored in a 3 M NaCl solution as recommended by the supplier. Before inserting REs into MFC compartments where pure cultures were present, REs were first cleaned with absolute ethanol and air-dried. All potentials reported in this study are vs. Ag/AgCl (+ 210 mV vs. SHE).

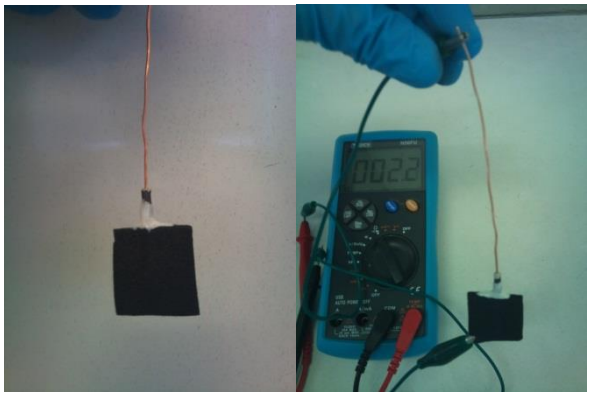

Figure 3-3: Electrodes used for electrochemical experiments; resistance measured was normally 2-3 Ω, as shown on the right.

Proton exchange membrane Nafion 117™ was supplied by Ion Power Inc. and pre-treated by boiling in 30% H_2O_2, subsequently in 0.5 M H_2SO_4 and then in deionised water, each for 1 h as described by Kim et al. (2007) and Li et al. (2009). Cation exchange membrane CMI-7000 and anion exchange membrane AMI-7001 were supplied by Membranes International Inc. and were stored in a 0.5 M NaCl solution before use.

3.3. Chemicals

All chemicals were used as supplied. In particular, Na-DL-Lactate (60% w/w syrup, L1375), Na_2-Fumarate (F1506) and Na-Acetate (S2889) were supplied by Sigma-Aldrich and were used when needed at the concentrations mentioned. Oven-dried potassium chromate ($K_2Cr_2O_7$) was used to make a 4 g-Cr(VI) L^{-1} stock solution. This solution was then spiked to give the initial concentrations indicated. Except where the addition of 1 μM riboflavin (R4500, Sigma Aldrich) is mentioned, all experiments were conducted without the use of artificial redox mediators.

Unless otherwise stated, the nutrient medium used in the WE chambers of half-cell reactors, in MFC bioanodes and biocathodes and in flasks, was a 50 mM phosphate buffer medium prepared in Millipore grade water (18.2 Ω·cm): KH_2PO_4; 21 mM, K_2HPO_4; 29 mM, NaCl; 70 mM, $MgCl_2$; 1 mM, NH_4Cl; 28 mM, $CaCl_2$; 0.7 mM, 0.1% v/v of a trace elements solution as reported by Rabaey et al. (2005b). The use of SO_4^{2-} salts was intentionally avoided, mainly to prevent bio-electrochemical sulphate reduction in the cathode. Heterotrophic consumption of sulphate in the anode might also be possible in this instance, along with the consequent bioelectrochemical oxidation of the produced sulphides. No vitamins or amino acids were added to the medium, which was also amended to contain various concentrations of Cr(VI), lactate, acetate and/or fumarate, as indicated. When pure cultures were used, the medium was autoclaved before use (121 ^{o}C, 15 min).

3.4. Microbiological techniques

Shewanella oneidensis MR-1 (NCIMB 14063) was received as a lyophilised culture and was revived aerobically at 30 ^{o}C in Tryptone Soya Agar (Oxoid, CM0131; pH 7.3). Twenty-four hours after revival, stock cultures were stored at 4 ^{o}C until use. MR-1 was normally grown to the required optical density at 600 nm (OD_{600}), aerobically and under agitation (200 rpm, 30 ^{o}C for 24 h) in Luria-Bertani (LB) medium (15 g L^{-1} tryptone water, 5 g L^{-1} NaCl, 5 g L^{-1} yeast extract-pH adjusted to 7.4 with 5 M NaOH). Following growth, the inoculum was separated by centrifugation (5,000 × g, 4 ^{o}C, 20 min) and washed using an autoclaved 100 mM NaCl-50 mM

phosphate buffer solution (pH 7.0) before inoculating the reactors or the flasks. Unless otherwise indicated, initial OD_{600} for potentiostatically controlled biocathode experiments was 0.2, for MFC biocathodes was 0.3 and for MFC bioanodes was 0.5. Because the biocathodes also involved an intermediate step where the chambers initially operated as anodes to form the biofilm and produce the redox mediators needed, additional growth occurred in these chambers during this intermediate period. Therefore, less biomass was initially added in the MFC biocathodes in order to balance the biomass concentrations present in the two MFC chambers. Work with the pure cultures, including all spiking and sampling, was conducted using sterilisation techniques and all media and glassware were autoclaved before use.

Primary, anaerobic and activated sludge were obtained from the wastewater treatment plant of Millbrook, Southampton, UK. The three different types of sludge were originating from the primary clarifier, the anaerobic digester and the aeration tank of the wastewater treatment plant, respectively. After initial screening for the removal of unwanted solids, the three inocula were used to inoculate the flasks and/or the bioelectrochemical reactors, as described in Chapter 4.4.

3.5. Electrical monitoring and control

3.5.1. MFC operation

During MFC operation, the cell potential E_{cell} and current I flowing through known external resistances R_{ext} were measured using a datalogger with the ability to collect and store data from multiple channels. A DT505 model with 15 bits effective resolution, 0.15% DC voltage accuracy and 0.25% DC current accuracy (at 25 ^{o}C) was used for voltage and current measurements. A similar model (DT50) has already been used in a similar application (Luo et al., 2010).

Polarisation curves (the plots of the cell voltage as a function of the current or the current density) were obtained after a stable open circuit voltage was recorded, under the gas conditions described in each experiment. A series of external resistances $R_{ext.}$ was then applied, from high to low resistance values, and values were taken after the potential was not substantially altering for each of the $R_{ext.}$ applied (normally after 4-5min). Current was then calculated using Ohm's law, normalised by the total apparent surface area and plotted versus voltage. From the polarisation graphs, the internal resistance can also be practically identified

as this will be equal to the external resistance that produced the peak power output (Logan, 2008).

3.5.2. Chronoamperometry (CA)

During half-cells operation, a portable potentiostat (PG580, Uniscan Instruments Ltd.) and its PC software UiEchem™ were used in a three-electrode configuration in order to keep the WE potential constant vs. the Ag/AgCl RE and to record the current flowing between the WE and the counter electrode (CE) (normally every 4 sec in CA experiments). For most of the cases where the CE chamber was separated from the WE chamber, the solution in the CE chamber was a 100 mM NaCl-50 mM phosphate buffer solution (pH 7.0).

In general, when an oxidation reaction is taking place at the WE, the potentiostat will drop the potential of the CE in order to drive a reaction able to accept the electrons and protons produced at the WE chamber. When no other electron acceptors are present in the CE chamber, protons will be utilised for hydrogen formation, according to the following reduction reaction:

$$2H^+ + 2e^- \rightarrow H_2 \text{ (R. 3.1)}$$

On the other hand, when a reduction reaction is taking place at the WE, the potentiostat will increase the potential of the CE in order to drive a reaction able to produce the electrons and protons to be consumed at the WE chamber. When no other electron donors are present in the CE chamber, water is oxidised according to the following oxidation reaction:

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2 \text{ (R. 3.2)}$$

In the case where the current is high and/or the pH does not allow the above reactions to take place and the potentiostat cannot provide sufficient electrons to keep the WE potential constant (WE-CE potential difference of over 4.0 V, current over 20 mA), a warning light is flashing on the instrument. Also, a warning light is flashing when the current is low and the accuracy in measurement is not high.

Following the conclusions from preliminary experiments, when an oxidation reaction was intended to be driven by the WE (e.g. lactate oxidation via bacterial metabolism), the WE potential was normally set at a potential as high as + 300 mV. On the contrary, when a reduction reaction was intended to be driven by the WE (e.g. Cr(VI) reduction), the WE potential was normally set at a potential as low as -500 mV.

3.5.3. Cyclic Voltammetry (CV)

The most common and straightforward technique to determine the mechanisms of the electrode reactions is CV (Zhao et al., 2009). With CV measurements the electrochemical activity of microbial strains or consortia can be assessed, the standard redox potentials of redox active components can be determined, and also the materials' performance can be tested (Logan et al., 2006). In general, CV requires a three-electrode configuration of the potentiostat and current is measured with voltage sweeps generally within the scan rate ranging from 1-100 mV sec^{-1} for MFC studies (Zhao et al., 2009).

In this study, CV was performed with a three-electrode configuration, normally from -600 mV to +600 mV and at room temperature. This potential range was sufficiently broad to allow observation of the cell's behaviour but also narrow enough to prevent the formation of H_2 and O_2 (at <-600 mV and >+600 mV respectively at neutral pH), which might have disturbed the microbial metabolism and hidden the effect of the reactions under investigation. Also within this potential range the participation of the Cr(VI)/Cr(III) couple in redox reactions was thermodynamically favoured (E_0' for the Cr(VI)/Cr(III) couple at 20^{o}C is approximately 220 mV vs. Ag/AgCl, calculated from the Nernst equation). The scan rate was selected to be 5 mV sec^{-1} and the potentiostat was recording current every 1 sec. This scan rate was selected to be low enough to allow the study of the redox mediators and the biofilm, but also high enough to avoid prolonged scanning times, which would give CV graphs that do not represent the conditions employed at the beginning of the experiment. Cyclic voltammetry experiments were repeated until consistent results were obtained (normally after 2 to 5 times) unless otherwise indicated (e.g. during non-stop consecutive CV scans). During the CV experiments, quiescent, mass transfer limited conditions were created by pausing any stirring of the solution and introducing the appropriate gas only on the surface of the solution. Stirring (250 rpm) and sparging of the solution with an appropriate gas were again applied in the WE chamber, only between the CV experiments.

3.6. Analytical techniques

3.6.1. pH

The pH of the media and samples was measured by using a calibrated Mettler-Toledo pH meter with a resolution of 0.01 pH units and an accuracy of ± 0.01 pH units. pH of the samples

was measured immediately after collection, after thoroughly rinsing the pH probe with deionised water.

3.6.2. Chromium

Hexavalent chromium was measured colorimetrically at 540 nm after reaction with 1,5-diphenylcarbazide in an acidic solution as described by the Standard Method 3500-Cr B (APHA, 2005). Proportional volumetric flask sizes (5-100 mL) and sample volumes were used, depending on the estimated mass of Cr(VI) to be measured. Samples were centrifuged at 14,000 rpm and Millipore grade water (18.2 Ω·cm) was used for the preparation of all standards and solutions. All glassware used were kept overnight in a solution containing HNO_3 (1 part), HCl (2 parts) and deionised water (9 parts), as indicated by APHA (2005), to avoid sorption of metals to the glass.

Total chromium (Cr_{tot}) was measured using atomic absorption spectrometry (SpectrAA-200) at the wavelength of 357.9 nm after filtration through a 0.45 μm filter and acidification with HNO_3 at a final concentration of 1 M. The concentration of trivalent chromium (Cr(III)) was considered as the difference between [Cr_{tot}] and [Cr(VI)].

3.6.3. Total Suspended Solids (TSS) and Total Volatile Suspended Solids (TVSS)

TSS and TVSS were measured according to the Standard Methods 2540-D and 2540-E respectively (APHA, 2005), using 0.45 μm pore size filters. TSS are determined by the weight difference between the filter and the dried residual after drying in an oven at 103-105 ^{o}C for 1 h. TVSS are determined as the difference between TSS and the residual ash after ignition in a furnace at 550 ^{o}C for 15 min (fixed suspended solids).

3.6.4. Volatile Fatty Acids (VFAs)

VFAs were determined using a gas chromatographer (Shimadzu, GC-2010) where helium was the carrier gas and a mixture of air and hydrogen were used to form the flame. Prior to analysis, samples were centrifuged (14,000 rpm) and formic acid (10% v/v) was added for acidification of the sample and of the standards used for calibration. Peak areas were then analysed using the instrument's software.

3.6.5. Lactic and acetic acid

Lactic and acetic acid were measured using Ion Chromatography (882 Compact IC Plus, Metrohm) after centrifugation (14,000 rpm). An ion-exclusion column (Metrosep Organic Acids, 250/7.8 mm, Metrohm) was used for separation and a 0.5 mM H_2SO_4 solution was used as an eluent. Flow was set at 0.6 mL min^{-1} and the peak areas of the solutions and the standards were further analysed using the instrument's software MagICNet™.

3.7. Calculations

3.7.1. Voltage, current and power

A complete guide on the formulas used in electrochemistry can be found in Bard and Faulkner (2001). Herein are presented the fundamental equations used for the purposes of this thesis, which can also be found in (Logan et al., 2006).

Unless directly measured, current *I* (A) was calculated using Ohm's Law:

$$I = \frac{E_{cell}}{R_{ext}} \quad \text{(Eq. 3.1)}$$

E_{cell}: voltage drop across R_{ext}, recorded at a fixed time interval (V)

R_{ext}: external resistor (Ω)

Produced power *P* (W) is given as:

$$P = \frac{{E_{cell}}^2}{R_{ext}} \quad \text{(Eq. 3.2)}$$

In order to make comparison between the different systems possible, current and power were normalised to the total apparent surface area *A* of the electrodes of interest (20 cm^2):

$$I = \frac{E_{cell}}{A \cdot R_{ext}} \quad \text{(Eq. 3.3)}$$

$$P = \frac{{E_{cell}}^2}{A \cdot R_{ext}} \quad \text{(Eq. 3.4)}$$

3.7.2. Coulombic efficiency

The total amount of Coulombs electrochemically produced were calculated based on current measurements at fixed time intervals (usually 4-20 sec for potentiostatically controlled experiments and 1-20 min for MFCs):

$$C_1 = \sum I\Delta t \quad \text{(Eq. 3.5)}$$

I: current (A) measured at fixed time intervals

t: time (s)

The total amount of Coulombs transferred to Cr(VI) for a complete Cr(VI) to Cr(III) reduction was calculated as follows:

$$C_2 = \frac{nFv\Delta[Cr(VI)]}{M} \quad \text{(Eq. 3.6)}$$

n: 3 moles of electrons exchanged mol^{-1} of Cr(VI) reduced assuming a complete Cr(VI) to Cr(III) reduction

F: 96,485.3 Coulombs mol^{-1} of electrons

v: catholyte volume (L)

Δ[Cr(VI)]: Cr(VI) concentration reduction (g L^{-1})

M: 52 g-Cr mol^{-1}-Cr

Cathodic efficiency (%) was calculated in this study based on the reduction equivalent, as the ratio of the amount given by Eq. 3.5 (charge delivered to the cathode) over the amount given by Eq. 3.6 (charge received by Cr(VI)) (Huang et al., 2010):

$$CE\ (\%) = \frac{C_1}{C_2} \times 100\% \quad \text{(Eq. 3.7)}$$

Based on the above, the cumulative mass of Cr(VI) (g) reduced due to current is calculated according to the cumulative electrical charge produced, as below:

$$\sum Cr(VI) = \frac{M \sum I\Delta t}{nF} \text{(Eq. 3.8)}$$

Chapter 4 Experimental set-up, results and discussion

4.1. Electrode fouling

4.1.1. Experimental set-up

In order to study the effect of the Cr(VI) reduction products on the electrochemical remediation process, potentiostatically controlled half-cells were assembled and tested. The general reactor set-up described in Section 3.2, with a Nafion 117™ proton exchange membrane, was used in these potentiostatically controlled experiments. The nutrient medium described in Section 3.3 (pH 7.0) was used in both the working and the counter electrode chambers and N_2 was continuously sparged to maintain anaerobic conditions in the working electrode chamber. The working electrode potential was set at -500 mV vs. Ag/AgCl during the chronoamperometry tests. During these tests, current that was initially produced and consisted of both Faradaic and capacitance current, was let to drop to the low background levels that were observed (approximately 5-7 μA in all cases), before Cr(VI) was added. Results presented are from the first Cr(VI) spike time-point onwards, and after background current was subtracted.

4.1.2. Results and discussion

When 20 mg-Cr(VI) L^{-1} were spiked in the abiotic cathode, current showed a high initial response, which however dropped rapidly (Figure 4-1), leaving 19 mg-Cr(VI) L^{-1} still in solution at the end of the 4 h operation period. Two consecutive runs were then performed, using the same electrode, which was cleaned with ultra-pure water between successive Cr(VI) additions, but replacing the catholyte each time. It can be seen in Figure 4-1 that the initial current response dropped sharply from 820 mA m^{-2} in the 1st run to less than 100 mA m^{-2} in the 2nd and 3rd runs. A recovery of the initial current response was observed in the 4th run, however, this was after the electrode was cleaned using sequential alkaline, acid and ultra-pure water washing, as described in Chaudhuri and Lovley (2003). The decrease in the current response observed in this set of experiments and the recovery after electrode cleaning indicated the formation and accumulation of inhibitory substances on the electrode surface. This inhibitory effect has also been observed when Cr(VI) was tested as a corrosion inhibitor on carbon, copper and platinum electrodes (Clark and McCreery, 2002, Hurley and McCreery, 2003).

According to these previous studies, the reason for this inhibitory effect was the formation of non-conductive Cr(III) oxyhydroxide monolayers on the electrode surface at neutral and near neutral pH, which prevent further reduction of Cr(VI).

Consecutive cyclic voltammogram experiments in abiotic cathodes also showed a decreasing Cr(VI)-reducing ability of the cathodes with time. As can be seen in Figure 4-2, the initial potential-depended Cr(VI)-reducing current produced at the low range of the CV scan is progressively inhibited, indicating an increase in the cathode overpotentials. In practice, this means that more negative applied potentials are required to maintain similar Cr(VI)-reduction rates, which is translated into more energy spent during the treatment. In other words, depending on the amount of Cr(VI) to be reduced, either more energy will have to be spent in the abiotic cathode, or a higher electrode surface area will have to be utilised. Both these solutions cannot be considered sustainable and other options need to be available.

Another fact that can be observed from the cyclic voltammograms presented in Figure 4-2 is the evolution of a reduction peak at approximately +10 mV vs. Ag/AgCl, that has an onset at approximately +150 to +200 mV vs. Ag/AgCl. This peak was present both during consecutive CV scans and after a chronoamperometry experiment at -500 mV vs. Ag/AgCl for 4 h. By using UiEchem software, the peak's height from the baseline was measured. As can be seen in Figure 4-3, the height of this peak was increasing after every cyclic voltammogram experiment and was highest after the chronoamperometry experiment during which approximately 1 mg L^{-1} Cr(VI) (4 μmoles) was reduced. This observation could indicate the presence of an adsorbed species that is progressively formed on the electrode surface. Because this peak is a reduction peak with increasing surface area, one can speculate that Cr species other than Cr(VI) might be reduced in this instance. Cr(III) has been reported in the literature as the only stable Cr(VI) reduction product. However, the considerably higher peak produced after a chronoamperometry experiment, could indicate the production of other Cr products (Cr(V) and Cr(IV)) formed during Cr(III) and/or Cr(IV) oxidation at high potentials (over +400 mV vs. Ag/AgCl). Therefore, the reduction peak observed could be due to the reduction of only partially reduced Cr species. Depending on the electrostatic charge of their final complex, reduction of Cr species other than Cr(VI) ones might be taking place prior to the reduction of the negatively charged Cr(VI).

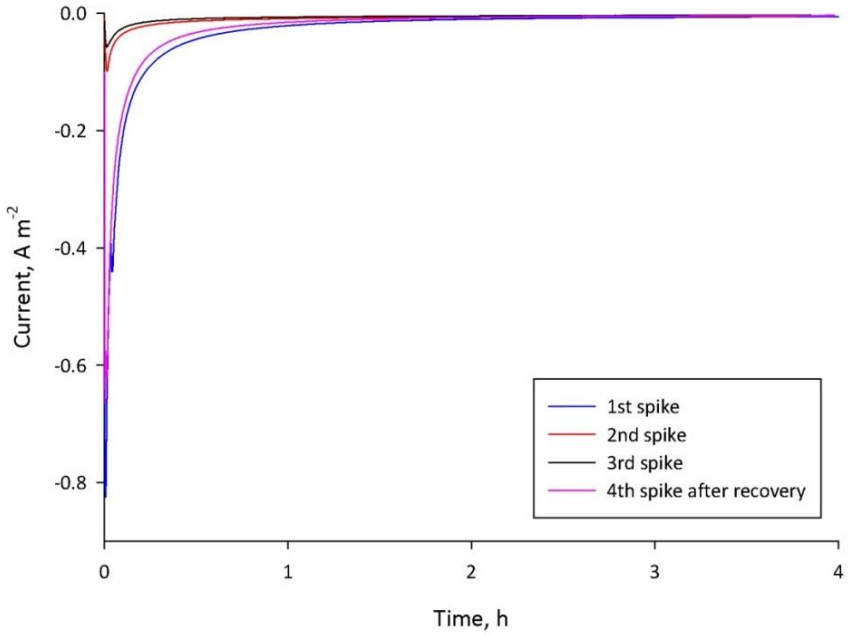

Figure 4-1: Current evolution after consecutive spikes, with deionised water cleaning in between spikes 1-3 and alkali-acid-deionised water cleaning before the 4th spike.

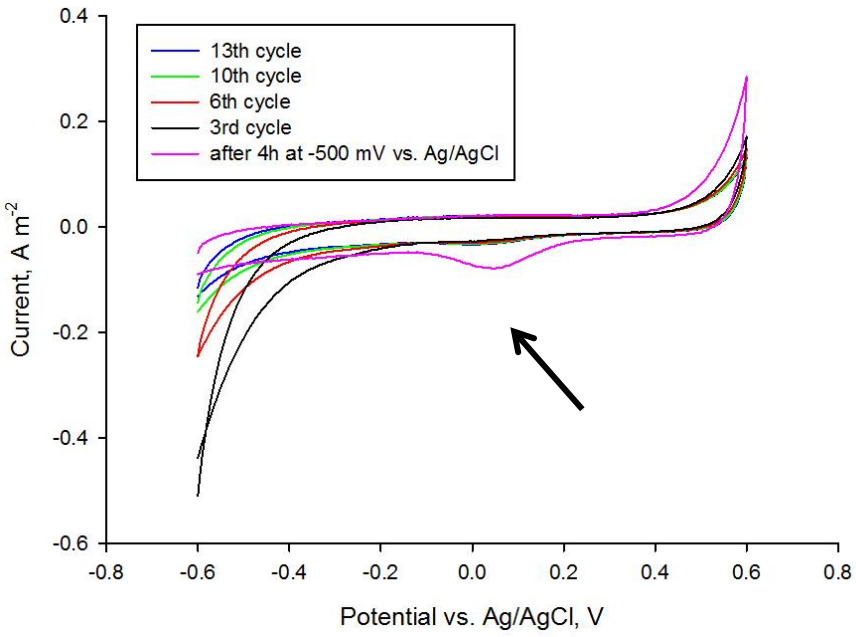

Figure 4-2: Evolution of the cyclic voltammograms with time, showing a decreasing cathodic current and a peak production (indicated by the arrow) at approximately +10 mV vs. Ag/AgCl. Scan rate is 5 mV sec^{-1}.

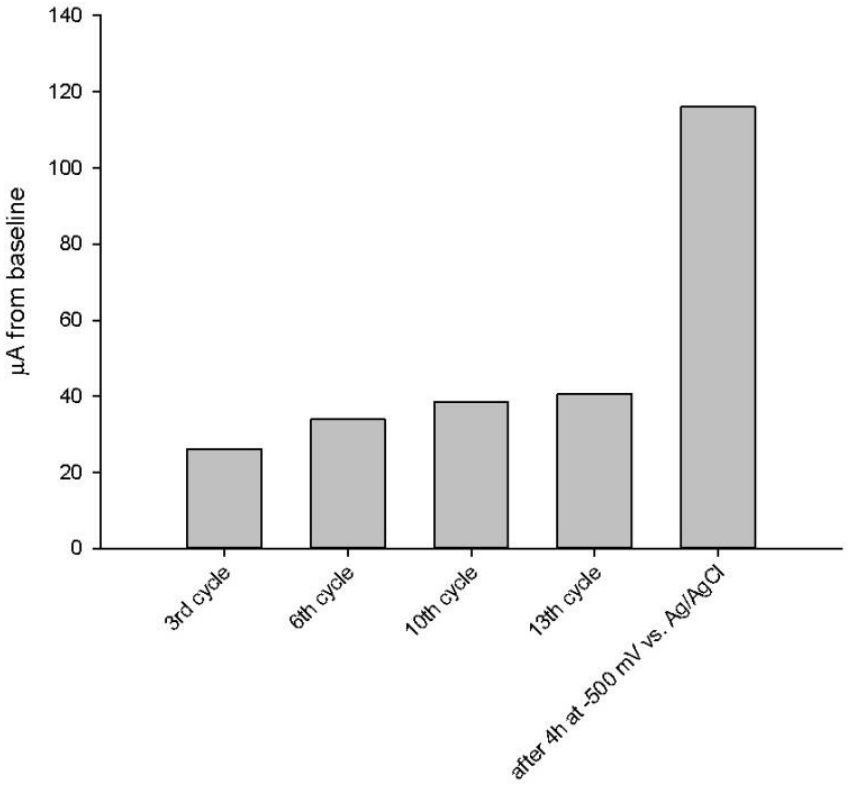

Figure 4-3: Increase of the peak produced at + 10 mV vs. Ag/AgCl with time.

4.2. Effect of metal chelators and riboflavin

4.2.1. Potentiostatically controlled cells

4.2.1.1. The effect of different metal chelators

Whilst at acidic pH Cr(VI) reduction mainly results in Cr(III) remaining in solution, at neutral and alkaline pH the process is severely inhibited by the formation of Cr(III) oxyhydroxide layers which rapidly form on the cathode's surface and deactivate it (Clark and McCreery, 2002, Hurley and McCreery, 2003). This self-inhibiting behaviour results in a rapid slow down of the process, as already shown in the previous section, and eventually in a poor ratio of Cr(VI) reduced per electrode surface area.

A solution to this problem would be to maximise the ratio of electrode surface area used per mass of Cr(VI) available for reduction, however this option would be impractical for real scale applications where the mass of Cr(VI) that needs to be reduced is high. A second option would be to screen the bacterial catalysts in the MFC cathodes, which have the ability to transform Cr(VI) to soluble Cr(III) (Huang et al., 2010), but this option would still be impractical in open

field remediation applications where bacterial selection is not easily controlled. To overcome the problem of Cr(III) oxyhydroxide layers deactivating the electrode, it was tested whether lactate, a metal chelator, which is known to form chelates with Cr(III) (Hamada et al., 2005, Puzon et al., 2005), can have a positive effect in the electroreduction process. Lactate was particularly chosen in this study because it occurs naturally in the environment, it exhibits low environmental toxicity (Bowmer et al., 1998), it is easily biodegradable by bacteria of environmental interest like *Shewanella* (Pinchuk et al., 2011) and can be used as an electron donor for Cr(VI) bioremediation (Brodie et al., 2011, Alam et al., 2006).

As described in Section 4.1, a rapid current drop was observed in all cases of abiotic cathodes without lactate addition, and this was independent of the fact that Cr(VI) was still present in the solution. By supplying 30 mM of lactate in an abiotic cathode (same reactor set-up as the one described in the previous paragraph), the initial current peak increased when compared to that of the cathode without lactate (Figure 4-4). However, the fall in current to background levels in this instance was also quick, and 17 mg L^{-1} of Cr(VI) was still left in solution at the end of the 4 h operation period (Figure 4-5). The improved initial current response caused by lactate addition can be partially explained by the ability of Cr(III) to form insoluble chelates with organic ligands (Hamada et al., 2005, Puzon et al., 2005), which may delay or even prevent the deactivation of the electrode's active sites, depending on the initial Cr(VI) and ligand concentration. If this is the case, then a possible mechanism could involve neutralisation of the metal's positive electrical charge by lactate. This would eventually reduce the metal's affinity to the negatively charged cathode. Lactate-Cr(III) chelates have been reported in the literature to be able to precipitate at neutral pH, but this is not the case for all Cr(III) chelated organic ligands (e.g. malate) (Hamada et al., 2005, Puzon et al., 2005). Acetate has also been reported capable of forming insoluble Cr(III) complexes (Puzon et al., 2005), but when acetate was supplied instead of lactate, cathodic performance showed no difference from that of the abiotic cathode without lactate; that was both in terms of current production (Figure 4-4), Cr(VI) reduction (Figure 4-5) and total charge production (Figure 4-6) and could be probably attributed to a weaker ligand formation.

Regarding Cr(VI) reduction, it generally had a positive correlation with current production, with approximately 5 mg-Cr(VI) L^{-1} reduced by the citrate cathode, 3 mg-Cr(VI) L^{-1} by the lactate cathode and only approximately 1 mg-Cr(VI) L^{-1} reduced by the acetate cathode and the cathode without any organic addition. As can be seen, citrate seemed to be performing better in the cathode; however, lactate, which also showed an enhanced performance, was selected

for the experiments that are described later in the text. That was because of the reasons mentioned earlier in this section, and mainly because lactate is a very important carbon source for *Shewanella* (as already discussed in Section 2.3), which is studied in many of the experiments described later on.

Despite the fact that the experiments presented herein lasted for only 4 h, the current curves obtained were sufficient to provide a good estimation of the total charge that could be produced during the process (Figure 4-6, Table 4-1). As can be seen in Table 4-1, exponential equations can describe the phenomena in all cases and the maximum charge that can be produced will be given for the exponential parts of the equation approaching zero. This exponential rise to a maximum charge that can be produced indicates that even in the presence of metal chelators, the process presents limitations that need to be overcome.

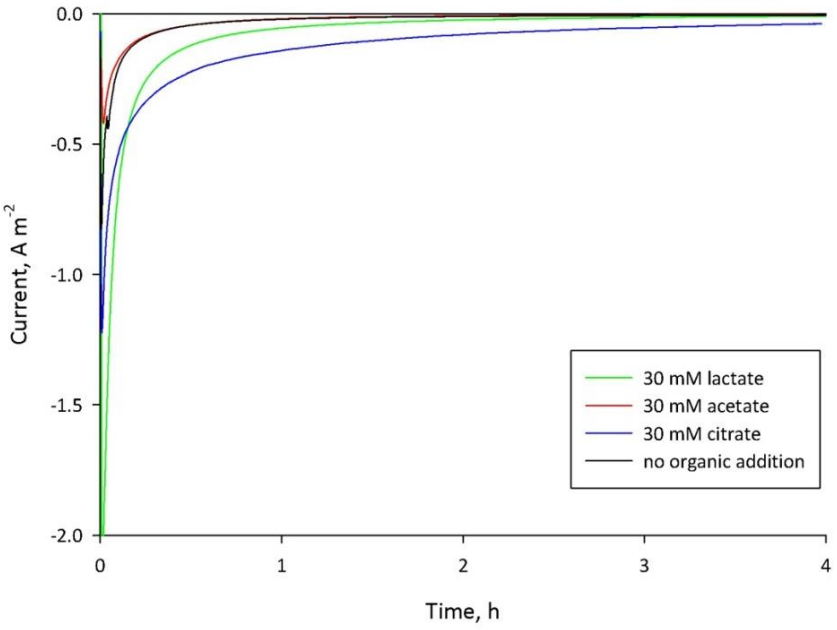

Figure 4-4: The effect of different metal chelators on current production.

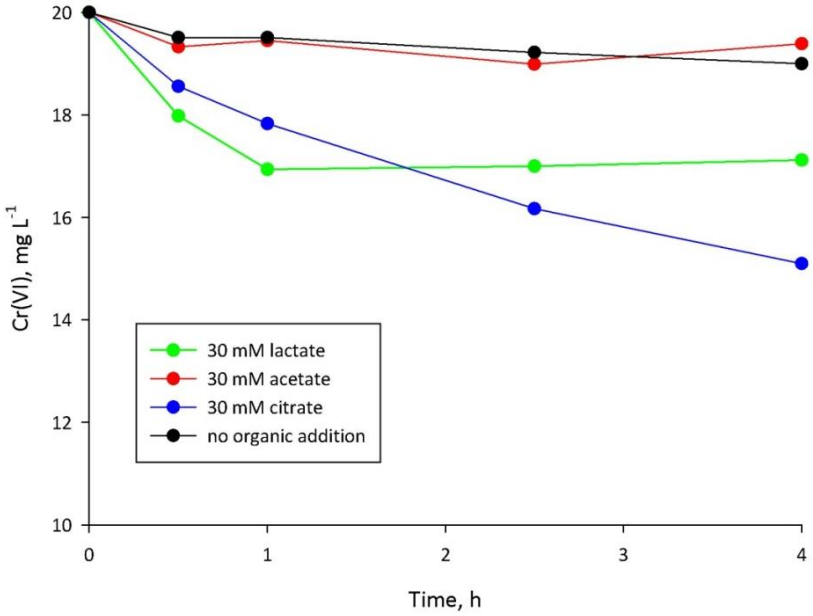

Figure 4-5: The effect of different metal chelators on Cr(VI) reduction.

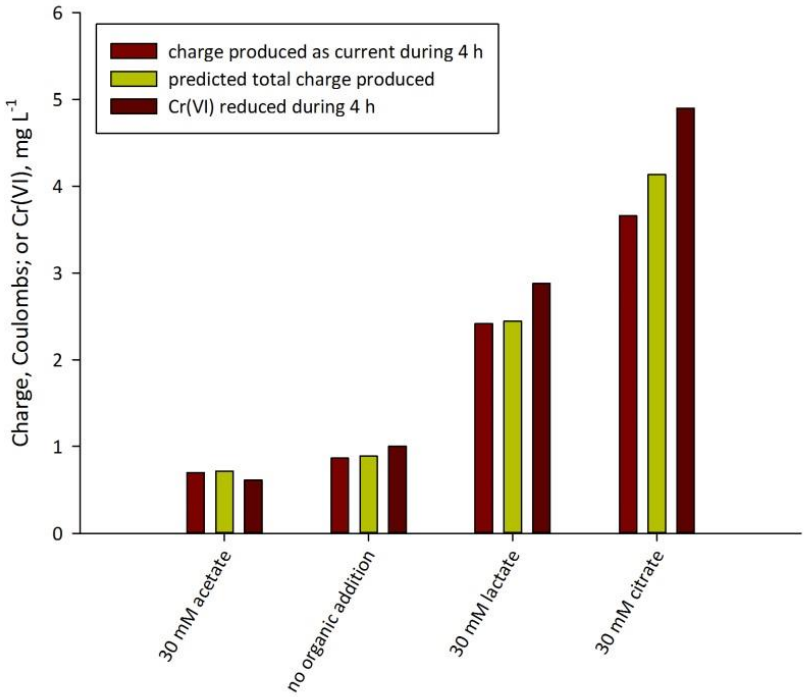

Figure 4-6: The effect of different metal chelators: charge produced and Cr(VI) reduced after 4 h, and maximum predicted charge that can be produced. Maximum charge was calculated by best fitting exponential charge rise to maximum equations of the form y=a*(1-exp(-b*x)+c*(1-exp(-d*x) to the charge actually produced, where y is the cumulative charge produced (C) and x is time (h).

Table 4-1: Regression parameters for the charge produced (based on current production), in the presence of different metal chelators. Equations are in the form y=a*(1-exp(-b*x)+c*(1-exp(-d*x), where y is cumulative charge produced (C), x is time (h), "a" and "c" are parameters representing a maximum possible charge produced for x→∞ (C), and "b" and "d" are parameters representing how fast the maximum possible charge produced can be approached (h^{-1}).

Metal chelator	Equation (y=)	R^2
None	0.43*(1-exp(-8.95*x))+0.46*(1-exp(-0.65*x))	1.00
Acetate (30 mM)	0.34*(1-exp(-6.20*x))+0.38*(1-exp(-0.71*x))	1.00
Lactate (30 mM)	1.27*(1-exp(-9.69*x))+1.17*(1-exp(-0.82*x))	1.00
Citrate (30 mM)	0.87*(1-exp(-5.45*x))+3.27*(1-exp(-0.48*x))	1.00

4.2.1.2. The effect of riboflavin

The performance of the lactate containing cathode has been shown in the previous paragraph to be enhanced, but still limited. The scope of the following experiments was to evaluate whether the presence of the redox mediator riboflavin (RF) could have a further positive impact on the cathode's performance alone, or in the presence of lactate. Riboflavin is a redox mediator produced by bacteria with environmental importance like *Shewanella*, and its effect on Cr(VI)-reducing cathodes could help us understand the electron transfer routes utilised by the bacteria for Cr(VI) reduction when solid surface electron donors (electrodes) are being utilised.

As shown in Figure 4-7, the presence of 1 μM RF alone did not have any positive effect on the cathode's performance and more than 19 mg-Cr(VI) L^{-1} was still left in solution by the end of the 4 h operation period. Although riboflavin has also been demonstrated to be capable of forming chelates with metal cations (Marsili et al., 2008), the concentrations of riboflavin used herein would be sufficient for the chelation (or even the reduction) of less than 0.05 mg L^{-1} (1 μM) of chromium ion assuming a 1:1 molar chelation ratio. However, in the presence of 30 mM lactate, increasing RF concentration in the catholyte presented an increasing current production and Cr(VI) reduction. As a result, when a concentration of 5 μM RF was used, more than 10 mg-Cr(VI) L^{-1} was reduced (Figure 4-8), with approximately 80% of the maximum possible charge produced within the 4 h operation period (Figure 4-9). Again, exponential-rise-to-maximum equations can be used to describe total charge produced, however this maximum is now higher with increasing RF concentrations.

This enhanced current production and Cr(VI) reduction with increasing RF concentrations indicates that RF can act as a redox mediator for Cr(VI) reduction, when an appropriate electrode potential is available. As a result of this electron mediation, from the electrode to Cr(VI) via riboflavin, an important fraction of Cr(VI) reduction takes place in the bulk solution by RF that is reduced on the electrode surface. Consequently, the electrode is active and can reduce Cr(VI) and RF for a longer time period (Figure 4-7 and Figure 4-8). Therefore, by exploiting the electrochemical properties of a redox mediator that is naturally produced by the bacteria, the remediation process could be more cost effective than the approach of employing high electrode surface areas.

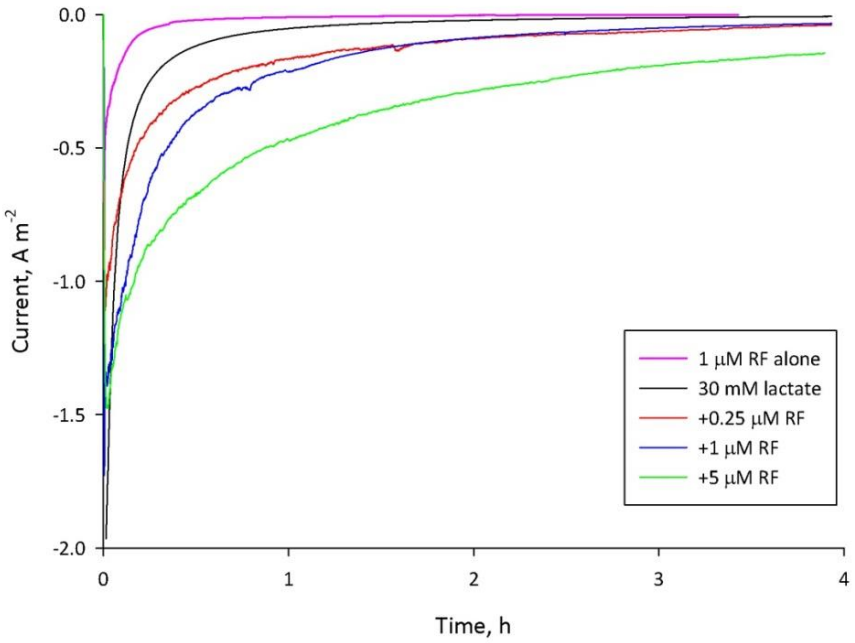

Figure 4-7: The effect of riboflavin concentration on current production.

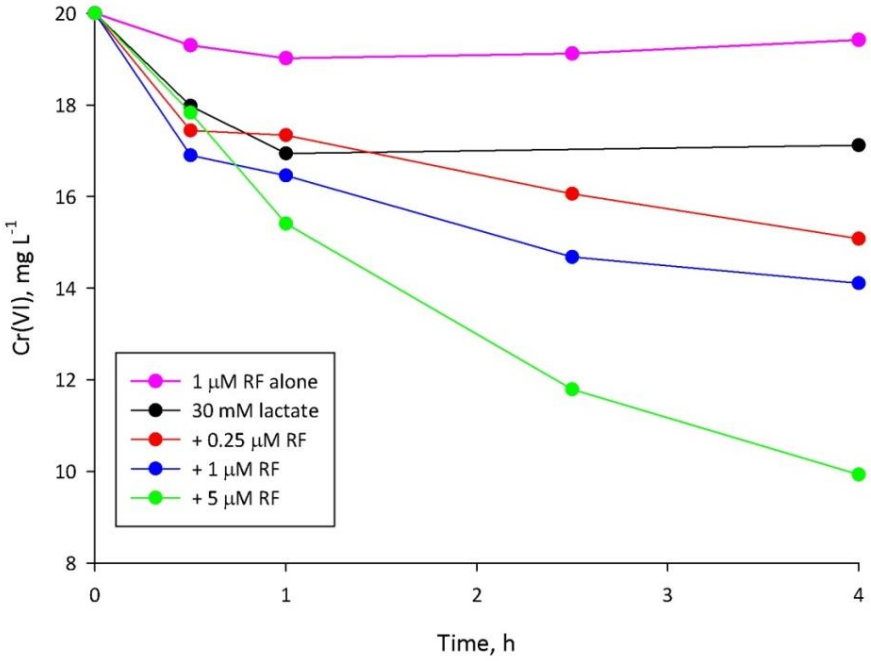

Figure 4-8: The effect of riboflavin concentration on Cr(VI) reduction.

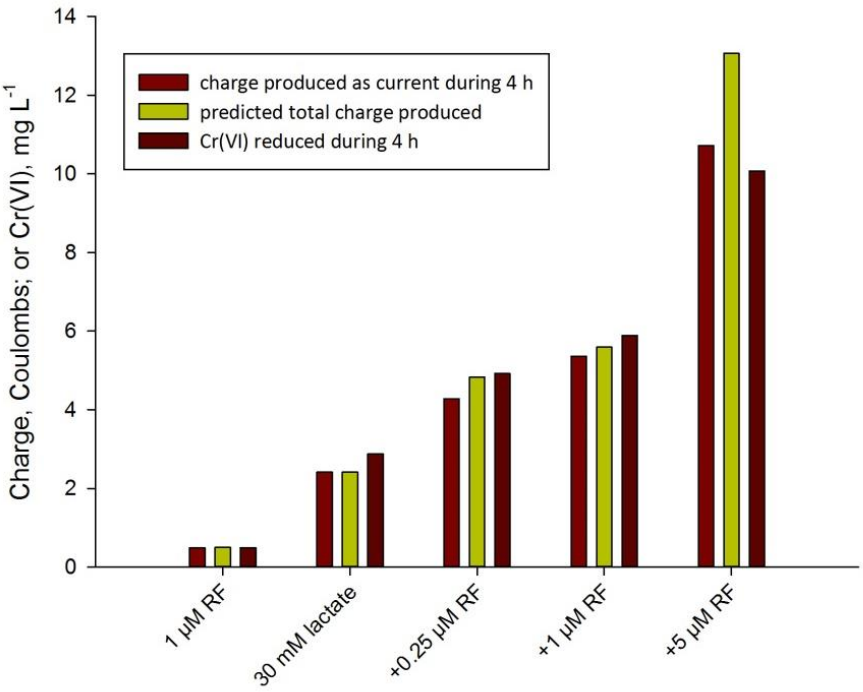

Figure 4-9: The effect of different riboflavin concentrations: charge produced and Cr(VI) reduced after 4 h, and maximum predicted charge that can be produced. Maximum charge was calculated by best fitting exponential charge rise to maximum equations of the form y=a*(1-exp(-b*x)+c*(1-exp(-d*x) to the charge actually produced, where y is the cumulative charge produced (C) and x is time (h).

Table 4-2: Regression parameters for the charge produced (based on current production), in the presence of different riboflavin concentrations. Equations are in the form y=a*(1-exp(-b*x)+c*(1-exp(-d*x), where y is cumulative charge produced (C), x is time (h), "a" and "c" are parameters representing a maximum possible charge produced for x→∞ (C), and "b" and "d" are parameters representing how fast the maximum possible charge produced can be approached (h^{-1}).

Metal chelator	Equation	R^2
1μM RF (no lactate)	0.29*(1-exp(-11.71*x))+0.21*(1-exp(-1.19*x))	1.00
0 μM RF+30 mM lactate	1.24*(1-exp(-10.61*x))+1.12*(1-exp(-0.92*x))	1.00
0.25 μM RF+30 mM lactate	1.08*(1-exp(-4.52*x))+3.75*(1-exp(-0.48*x))	1.00
1 μM RF+30 mM lactate	1.75*(1-exp(-4.24*x))+3.84*(1-exp(-0.67*x))	1.00
5 μM RF+30 mM lactate	1.55*(1-exp(-3.12*x))+11.51*(1-exp(-0.41*x))	1.00

Cyclic voltammograms produced at the end of the 4 h operation period (Figure 4-10, Figure 4-11), revealed that in the presence of RF, current is mainly produced at low potentials (<-400 mV vs. Ag/AgCl). As can be seen in Figure 4-11, two current acceleration points were observed in the presence of riboflavin, one at approximately -330 mV and one at approximately -470 mV vs. Ag/AgCl. Riboflavin is a molecule that accepts one or two electrons at similar potentials during reduction, and this observation is a proof of the electrochemical redox cycles that involve riboflavin reduction at the electrode and oxidation by Cr(VI).

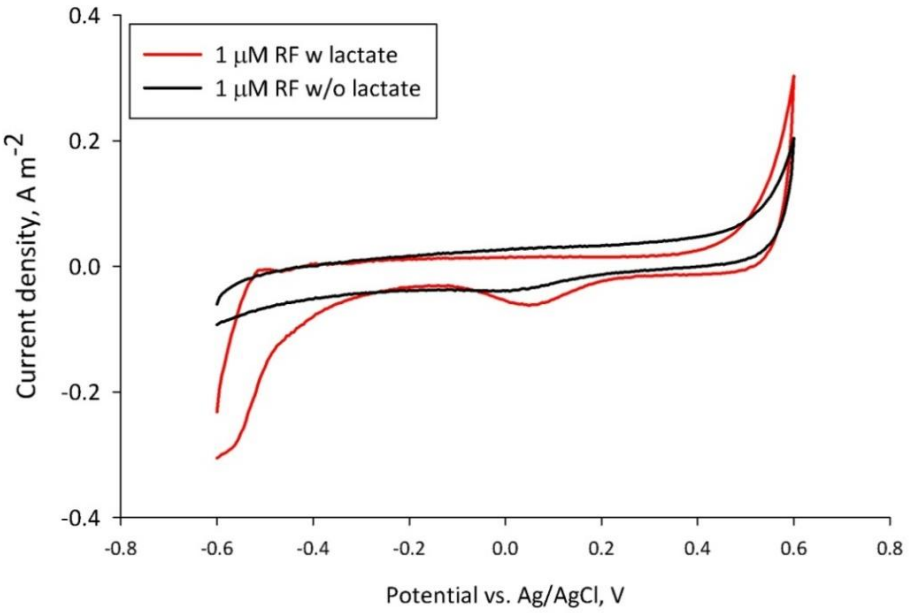

Figure 4-10: Cyclic voltammograms of riboflavin in the presence of Cr(VI), with and without lactate. Scan rate is 5 mV sec^{-1}.

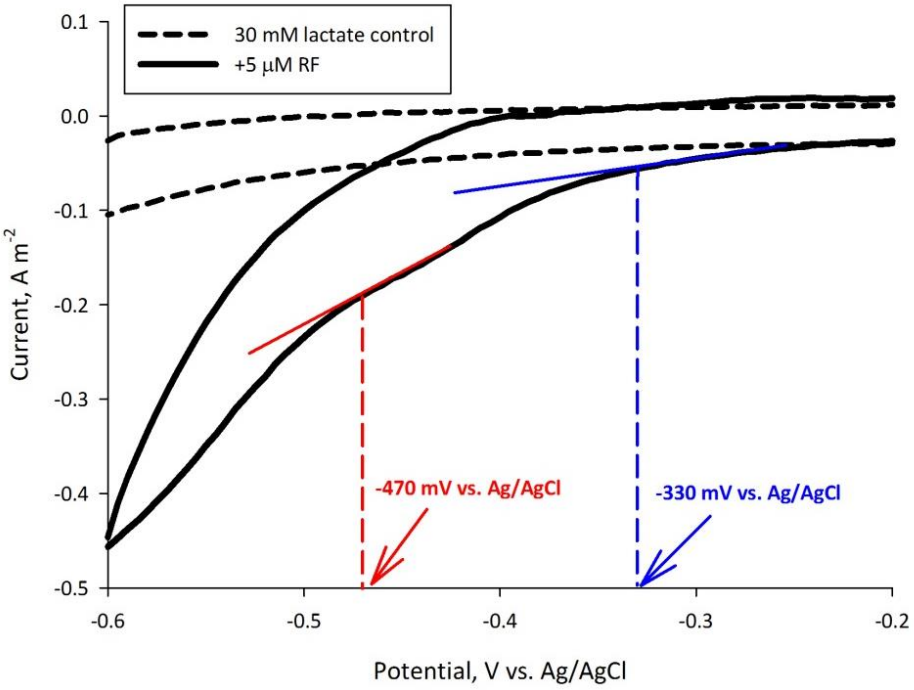

Figure 4-11: Magnified region of the cyclic voltammogram with 30 mM lactate and 5 µM riboflavin, showing current increase acceleration due to the presence of riboflavin. Scan rate is 5 mV sec^{-1}.

4.2.1.3. Conclusions

Minimal medium containing lactate and riboflavin showed an enhanced performance as a result of the presence of the redox mediator, the concentration of which was also important. In the absence of lactate, however, the catalytic activity of riboflavin could not be expressed due to the rapid fouling of the electrode. Cathodic current was minimal in all potentiostatically controlled cells, which did not contain an efficient metal chelator, despite the presence of the redox mediator riboflavin. By comparing the CV graphs of these abiotic experiments, it is clear that the cathodic catalytic current produced in the presence of riboflavin was only effective at low potentials after 4 h of potentiostatic control. Also, in the absence of an effective metal chelator like lactate, the performances of the reactors with and without riboflavin become comparable. The above results indicate that the effective expression of cathodic current, under the conditions described, required the presence of both lactate and redox mediators.

4.2.2. Cr(VI) reduction in abiotic cathode MFCs with lactate as a metal chelator

4.2.2.1. Experimental plan

4.2.2.1.1. Chemicals

Anodes were filled up with 200 mL of pH 7 phosphate buffer minimal medium described in Section 3.3. A specific volume of a 1.2 M Na-Acetate stock solution was added to the mediatorless MFC anodes to increase acetate concentration to approximately 40 mM before each experiment. The catholyte was a 50 mM phosphate buffer saline (PBS) containing (mM): KH_2PO_4 (44), K_2HPO_4 (6) at pH 6; KH_2PO_4 (21), K_2HPO_4 (29) at pH 7; KH_2PO_4 (3), K_2HPO_4 (47) at pH 8 and KH_2PO_4 (0), K_2HPO_4 (50) at pH 9. The pH in the reactors was corrected by using 5 M HCl or NaOH solutions. The $K_2Cr_2O_7$ stock solution described in Section 3.3 was then spiked in the cathodes to give an initial concentration of 10 mg L^{-1} unless otherwise indicated. Catholyte also contained Na-DL-Lactate at the following concentrations: 0, 2, 20, 200 mM. To equalise Na^+ concentration in the catholyte and to keep the solution's ionic strength at similar levels, NaCl was added to give a final Na^+ concentration of 200 mM in all cathodes.

4.2.2.1.2. Inoculation and start-up

Anaerobic sludge was obtained from the wastewater treatment plant of Millbrook, Southampton, UK and was used to inoculate (5 mL) a MFC fed with acetate in a fed-batch mode for more than 3 months (Figure 4-12). Fifteen mL of this MFC effluent was then used to inoculate the anodes in each of the 3 MFCs used which were initially running under continuous air supply in the cathode and were connected with an external resistor of 1,000 Ω. After biofilm formation in the anode and current stabilisation at approximately 61 ± 4 mA m^{-2} for over 3 d (Figure 4-13), the circuit was opened, the catholyte was replaced with anaerobic catholyte containing 10 mg-Cr(VI) L^{-1} and the experimental period started by connecting the two chambers with an $R_{ext.}$ of 1,000 Ω.

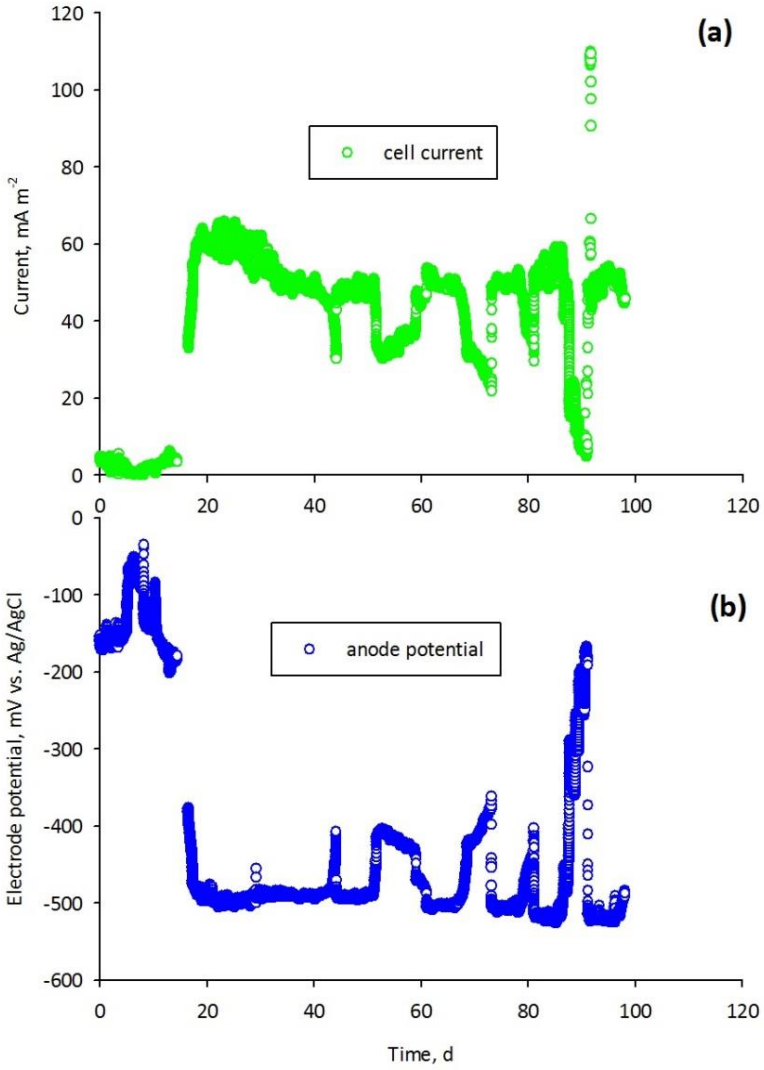

Figure 4-12: Cell current (a) and anode potential (b) during start-up of the "parental" reactor for the abiotic cathode MFCs; data missing from day 16 to day 18 is due to power disruption in the lab.

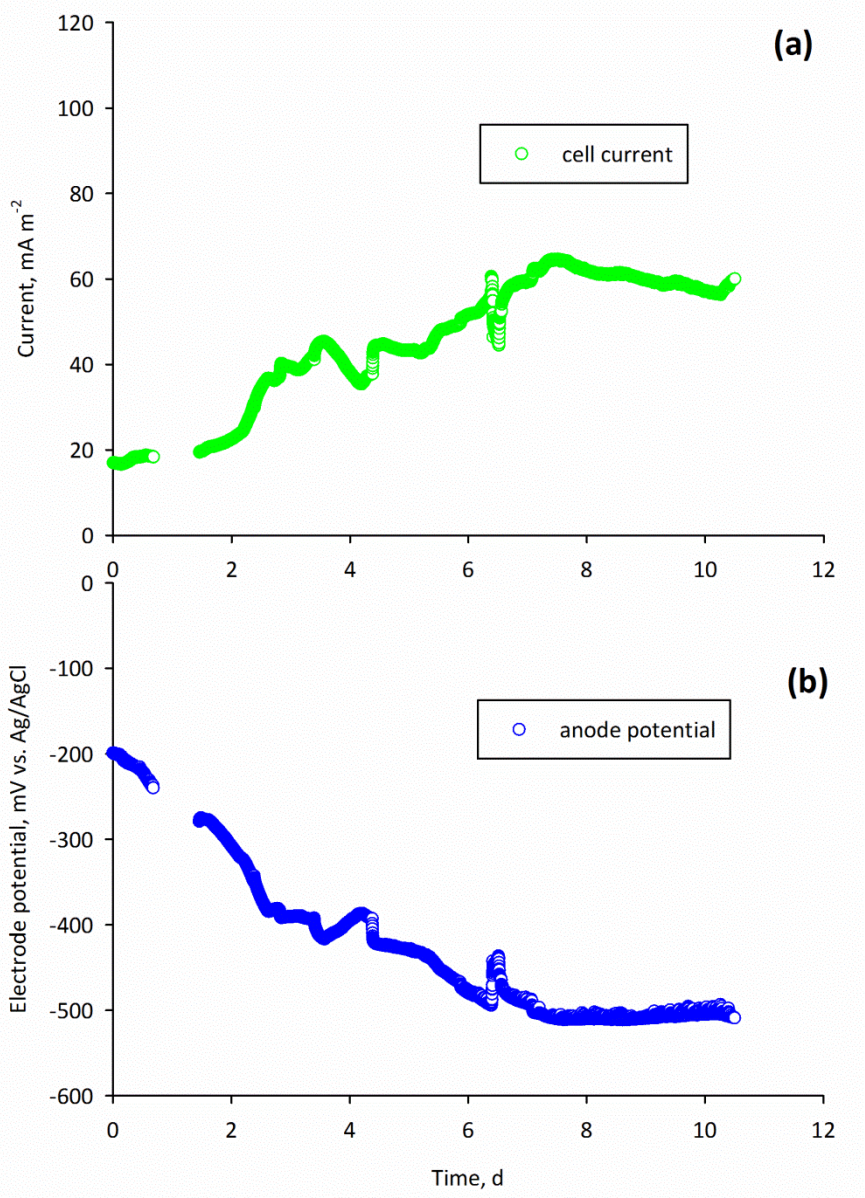

Figure 4-13: Cell current (a) and anode potential (b) during start-up of the abiotic cathode MFCs. Curves represent the average values from triplicate reactors; data missing during day 1 is due to power disruption in the lab.

4.2.2.2. Results and discussion

4.2.2.2.1. Lactate concentration effect

To demonstrate how lactate concentration in the cathode can affect Cr(VI) reduction, four lactate concentrations were studied, i.e. 0, 2, 20 and 200 mM. Cathodic pH was chosen to be 7 to allow comparison with other studies with Cr(VI) in the cathode of MFCs. Acetate was available at all times in the anode and the anodic potential remained low at -542 mV ± 20 mV

vs. Ag/AgCl under closed circuit conditions throughout operation. Also, anodic pH increased during start-up and remained at approximately 7.9 ± 0.2 throughout operation in all experiments.

Figure 4-14 shows that an increased current stability was obtained with increasing lactate concentration, allowing more current to be produced. Anodic potentials showed no considerable differences between the reactors (Figure 4-15), therefore it is more likely that the observed differences are due to the different conditions applied in the cathodes. When no lactate was added in the cathode, current density dropped from 43.8 mA m^{-2} to 3.5 mA m^{-2} within 7 h of operation and this was independent of 8.7 ± 0.2 mg-Cr(VI) L^{-1} still available in solution by the end of the 72 h operation period (Figure 4-16). On the contrary, when 200 mM lactate was added in the cathode, current dropped from 59.2 mA m^{-2} to 3.6 mA m^{-2} only after 70 h of operation and when there was only 0.3 ± 0.3 mg-Cr(VI) L^{-1} still in solution. Whilst current production at the end of the 72 h operation period was below 3.7 mA m^{-2} in all cases (equivalent to electrochemical Cr(VI) reduction at a rate of less than 0.59 mg-Cr(VI) L^{-1} day^{-1}), there was still 2.5 ± 0.7 and 7.1 ± 0.4 mg-Cr(VI) L^{-1} in solution when 20 and 2 mM lactate were added in the cathode respectively. This is translated into increased rates and overall reducing ability of the cathode with increasing chelate concentration in the cathode and is also clearly shown in Figure 4-17. Organic ligands can increase the redox potentials of the catholyte when they bind with the reduced form of the metal (Miller et al., 1990) and this could also be a factor for the increased performance observed in the presence of lactate. An increased current production was also recently observed by Chung et al. (2012), who complexed iron with citrate and EDTA to manipulate the redox potentials of the Fe^{3+}/Fe^{2+} couple and increase the performance of a MFC- chemical FC configuration.

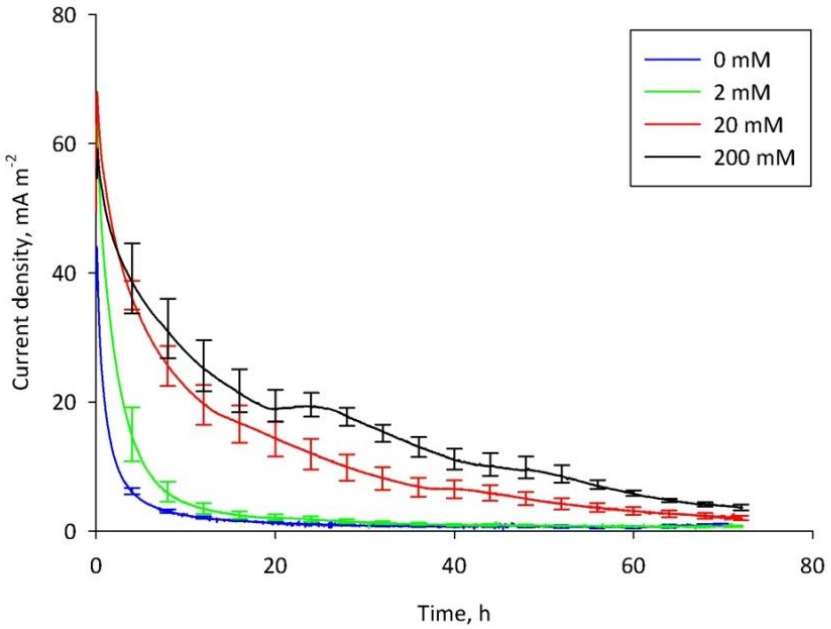

Figure 4-14: The effect of different lactate concentrations present in the cathode (pH 7); current evolution- averages from triplicate experiments, error bars added every 4 h represent the min and max of the measurements from the three reactors.

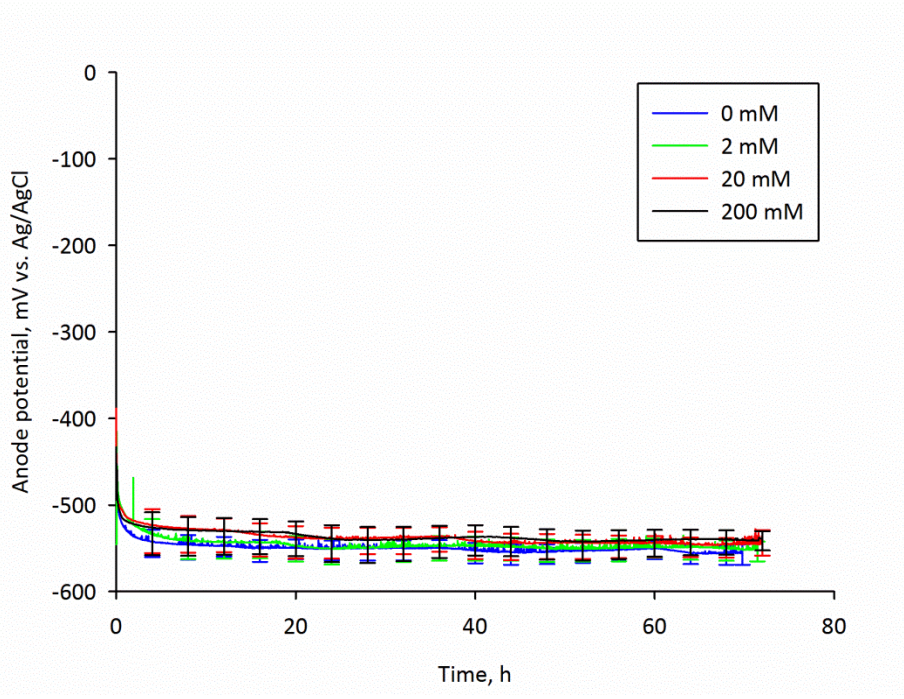

Figure 4-15: The effect of different lactate concentrations present in the cathode (pH 7); anodic potentials- averages from triplicate experiments, error bars added every 4 h represent the min and max of the measurements from the three reactors.

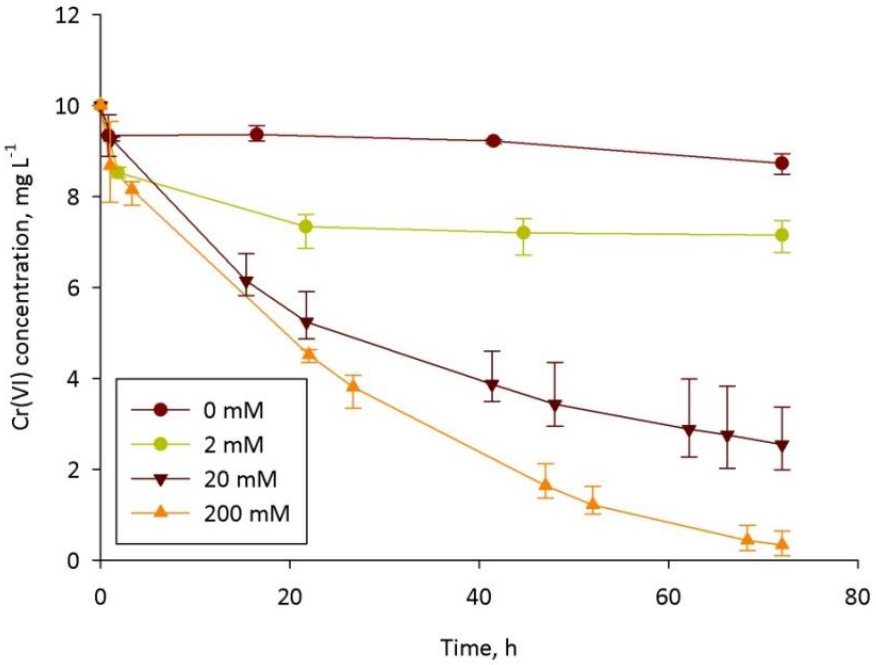

Figure 4-16: The effect of different lactate concentrations present in the cathode (pH 7); Cr(VI) remaining in solution- averages from triplicate experiments, error bars added represent the min and max of the measurements from the three reactors.

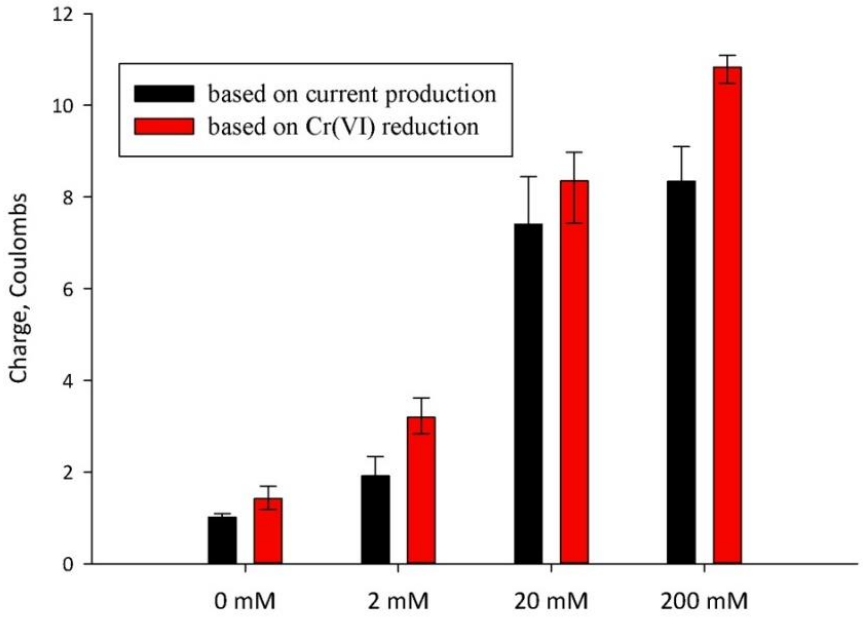

Figure 4-17: The effect of different lactate concentrations present in the cathode (pH 7); charge produced by the end of the 72 h operation period- averages from triplicate experiments, error bars added represent the min and max of the measurements from the three reactors.

Total Cr not retained by the electrode and the 0.45 μm filter remained above 8.8 mg-Cr L^{-1} in all cases, however its toxic Cr(VI) form decreased from 86% with 0 mM lactate to only 3% with 200 mM lactate. Cr(III) remained in the catholyte even in the absence of lactate, indicating that perhaps only a small quantity of Cr(III) is enough to inhibit the process; the rest of Cr(III) that forms during the process simply remains in the catholyte. This is in accordance with the observation by Clark and McCreery (2002) who noticed that only 8.4 nmol Cr(III) cm^{-2} (8.7 μg-Cr(III) 20 cm^{-2}) was enough to cause a kinetic overpotential of over 1.0 V and to inhibit any further Cr(VI) reduction on a glassy carbon electrode.

It should be noted here that the results presented herein seem to contradict the results observed by Puzon et al. (2005) and Hamada et al. (2005), who characterised the lactate-Cr(III) complexes as insoluble. An explanation for this could be that the authors analysed their samples after centrifugation and filtration though 0.22 μm pore size filters. On the contrary, 0.45 μm pore size filters were used in this study, as also indicated by APHA (2005), and it is possible that the Cr(III)-lactate complexes managed to pass through these filters but not through the 0.22 μm ones.

4.2.2.2.2. Cr(VI) concentration effect

To evaluate the limits of Cr(VI) reduction, two more experiments were conducted with 2 and 50 mg-Cr(VI) L^{-1}, both with 200 mM lactate and pH 7 (Figure 4-18). Using 2 mg-Cr(VI) L^{-1} as initial concentration, Cr(VI) was totally reduced to undetectable levels within 60 h and current was decreased from a maximum of approximately 30 mA m^{-2} to less than 0.3 mA m^{-2}. With an initial concentration of 50 mg-Cr(VI) L^{-1} on the other hand, current dropped from approximately 90 mA m^{-2} to approximately 5 mA m^{-2} within 189 h of operation, however Cr(VI) was only incompletely reduced by the end of the operation period; 17.6 mg-Cr(VI) L^{-1} was reduced within the first 92 h and only 1.2 mg-Cr(VI) L^{-1} was reduced within the last 97 h. This is in compliance with our previous results showing increasing Cr(VI) reduction with increasing chelate concentration and stresses the importance of the chelate to Cr(VI) ratio. It also shows that, given a specific electrode surface area and chelate to Cr(VI) ratio, the electrode can reduce only a certain amount of Cr(VI) before the kinetics become prohibitive for any further Cr(VI) reduction.

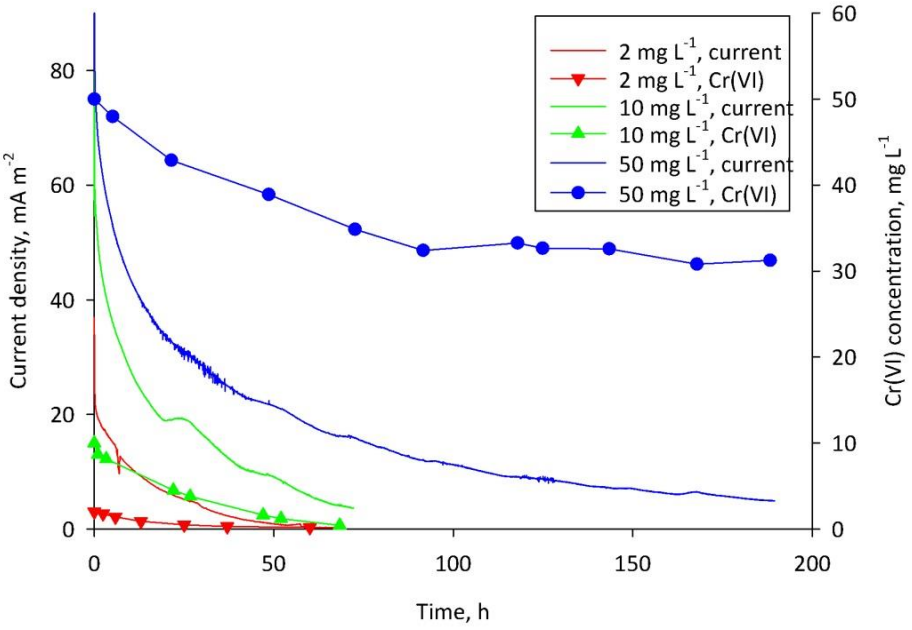

Figure 4-18: Effect of initial Cr(VI) concentration on current evolution (pH 7, 200 mM lactate).

4.2.2.2.3. pH effect

Since 200 mM lactate gave the higher Cr(VI) reduction rates, the same concentration was used to study the pH effect in the cathode. A pH range of 6 to 9 was chosen in order to study how changing the pH from slightly acidic to alkaline would affect the MFC performance. Figure 4-19 demonstrates the higher current production by the MFCs operating with the cathode at pH 8, resulting in faster Cr(VI) reduction compared to other pH levels (Figure 4-20). The pH 8 cathodes exhibited more efficient Cr(VI) reduction even compared to pH 6 cathodes, regardless of the fact that both systems had an initial current production of 70 mA m^{-2}. As a result, Cr(VI) levels in pH 8 MFCs fell to less than 0.3 ± 0.3 mg L^{-1} within the first 43 h of operation, whilst at pH 6 and 7 the same levels of Cr(VI) remaining in solution was only achieved at the end of the 72 h operation period. At pH 9, Cr(VI) was continuously reduced, however reduction rates were slower and there was still 6 ± 0.4 mg L^{-1} available at the end of the operation period.

Anodic potentials did not alter much and were -531 ± 49 mV at pH 6, -536 ± 7 mV at pH 7, -542 ± 21 mV at pH 8 and -558 ± 4 mV at pH 9. Also, anodic pH and temperature did not alter considerably throughout operation, and therefore cathode-related factors are thought to have been the main reasons resulting in the higher current production observed at pH 8. It might be

assumed that an increased stability of the Cr(III)-lactate complex was maintained with increasing pH, as a result of a stronger deprotonation of the lactic ion. However, with a low pK_a value for the carboxyl (3.86) and a high pK_a value for the hydroxyl (15.1) groups (Silva et al., 2009), the protonation state of the lactic ion would not alter much within the pH range studied.

Another more likely explanation would involve the speciation of Cr(III)-lactate complexes with varying pH. In an aquatic environment, four Cr(III) species are dominant within the pH range 6-9 (Sun et al., 2010): $Cr(OH)^{2+}$ and $Cr(OH)_2^+$ at pH 6; $Cr(OH)_2^+$ at pH 7; $Cr(OH)_2^+$ and $Cr(OH)_3$ at pH 8; $Cr(OH)_2^+$, $Cr(OH)_3$ and $Cr(OH)_4^-$ at pH 9. Considering a 1:1 lactate-Cr(III) chelation (Hamada et al., 2005), complexes at lower pH might have retained a positive charge, which attracted them on the negatively charged electrode surface. Cr(III) precipitants have been observed even after a reduction at pH as low as pH 2 (Zhang et al., 2012), however the higher H^+ availability at lower pH is probably counteracting this inhibition effect by considerably increasing the cathode's reduction potential.

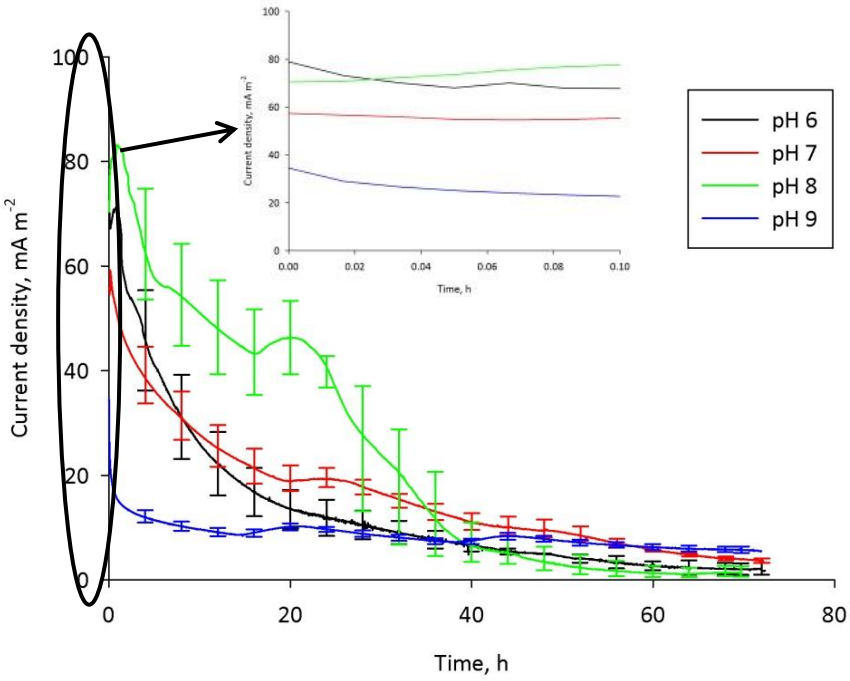

Figure 4-19: The effect of cathodic pH (200 mM lactate); current evolution- averages from triplicate experiments, error bars added every 4 h represent the min and max of the measurements from the three reactors. Magnified area shows the same graph during the first 6 min of operation; the pH 6 cathode MFC started with the highest current production, however current rapidly decreased afterwards in this instance.

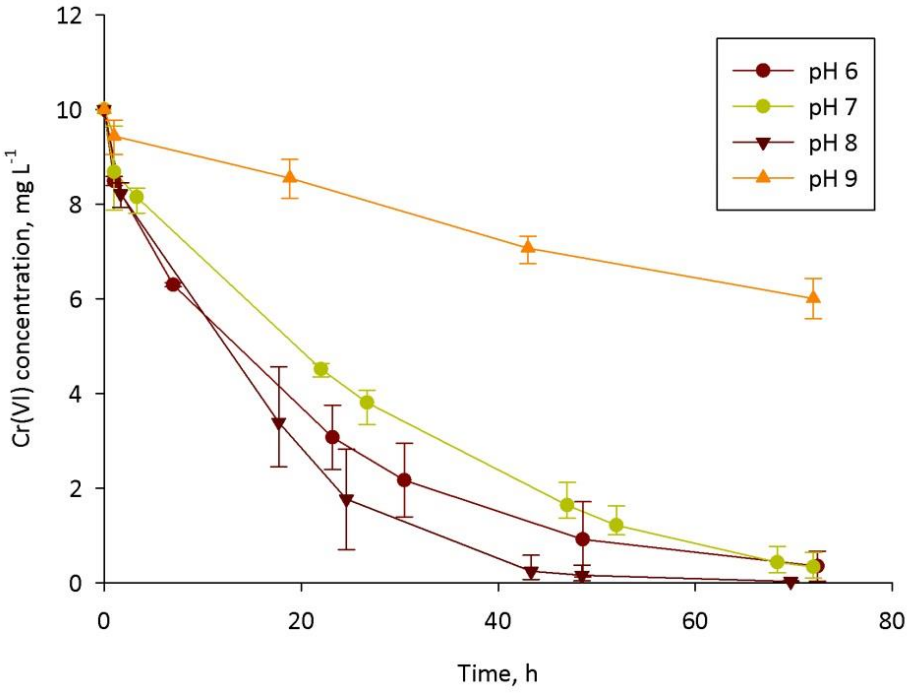

Figure 4-20: The effect of cathodic pH (200 mM lactate); Cr(VI) remaining in solution- average values from triplicate experiments, error bars added represent the min and max of the measurements from the three reactors.

Another explanation for the enhanced electrochemical behaviour observed at pH 8 could be that at lower pH, partial Cr(VI) reduction by non-electrochemical pathways was observed. Whilst cathodic efficiencies were close to 100% at pH 8 and 9, at pH 7 and 6 they dropped to 77% and 63% respectively (Figure 4-21), indicating that another Cr(VI) removal pathway was present at lower pH. With Cr(VI) at neutral pH being in the negative chromate ion (CrO_4^{2-}) form, Cr(VI) cannot be electrostatically removed by the negatively charged cathode (Farmer et al., 1997) or pass through the Nafion proton exchange membrane that was used. A more possible mechanism, which explains this decrease in Coulombic efficiency would be that of chemical Cr(VI) reduction by the lactic ion (Deng and Stone, 1996). Finally, almost all Cr_{tot} was measured in solution by the end of the operation period, and as discussed previously, the filter's pore size chosen for Cr_{tot} determination might have been a reason for this.

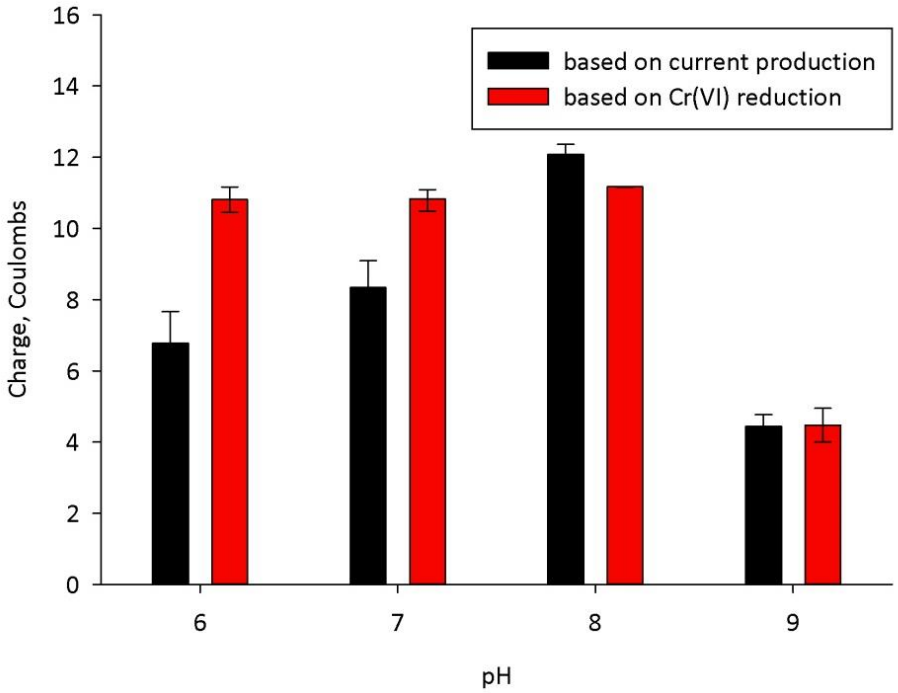

Figure 4-21: The effect of cathodic pH (200 mM lactate); charge produced by the end of the 72 h operation period- average values from triplicate experiments, error bars represent the min and max of the measurements from the three reactors.

Polarisation curves were obtained before start of the experimental period to assess power and current production at that time (Figure 4-22). Power production at pH 8 was maximum at the current density of 104 mA m^{-2}, when 21 mW m^{-2} were produced. Whilst power was increased at slightly acidic pH 6 (25 mW m^{-2} at 112 mA m^{-2}) as can be explained by the Nernst equation, power densities at pH 7 and 9 were considerably lower compared to the pH 8 cathodes (9 mW m^{-2} at 48 mA m^{-2} and 2 mW m^{-2} at 15 mA m^{-2} respectively). However, it should be noted at this point that polarisation graphs obtained must have also been affected by the formation of Cr(III) oxyhydroxides on the electrode surface. These tests were normally completed 30 minutes after start and they represent a temporal effect; the longer cathode conditions were exposed to pH 8, the better the performance, as already discussed. This could probably be the reason why the pH 8 catholyte gave higher current and power than the pH 6 catholyte did when the same external resistor values were used by the end of the experiments (Figure 4-22).

Until now, the only cases of Cr(VI) reduction at neutral- slightly alkaline pH had focused on the use of Cr(VI) reducing bacterial catalysts in the cathode. In Table 4-3, maximum power densities recorded in the literature were normalised to the total apparent surface area of the cathode, rather than for instance to the working volume of the cathode. This is because it has

now become clear that the Cr(VI) reduction reaction in MFCs and other electrochemical configurations are to a great extent limited by the processes occurring on the electrode surface and thus highly depended on the electrode's surface area. At pH 6, power production was comparable to previous studies (Wang et al., 2008, Li et al., 2008), however only 1/5 to 1/10 of the concentration used elsewhere was used in this study. At pH 7, the maximum power recorded was up to 44 times higher than the power observed elsewhere in abiotic cathodes (Huang et al., 2010, Huang et al., 2011b) and at similar levels to the power produced when bacterial catalysts were employed (Huang et al., 2010, Huang et al., 2011b, Huang et al., 2011a, Tandukar et al., 2009). At pH 8 and 9, maximum power produced by 10 mg-Cr(VI) L^{-1} was 31 and 3 times higher respectively than the power produced in a biocathode MFC running with 20 mg-Cr(VI) L^{-1} at pH 8 (Huang et al., 2011b). However, further work would be needed for this comparison.

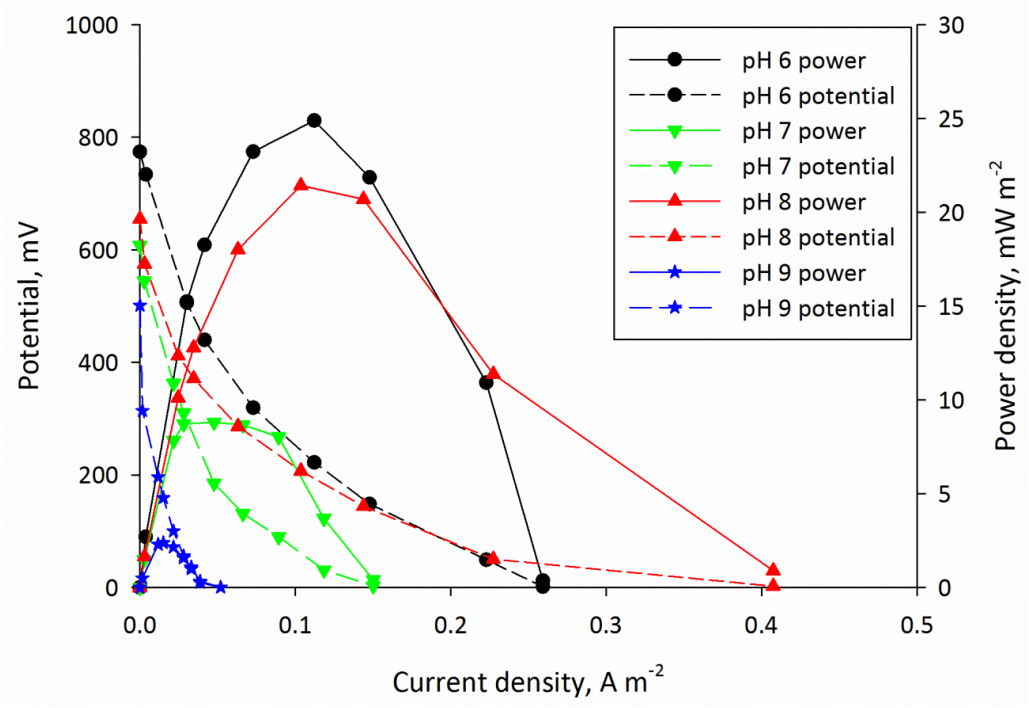

Figure 4-22: Polarisation graphs obtained before start of the experimental period at different pH values (200 mM lactate).

Table 4-3: Comparison of this study (200 mM lactate) with other studies at near neutral to alkaline pH.

pH	Maximum power density observed (mW m^{-2})	Current for maximum power density (mA m^{-2})	[Cr(VI)] (mg L^{-1})	Biocatalyst in the cathode	Electrode surface area per cathodic working volume (m^2 m^{-3})	Reference
6	23.0	-	100	No	15	Wang et al. (2008)
	24.9	**112.0**	**10**	**No**	**10**	**This study**
	29.6	-	50	No	10	Li et al. (2008)
7	0.2	2.3	39	No	620 (340-900)	Huang et al. (2010)
	0.2	2.4	20	No	9600	Huang et al. (2011b)
	2.5	9.5	20	Yes	800	Huang et al. (2011b)
	3.9	11.1	39	Yes	620 (340-900)	Huang et al. (2010)
	7.9	46.6	22	Yes	16	Tandukar et al. (2009)
	11.0	54.8	31			
	24.7	83.2	40			
	55.5	123.4	63			
	8.8	**47.6**	**10**	**No**	**10**	**This study**
	10.3	33.9	20	Yes	620 (340-900)	Huang et al. (2011a)
8	0.7	2.1	20	Yes	9600	Huang et al. (2011b)
	21.4	**103.5**	**10**	**No**	**10**	**This study**
9	**2.4**	**15.0**	**10**	**No**	**10**	**This study**

4.2.2.3. Conclusions

By using lactate as a chelating agent, it was possible to overcome, up to a certain extent, a major drawback of Cr(VI) reduction in electrochemical systems, i.e. that of electrode deactivation by Cr(III) precipitates. Current and power produced were at the same levels as those of microbially catalysed cathodes and maximum efficiency was achieved at alkaline pH 8. Lactate is believed to be enhancing the process by forming complexes with Cr(III), with a net charge, which delays their adsorption on the cathode. Since organic acids can be excreted by bacteria (Vollbrecht et al., 1978), which may be present in mixed bacterial biocathodes, the chelation effect could have also played an important role in previous studies on Cr(VI)

biocathodes. Unfortunately, this effect had not been adequately addressed by researchers in the BES field, until now.

4.3. Study on *Shewanella oneidensis MR-1* as a biocatalyst

4.3.1. *Shewanella oneidensis* MR-1 as a Cr(VI) reducer

4.3.1.1. Experimental plan

To investigate the ability of MR-1 for Cr(VI) reduction, two sets of flask experiments were conducted, one with low and another one with high biomass concentration. For this reason, MR-1 was initially grown aerobically in 100 mL of LB medium (pH 7.4) for 24 h, when an OD_{600} of 3.5 was reached in the rich LB medium (value corrected after serial dilutions to an OD_{600} limit of 0.4). After initial growth, the flasks' content was centrifuged and washed twice in a 100 mM NaCl- 50 mM buffer solution (pH 7.0). After washing, the biomass was inoculated in Erlenmeyer flasks to give optical densities of 0.5 and 0.8 in 200 mL of the medium described in Section 3.3. The nutrient medium of each flask was amended to contain adequate concentrations of lactate and fumarate and all experiments were conducted in duplicate. For low biomass concentration, lactate and fumarate (when supplied) had a concentration of 30 mM and 35 mM respectively. For high biomass concentration, the above concentrations were 60 mM and 60 mM respectively. The content of each flask was amended to contain adequate concentrations of lactate at all times and Cr(VI) was supplied each time after depletion, using the same stock solution described in Section 3.3. The content of each flask was flushed with filter-sterilised oxygen-free N_2 for 20 min before the first Cr(VI) addition and for 5 min after each Cr(VI) addition. After each Cr(VI) addition, flasks were closed using rubber stoppers and incubated at 22 ± 1 ^{o}C in an orbital shaker incubator (200 rpm).

4.3.1.2. Results and discussion

As can be seen in Figure 4-23, Figure 4-24, Figure 4-25 and Figure 4-26, Cr(VI) reduction took place at higher rates at the beginning of the experiments, however reduction rates slowed down considerably after the first 3-4 days of operation. Also, Cr(VI) reduction rates and maximum Cr(VI) reduction were increased with increasing biomass concentrations (Figure 4-27). When Cr(VI) was provided under anaerobic conditions, 4.2 ± 0.1 and 8.1 ± 0.0 mg-Cr(VI) was reduced by MR-1 at low and high biomass concentrations respectively (Figure 4-23, Figure 4-27). The presence of oxygen inhibited Cr(VI) reduction by 1.7 times with low MR-1 concentration and by 4.1 times with high MR-1 concentration and this had also been observed

before by Middleton et al. (2003) (Figure 4-24, Figure 4-27). In this instance, the difference in efficiency between low and high MR-1 biomass concentration was more obvious and an almost 5 times higher Cr(VI) reduction was observed by the end of the operation period when MR-1 was added at a high biomass concentration (4.7 ± 0.1 mg-Cr(VI) vs. 1.0 ± 0.1 mg-Cr(VI)). This could be attributed to the presumably lower oxygen levels present at higher MR-1 concentrations, which would force MR-1 to utilise Cr(VI), the only other electron acceptor present in the medium. On the contrary, fumarate did not seem to have a negative effect on Cr(VI) reduction (Figure 4-25, Figure 4-27). This had also been previously observed by Viamajala et al. (2002) and can be related to the distinct locations of the fumarate and Cr(VI) reductase activities (Myers et al., 2000). The "plateu" regarding Cr(VI) reduction, which was observed in all experiments after some time, has also been observed by Middleton et al. (2003) and represents an upper limit ("transformation capacity") that is reached by MR-1 during heterotrophic Cr(VI) reduction with lactate as an electron donor. Control experiments in the absence of lactate and oxygen revealed a minimal Cr(VI) reduction of 1.0 ± 0.0 mg-Cr(VI) and 3.0 ± 0.2 mg-Cr(VI) for low and high MR-1 concentrations respectively (Figure 4-26, Figure 4-27), indicating that some organic matter must have been stored by MR-1 cells, and was not removed during washing. Previously, organic carbon stored by *Shewanella* cells or released by the cell lysis, was considered partially responsible for lowering the cathodic efficiency in oxygen (Freguia et al., 2010) and Cr(VI) biocathodes (Hsu et al., 2012), respectively.

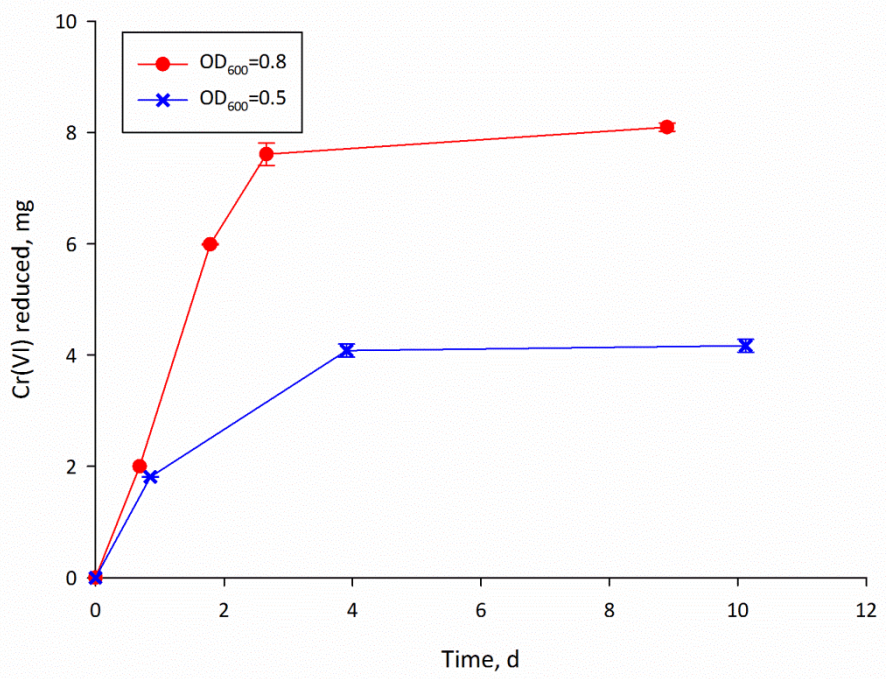

Figure 4-23: Cr(VI) reduced anaerobically in the presence of lactate (average values from duplicate experiments are reported).

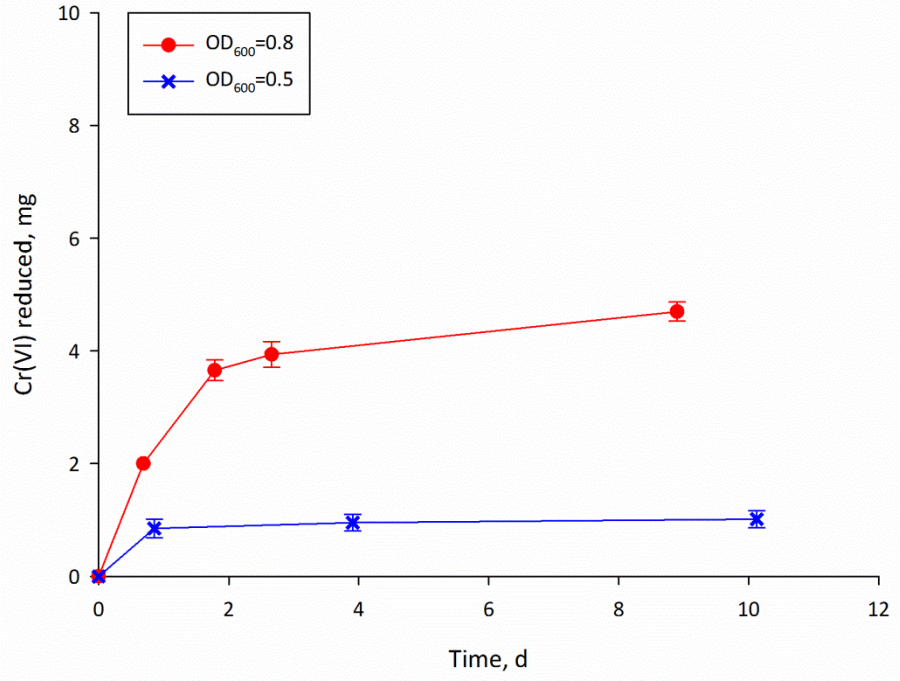

Figure 4-24: Cr(VI) reduced in the presence of air and lactate (average values from duplicate experiments are reported).

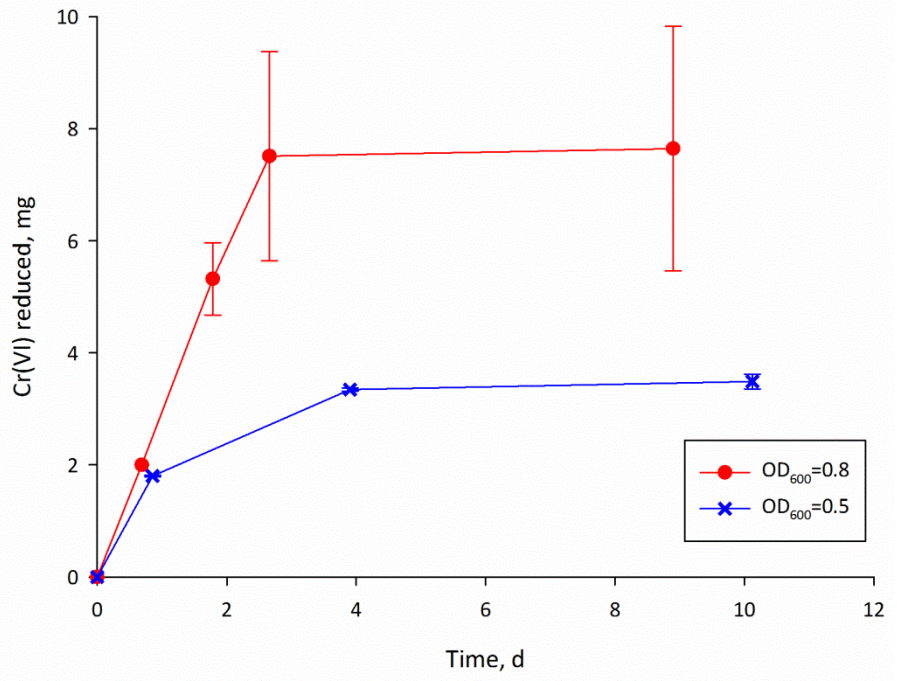

Figure 4-25: Cr(VI) reduced anaerobically in the presence of lactate and fumarate (average values from duplicate experiments are reported).

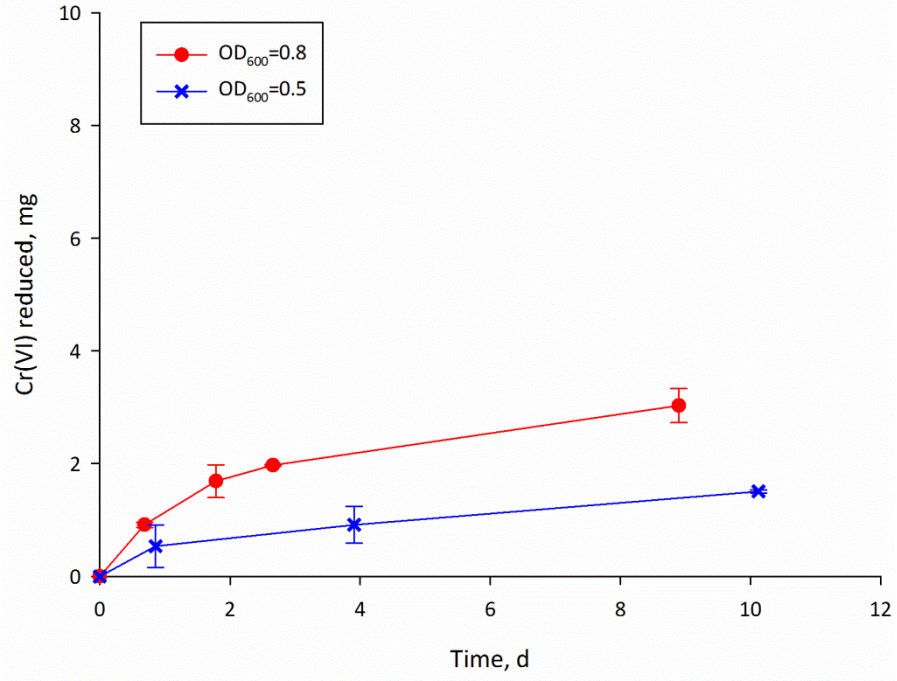

Figure 4-26: Cr(VI) reduced anaerobically in the absence of lactate (average values from duplicate experiments are reported).

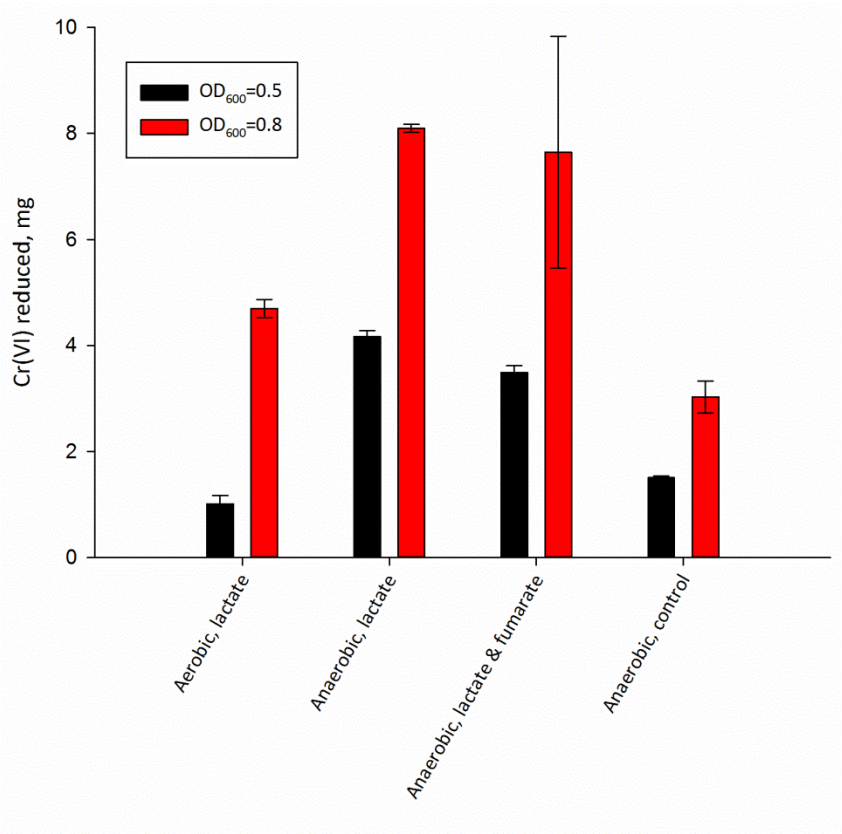

Figure 4-27: Comparison of Cr(VI) reduction by the end of the runs (average values from duplicate experiments are reported).

4.3.2. *Shewanella oneidensis* MR-1 as an anodophilic biocatalyst

4.3.2.1. Anodic current production during growth

As a first estimation of the MR-1 ability to produce anodic current, several MR-1 colonies were inoculated into the rich Luria-Bertani medium and were allowed to grow in the presence of a poised electrode (+300 mV vs. Ag/AgCl). No gas was actively flushed into the WE chamber, but MR-1 was exposed to oxygen that was present in the medium after autoclaving. As can be seen in Figure 4-28, current started to be produced exponentially after a lag period of 4 h. This exponential current increase was followed by a current peak period of 1 h and then a rapid decline in the current produced. No growth was visually observed before the initial 4 h period, and these results, in accordance with the existing literature, are a proof that MR-1 is capable of anodic current production. In this instance, MR-1 was grown with limited oxygen availability and this might be the reason why current dropped rapidly after the peak period at 8 h. As is also discussed in the next paragraph, MR-1 is known to be more electroactive when limited oxygen is available and this could be one reason for this rapid current drop observed. Nutrients

consumption could be another reason, however LB medium is a very rich substrate that can support aerobic growth of MR-1 for considerably longer periods and at higher biomass concentrations (OD_{600} over 1.5) than the ones observed here (OD_{600} was between 0.3 and 0.5).

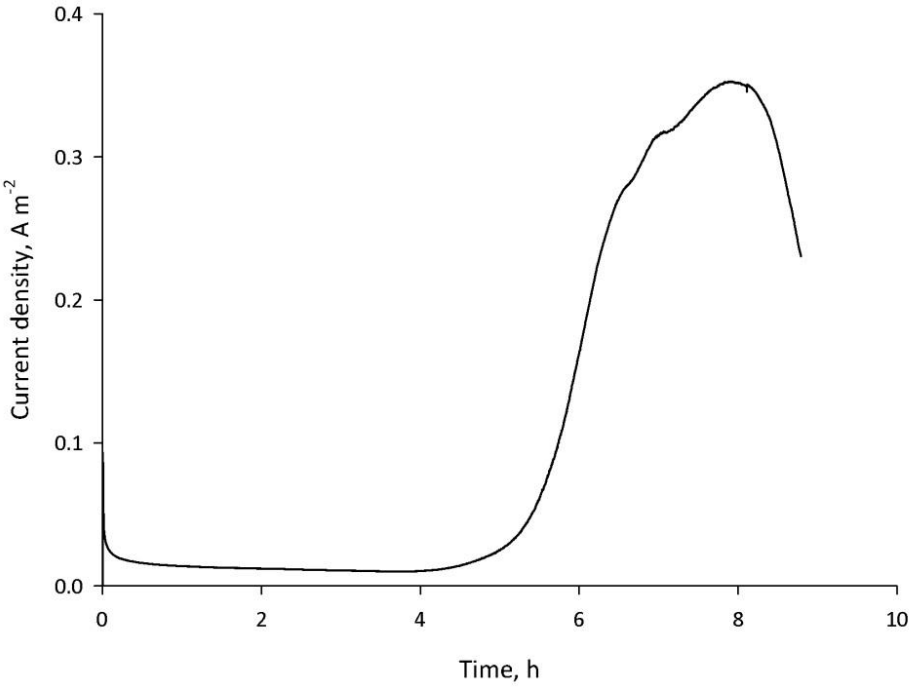

Figure 4-28: Chronoamperometry profile showing current production as MR-1 is growing in Luria-Bertani medium, in the presence of a poised electrode at +300 mV vs. Ag/AgCl and limited oxygen availability (average data from six experiments).

4.3.2.2. Generic performance of Cr(VI) reducing MFCs with MR-1 in the anode

To investigate how a Cr(VI) reducing MFC behaves under several stress conditions, a dual-chamber MFC with a proton-exchange membrane (Nafion 117™), a 100 Ω load and MR-1 in the anode was set up and operated for 24 d. The pH in the cathode was kept at approximately 2 and Cr(VI) was initially supplied at 100 mg L^{-1}. In this setup, MR-1 was supplied at high concentrations in the anode (OD_{600} approximately 1.5) and before start it was allowed overnight to stabilise under open circuit conditions. No gases were actively flushed into the anode but air was let to passively diffuse through a 0.2 μm filter in this instance. The cathode was flushed with N_2 at start and at 1 d intervals.

After 2 d of operation, Cr(VI) was depleted and current dropped sharply (Figure 4-29), also increasing the potential measured on the anode (Figure 4-30). After Cr(VI) addition, current was restored and followed a decreasing trend with decreasing lactate concentrations. Anodic potential was also affected (increased) during lactate depletion, stabilising at approximately -

180 mV vs. Ag/AgCl. This potential is higher than in the presence of lactate, however only by approximately 220 mV; this relatively not very large difference can be attributed to the presence of acetate in the solution under anoxic/microaerophilic conditions. Current did not increase after anolyte replacement on the 9th day, however the anodic potential dropped again to the previous levels within 1 day of operation. Low current production along with low anodic potential could indicate cathodic limitations due to accumulation of Cr(III) compounds on the cathode surface. After 3 more days, new biomass was supplied in the anode and cathode pH was decreased from 3 to 2. Whilst anodic potential did not alter considerably, current production showed a sharp increase; this is an indication that the cathode limitations were surpassed by this cathode pH decrease, probably by solubilising the Cr(III) precipitates of the cathode and/or by increasing the cathodic potential. Lactate concentration increase from 14 to 3,040 mg L^{-1} only slightly increased the current on day 14, supporting the argument that other factors like accumulation of Cr(III) compounds on the cathode are playing an important role in the process. Lactate concentration increase from 0 to 2,800 mg L^{-1} on day 19 increased current production, however at only approximately 30% of the levels initially observed.

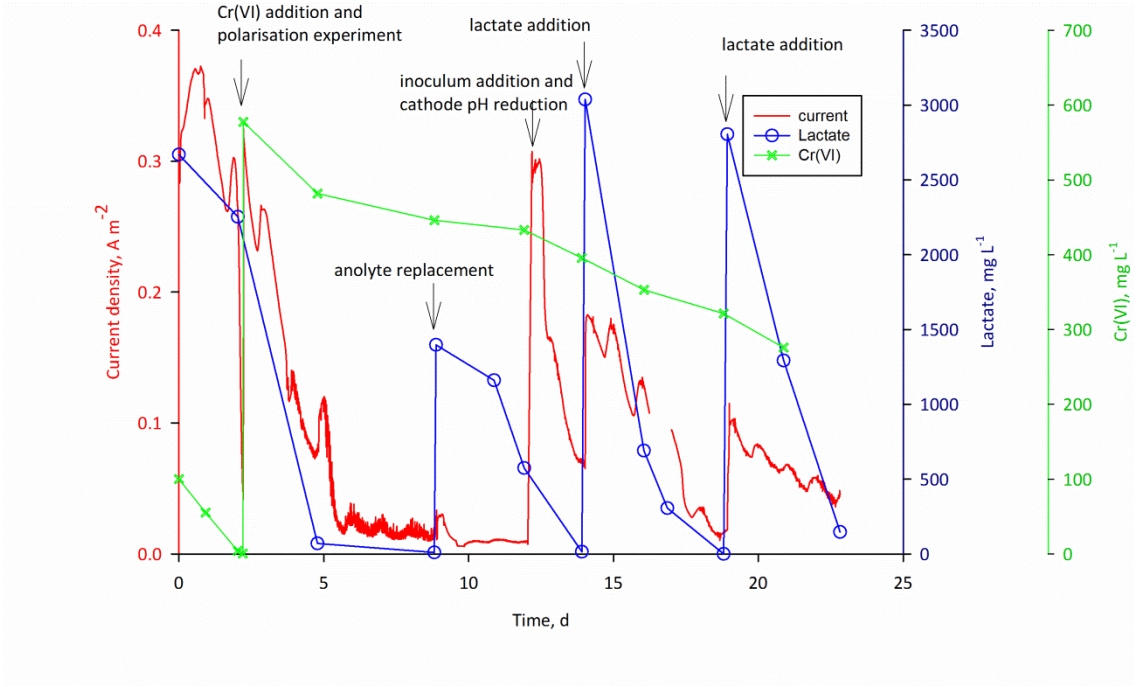

Figure 4-29: The effect of various stress conditions on current generation by the dual-chamber MFC that was assembled to study the generic performance of the system.

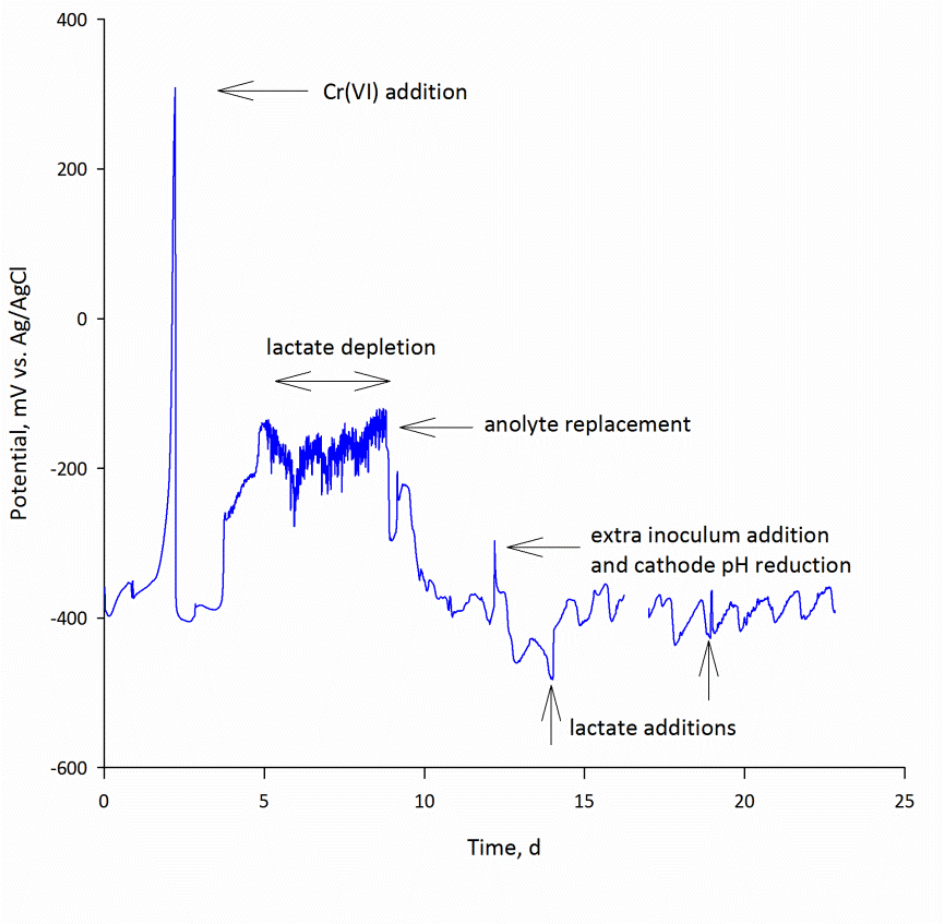

Figure 4-30: The effect of various stress conditions on the anodic potential of the dual-chamber MFC that was assembled to study the generic performance of the system.

Coulombic yield calculated based on both current production and Cr(VI) reduction are presented in Figure 4-31. As can be seen, Coulombs were produced at a constant rate until lactate depletion when current production was inhibited. However, Cr(VI) reduction and current generation were restored after the addition of new biomass on the 12th day.

A polarisation curve produced on the 2nd day of operation, immediately after Cr(VI) addition, can be seen in Figure 4-32. A maximum of 48 mW m^{-2}, with a corresponding current value of 114 mA m^{-2} was produced using a 1890 Ω resistance. The internal resistance would be expected to have a similar value and this falls within a typical range for simple H-type MFCs (Logan and Regan, 2006). Also, as previously shown in Table 2-3, such maximum power density values fall within the range previously reported for similar cathodic pH values and membrane surface areas (Liu et al., 2011, Pandit et al., 2011, Wang et al., 2008). This indicates that MR-1 can successfully compete the performance of mixed cultures in the anodes of fed-batch systems.

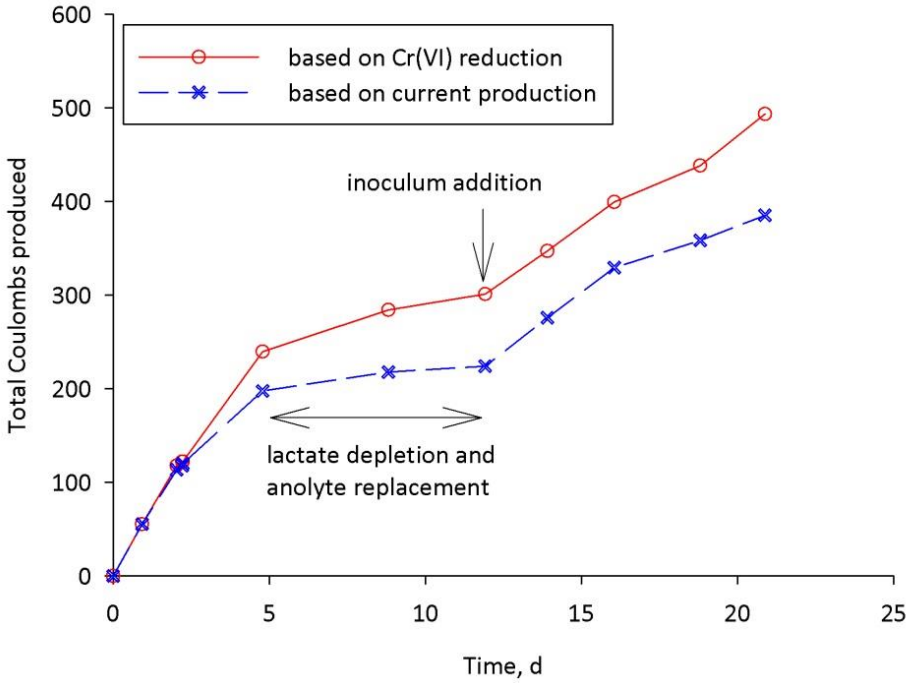

Figure 4-31: Coulombs produced by the dual-chamber MFC that was assembled to study the generic performance of the system.

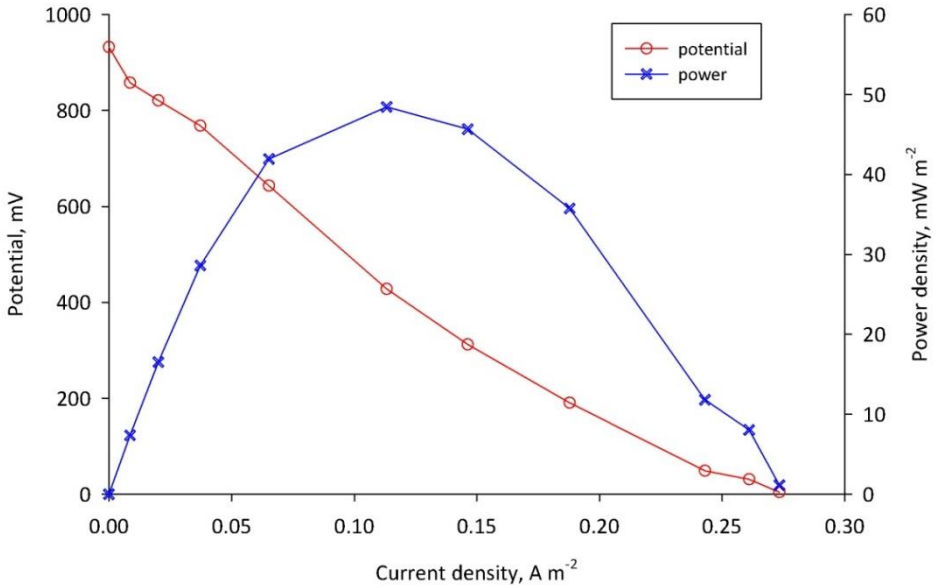

Figure 4-32: Polarisation (red) and power (blue) curves produced by the dual-chamber that was assembled to study the generic performance of the system. Curves were obtained on the second day of operation, after 580 mg-Cr(VI) L^{-1} was added in the cathode.

To evaluate the amount of Cr(VI) being removed without the presence of an anodic biocatalyst, a dual-chamber MFC was set-up as an abiotic control, with a proton exchange membrane (Nafion 117™) fixed between both chambers. The catholyte contained Cr(VI) at an initial pH 2, whilst the anolyte was a lactate containing anodic solution at pH 7. The reactor was operated for 56 d under agitation (as described in Chapter 3) and with 100 Ω external load. As seen in Figure 4-33, Cr(VI) concentration was reduced by approximately 45 mg L^{-1} by the end of the run. No Cr(VI) was detected in the anode, indicating that the membrane could sufficiently keep Cr(VI) only in the cathode chamber. A current of approximately 3 μA was estimated throughout the run, corresponding to only 13 mg Cr(VI) L^{-1} electroreduction (14.5 C) within the 56 d (assuming all electrons are utilised for Cr(VI) reduction). This indicates that other abiotic reactions were responsible for the reduction of the remaining 32 mg L^{-1} (0.123 mmoles), which would also explain the deviation observed in Figure 4-31 between the two different ways of calculating the total Coulombs produced. For example, such reactions could involve Cr(VI) reduction by trace elements that managed to cross the membrane. In addition, at a pH 2 or lower, the Cr(VI)/Cr(III) couple has a redox potential equal to or higher than that of the H_2O/O_2 couple (Figure 2-1), therefore at high Cr(VI) concentrations and low O_2 concentrations, water oxidation to O_2 by Cr(VI) would be thermodynamically possible. Finally, the pH in this reactor dropped from 7 to 6.1 in the anode and increased from 2 to 3.3 in the cathode by the end of the run, indicating the need for chemical pH control at this high pH gradient between the anode and the cathode.

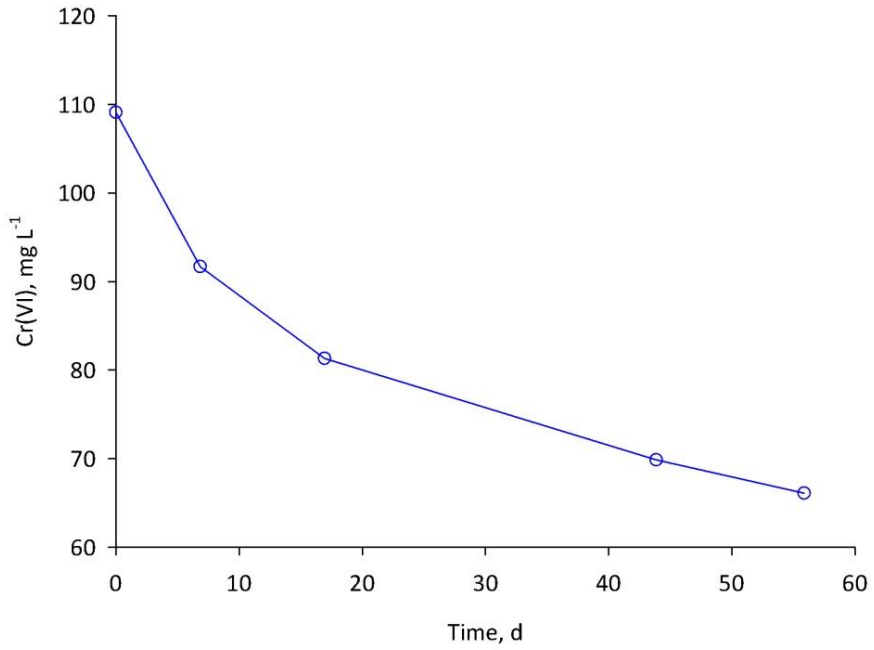

Figure 4-33: Cr(VI) removal in an dual-chamber abiotic MFC that was assembled to assess Cr(VI) removal in the absence of a bacterial "catalyst" in the anode.

With a lower cathodic pH increasing the cathodic potential (Figure 2-1), cathodic pH is a crucial parameter determining MFC performance. To evaluate the effect of increasing pH in Cr(VI) cathodes, a dual-chamber MFC with a proton-exchange membrane (Nafion 117™), a 100 Ω load, *S. oneidensis* MR-1 in the anode, and agitation as described in Chapter 3, was setup and operated without fixing the pH in the cathode. As pH increased from 2 to 2.6, the cathodic potential decreased from +500 mV vs. Ag/AgCl to even negative values, clearly affecting current generation (Figure 4-34). Cr(VI) depletion was not a reason in this instance, as Cr(VI) concentration in the cathode was over 230 mg L^{-1} at all times. After a pH decrease from 2.6 to 1.7 on the fourth day, current increased from 0.1 A m^{-2} to 0.8 A m^{-2} immediately after decreasing the pH (using a 5 N HCl solution), indicating that a pH increase of even less than 1 pH unit in the cathode can be a major limiting factor for current generation.

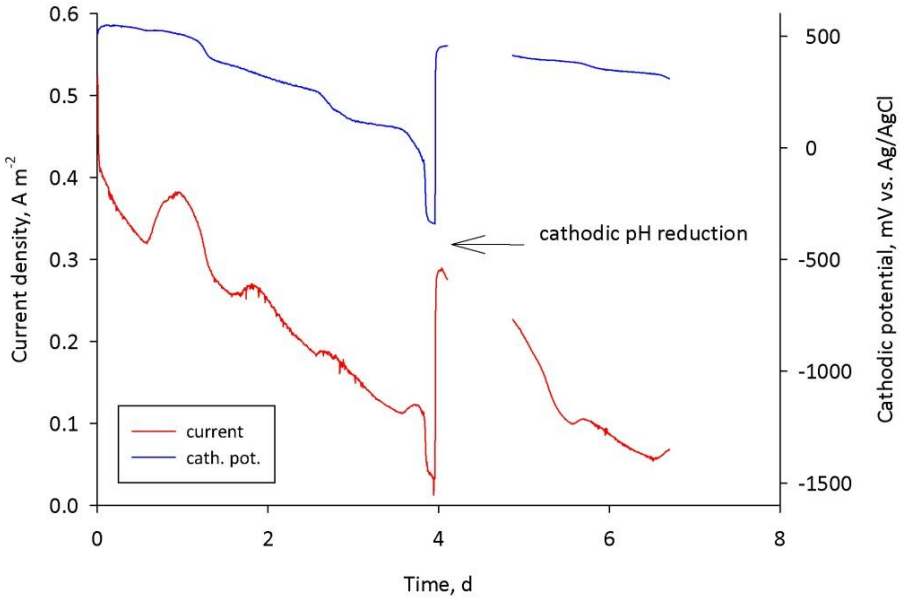

Figure 4-34: Effect of cathodic pH reduction from 2.6 to 1.7 on the current production and the cathodic potential of a dual-chamber MFC; gaps from day 4 to day 5 are due to a power disruption in the lab.

4.3.2.3. Assessing the various conditions for anodic current production by MR-1

In MFCs it is important to evaluate the form (planktonic or attached cells) in which the anodic biomass is more electrochemically active. To demonstrate this more clearly and without the restrictions imposed by the cathode, MR-1 was grown aerobically and then operated overnight at open circuit in a half-cell reactor with a proton exchange membrane (Nafion 117™) to consume all the oxygen present. Afterwards, the WE was constantly poised at +300 mV vs. Ag/AgCl (time zero) and the system was operated for 9 d until lactate reached approximately 700 mg L^{-1} in the anode (Figure 4-35). On the 9th day the anolyte was replaced with fresh medium containing 2,000 mg L^{-1} lactate. If the biofilm formation was a major reason for current generation, then a positive current response could be expected. However, this did not happen and electrochemical activity did not recover even after 3 d of operation, probably because electron transport to the electrode was mainly conducted by planktonic MR-1, and/or that the redox active compounds necessary for MR-1 respiration (e.g. flavins) could not be regenerated after the medium replacement. In addition, even though generally MR-1 does not form a thick biofilm, no biofilm formation could be visually observed after reactor disassembly. These findings are in agreement with previous studies showing that MR-1 is mainly present in planktonic form during MFC operation (Lanthier et al., 2008, Biffinger et al., 2009).

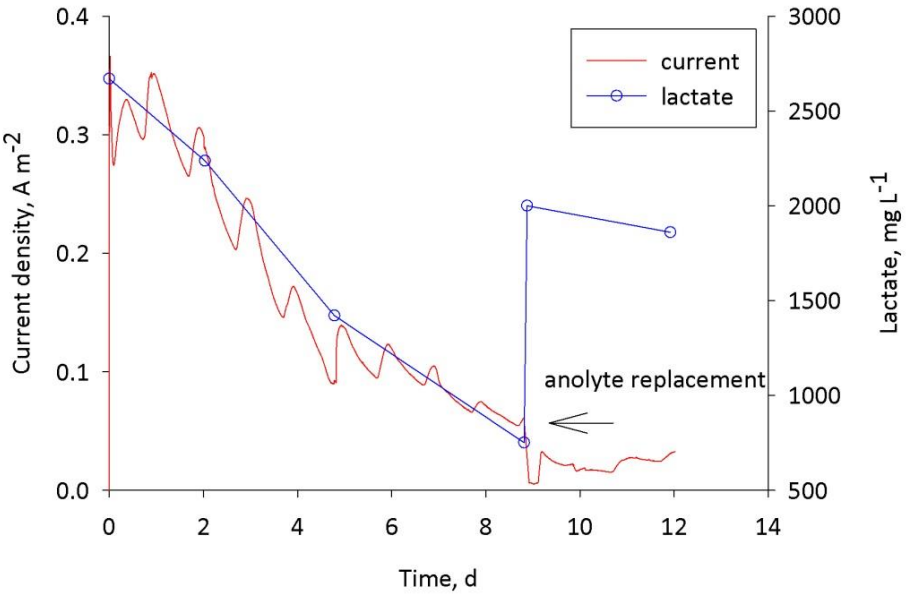

Figure 4-35: Current evolution at +300 mV vs. Ag/AgCl and the effect of anolyte replacement, in a reactor studying the effect of various conditions on anodic current production by MR-1.

In order to further assess the ability of MR-1 to produce anodic current, various operational conditions were applied in the WE of potentiostatically poised BES reactors, where MR-1 was allowed for 14-18 h to produce the metabolites needed for electron transfer and to form a biofilm. Further on, this operation period is notated as phase 1, to distinguish it from phase 2 described in Section 4.3.3, where MR-1 was assessed for its ability to produce cathodic current with Cr(VI) as the terminal electron acceptor. A poised potential of +300 mV vs. Ag/AgCl was applied to all working electrodes, except for some tests where a potential of -500 mV vs. Ag/AgCl was applied for comparison. At the end of phase 1, CV tests were conducted before the potentiostat potential was set at -500 mV (phase 2), so that the electrode could act as the electron donor for Cr(VI) reduction.

In Figure 4-36a it is shown that the pre-treatment conditions applied to MR-1 had a considerable effect on anodic current production. When MR-1 was kept under anaerobic conditions without any external redox mediator addition, lactate consumption caused a current increase during the first 3 h which then stabilised at a level of 0.04 A m^{-2}. Air flushing had a positive effect, and following a lag period of approximately 1 h, anodic current production constantly increased and reached 270 mA m^{-2} by the end of phase 1. This was probably due to the production of electrocatalysts by MR-1 under aerobic conditions in the presence of lactate (Biffinger et al., 2008a, Rosenbaum et al., 2010) and also probably due to a thicker biofilm that was visually observed on the electrode surface. Enhancement of anodic

current by redox mediators was confirmed when a 7-fold higher value (compared to that of the anaerobic reactor without redox mediators) was produced and remained stable when 1 μM of riboflavin was added under anaerobic conditions. On the other hand, anodic current remained close to background levels (10 to 20 mA m^{-2}) when lactate was not supplied to MR-1 under either anaerobic or aerated conditions. The presence of 20 mg L^{-1} Cr(VI) totally inhibited anodic current production, and this was reflected in the high open circuit potential observed after phase 1 (+150 mV vs. -350 mV in the anaerobic reactor with lactate, -400 mV in the aerated reactors with lactate and -430 mV in the anaerobic reactor with 1 μM RF and lactate; potentials recorded are vs. Ag/AgCl and under N_2 flushing anaerobic conditions).

When MR-1 was aerated and the electrode was poised at -500 mV, oxygen electroreduction was inhibited, as a result of the heterotrophic oxygen consumption (Figure 4-36b). This inhibition occurred even in the absence of lactate and this is in accordance with the previous findings of Freguia et al. (2010) that carbon stored in the cells was enough to inhibit cathodic oxygen reduction.

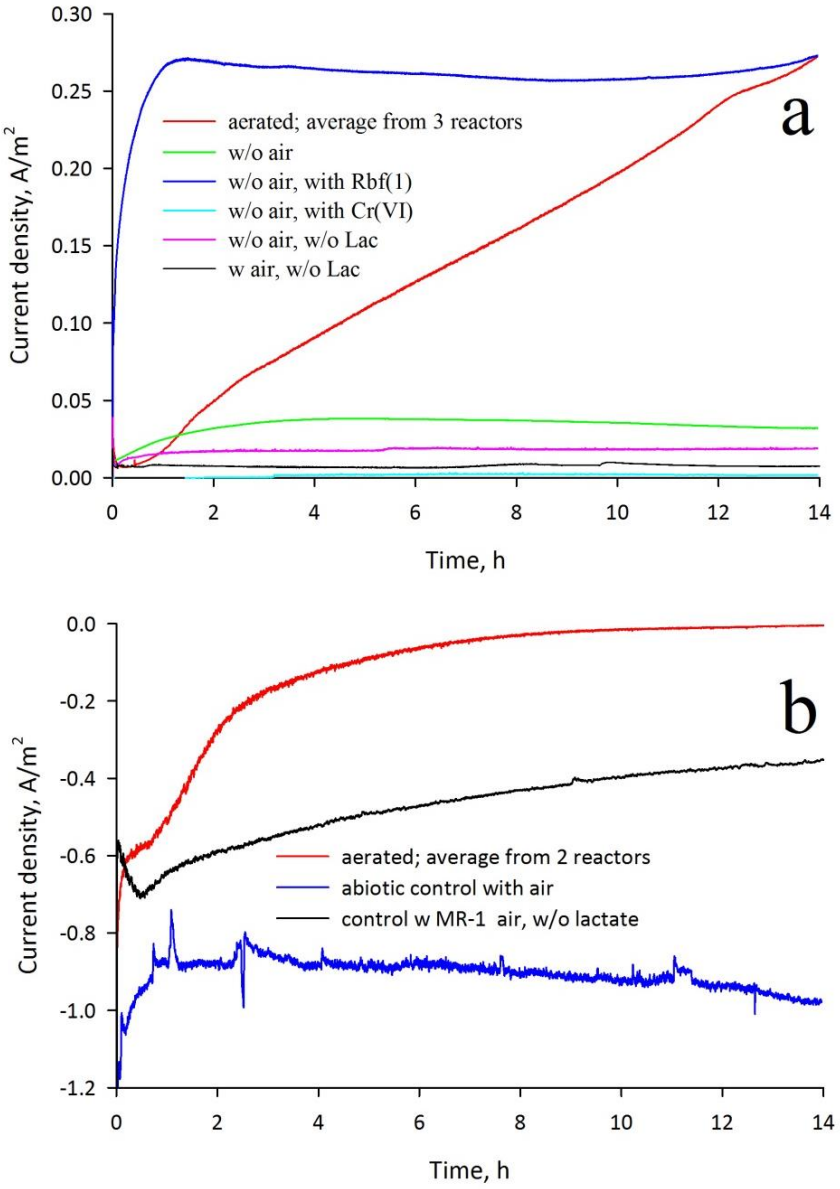

Figure 4-36: Chronoamperometry profiles at +300 mV (a) and at -500 mV (b) during phase 1 of the potentiostatically controlled experiments.

CV experiments at the end of phase 1 showed the onset of anodic current at -0.45 to -0.40 mV in the presence of MR-1 (Figure 4-37a), which is in agreement with the midpoint potentials of flavins (Marsili et al., 2008). Also, poising the electrode at +300 mV under aerated conditions during phase 1 gave CV graphs with higher anodic current than when the electrode was poised at -500 mV under aeration or when phase 1 was kept anaerobic (Figure 4-37b). Prolongation of the aeration step in the presence of the poised potential (+300 mV vs. Ag/AgCl) for 10 more hours gave a CV graph with considerably higher anodic catalytic current but with the same current onset (Figure 4-37b), indicating higher concentrations of the produced electrocatalysts

and probably a higher biofilm growth. Also, when an anaerobic reactor running with MR-1 and lactate for 14 h was supplied with 1 μM riboflavin, cyclic voltammograms gave almost the same catalytic current as the current of the reactors running with riboflavin since the beginning of the experiment (Figure 4-38). Finally, comparison of the first derivatives of the current produced during the cyclic voltammetry experiments, clearly shows that the compounds produced by MR-1 during aeration (either in flasks or in the bioelectrochemical reactors), gave catalytic currents at similar potentials (similar redox centres) with riboflavin (Figure 4-39).

As already discussed in Section 4.2.1.2 and will be emphasised later on in Section 4.3.3, the presence of such bacterial products proved useful during Cr(VI) electroreduction, where they were able to participate in reduction-oxidation cycles with Cr(VI) as the final electron acceptor. As shown later on in Section 4.3.3, MR-1 demonstrated the ability to enhance electroreduction of its own soluble metabolic products, even when lactate was present, and it is possible that these products could later participate in reduction-oxidation cycles with Cr(VI) as the final electron acceptor. From a metabolic point of view this possibility is remarkable, considering the interconnected reduction pathways for Cr(VI) and flavins.

Although the exact electron transfer pathway involved in Cr(VI) bioreduction is not yet clear, it is well known that the Mtr pathway plays a major role both in the reduction of flavins (Coursolle et al., 2010) and in enzymatic Cr(VI) reduction (Belchik et al., 2011). Also, Marsili et al. (2008) showed that (anodic) electron transfer was mediated by flavins even when a biofilm was formed on the electrode, emphasising the importance of this pre-treatment step (phase 1), where redox mediators are produced.

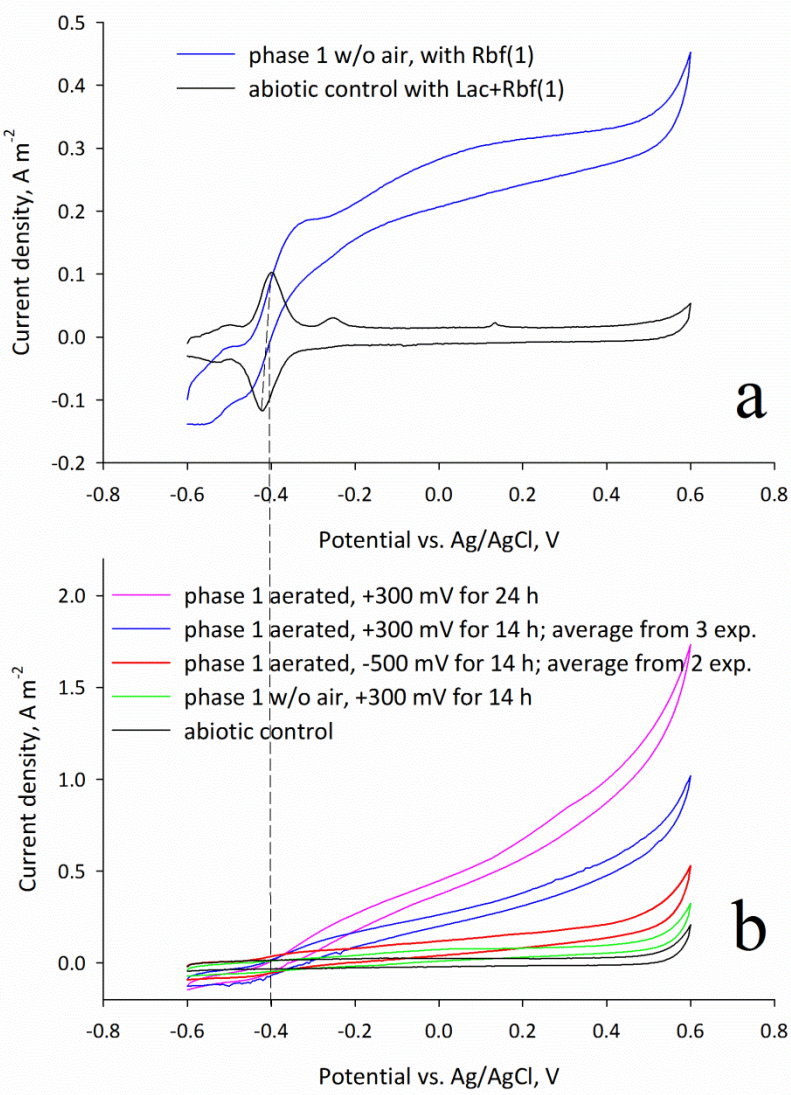

Figure 4-37: Cyclic voltammograms at the end of phase 1 of the potentiostatically controlled experiments. Scan rate is 5 mV sec^{-1}. (a) experiments with riboflavin addition; (b) mediatorless experiments with MR-1 under different pre-treatment conditions.

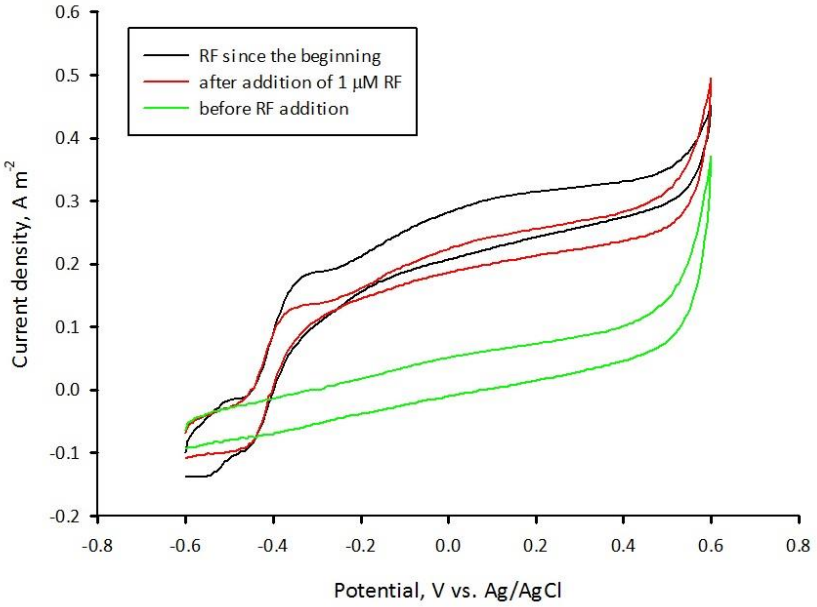

Figure 4-38: Cyclic voltammograms showing current production in a reactor before and after the addition of 1 µM riboflavin and in another reactor running with 1 µM riboflavin since the beginning of the experiment. Scan rate is 5 mV sec^{-1}.

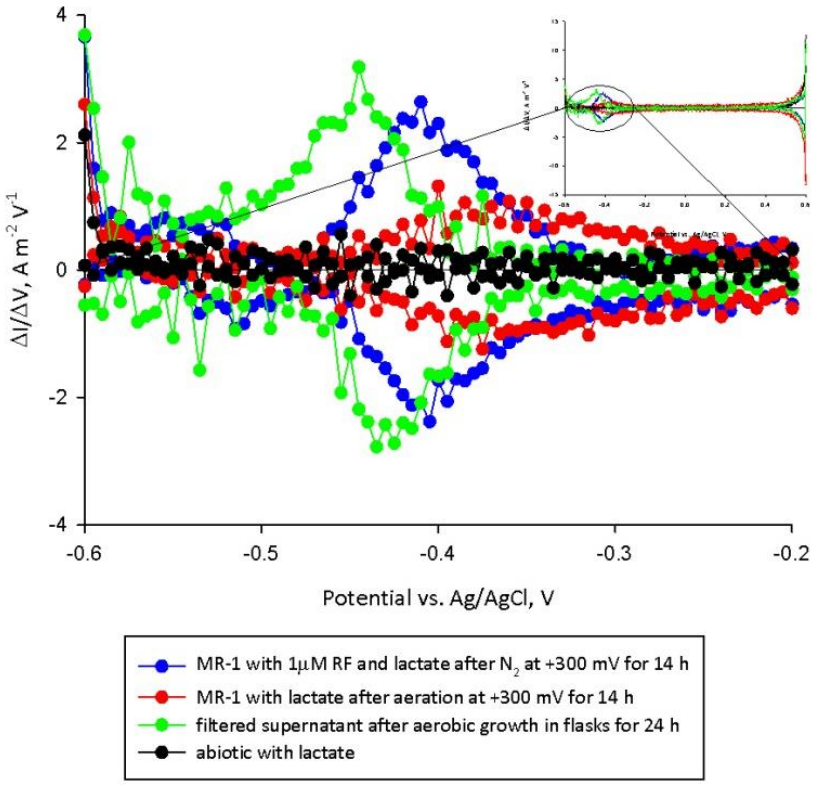

Figure 4-39: Comparison of the first derivatives of current produced during the cyclic voltammogram experiments conducted at the end of phase 1, before Cr(VI) addition. Scan rate is 5 mV sec^{-1}.

4.3.2.4. Current inhibition by Cr(VI) present in the anode

In the previous section, MR-1 has been shown to be capable of anodic current production even in the presence of an electron acceptor other than the electrode (oxygen). For Cr(VI) reducing MFCs however, it is important to evaluate if and how the presence of Cr(VI) in the anode can affect current production. This is important because under certain conditions, Cr(VI) can migrate to the anode, for example when using anion-exchange membranes or when applying MFC technology for Cr(VI) bioremediation of polluted sites.

In the previous section it was shown that when 20 mg-Cr(VI) L^{-1} was present in the anode, MR-1 was unable to produce anodic current when a poised potential was available as a potential electron acceptor (+300 mV vs. Ag/AgCl). However, Cr(VI) was provided since the beginning in that instance and therefore the Cr(VI) inhibition effect was not clearly shown. To demonstrate the effect of Cr(VI) supply in a chamber already operating as an anode, two dual-chamber MFCs (200 mL working volume in each chamber) were assembled, each with a cation-exchange membrane (CMI-7000), a 100 Ω load and MR-1 (OD_{600} 0.7 at the beginning) with lactate in the anode. Cr(VI) was added as the electron acceptor in the cathode (500 mg L^{-1}, pH 2) and the anode was flushed with N_2 for 30 min before operation. Subsequently, the two chambers were connected and the system was allowed to produce current for 20 h before the anode received Cr(VI) spikes, as shown in Figure 4-40. After each Cr(VI) spike in the anode, current was allowed to stabilise for several hours before the next spike. Lactate in the anode was kept at concentrations higher than 712 mg L^{-1} (8 mM) at all times (Figure 4-41), and pH in the anode was adjusted to 7 every 1-2 days to avoid pH in the anode dropping lower than 6.5.

As seen in Figure 4-40, current immediately dropped from 0.41 A m^{-2} to 0.11 A m^{-2} after the first 10 mg-Cr(VI) L^{-1} spike, which corresponds to a 73% drop. Current recovered slowly after complete Cr(VI) reduction but did not exceed 0.22 A m^{-2}, even after 2 d of operation. After the second spike, current dropped from 0.22 A m^{-2} to 0.08 A m^{-2}, corresponding to a 67% drop. After the current had stabilised, a third spike decreased current by 67% again, from 0.11 A m^{-2} to 0.04 A m^{-2}. After this drop, the current did not recover but stabilised to 0.04 A m^{-2}, even after the fourth spike and Cr(VI) concentration reduction to undetectable levels. As a consequence of current dropping each time, Cr(VI) reduction in the cathode proceeded at slower rates (Figure 4-42).

The immediate drop of current as a response to Cr(VI) additions in the anode could be due to the alternative electron acceptor present in the anode and the increase of the redox potential

in the anolyte. However, as demonstrated earlier, this was not the case when oxygen was continuously supplied in the anode, and a potential difference of around 660 mV between the anolyte and the catholyte should have remained present even after Cr(VI) addition in the anode (Nernst equation (Eq. 2.3)). In addition, the presence of an alternative electron acceptor in the anode would not explain the slow recovery (or the absence of any recovery) observed after Cr(VI) depletion. As an example, charge delivered by MR-1 during the first 24 h was 61 C; however, this dropped to only 37 C (including 11 C of charge delivered to 10 mg-Cr(VI) L^{-1}) during the second 24 h, after the first Cr(VI) addition. Current was not fully recovered after each Cr(VI) addition, probably indicating a reduced ability to utilise lactate for anodic current production after exposure to Cr(VI). Passivation of the electroactive components by Cr(III) precipitates on the bacterial cell surface could also be a reason for the reduced electroactivity of MR-1 in the anode, however this would not explain the inhibition effect observed in the previous section where the poised electrode and Cr(VI) were both provided since the beginning of the experiment. On the other hand, a minimum current of at least 0.04 A m^{-2} (80 μA) was sustained during all four Cr(VI) additions, indicating that MR-1 could (up to some minimal point) utilise both the electrode and Cr(VI) as the electron acceptors, under the conditions described in this paragraph.

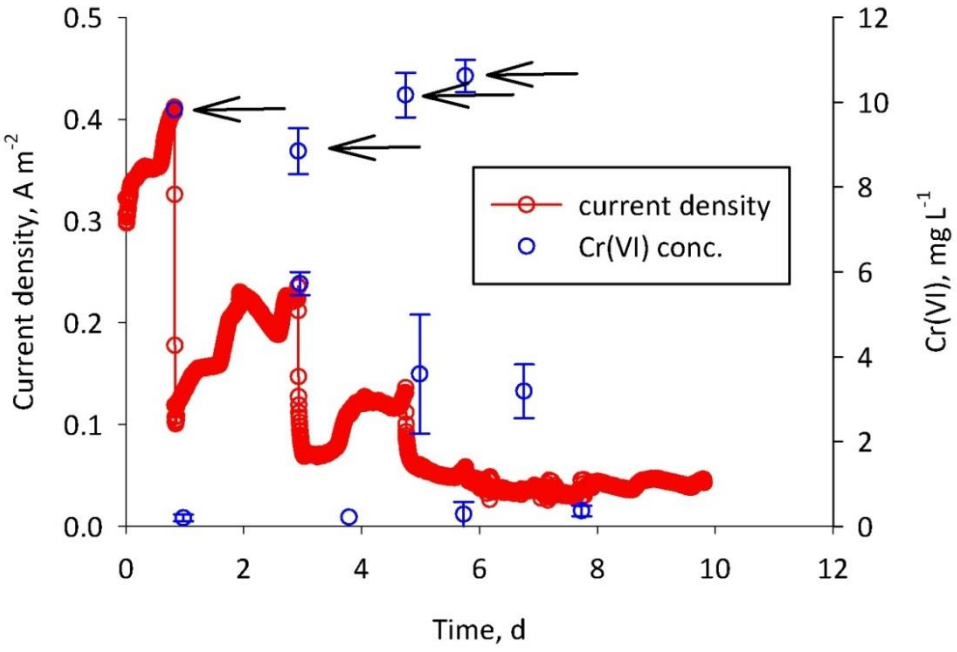

Figure 4-40: The effect of Cr(VI) presence in the anode; arrows indicate Cr(VI) spikes (average values from two reactors are reported; error bars represent min and max of the measurements). Electron donor concentrations are given in Figure 4-41.

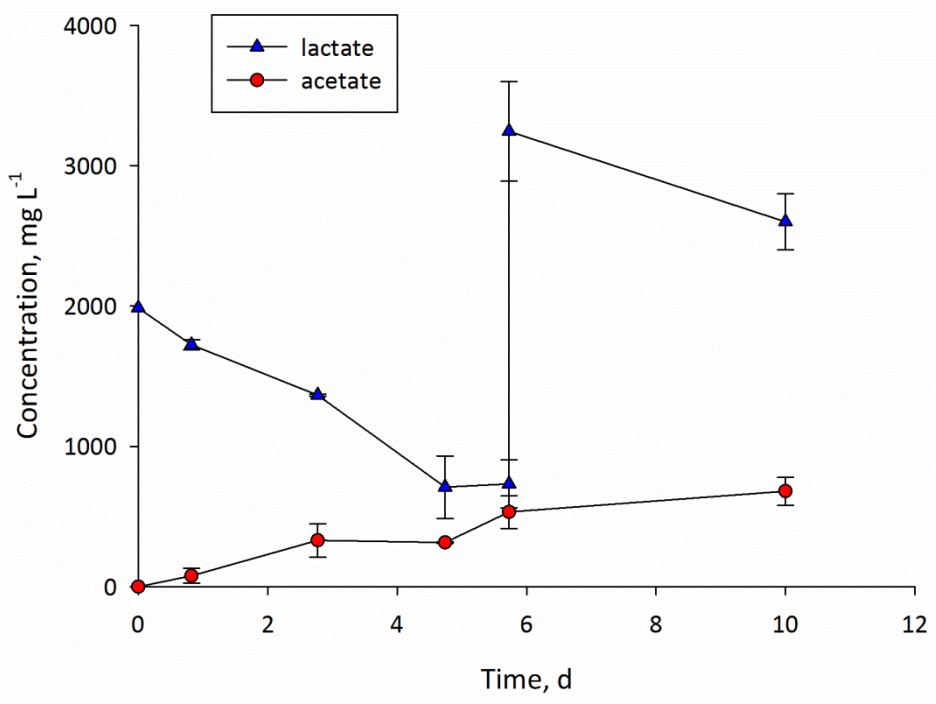

Figure 4-41: Lactate and acetate concentrations during the Cr(VI) inhibition test (average values from two reactors are reported; error bars represent min and max of the measurements).

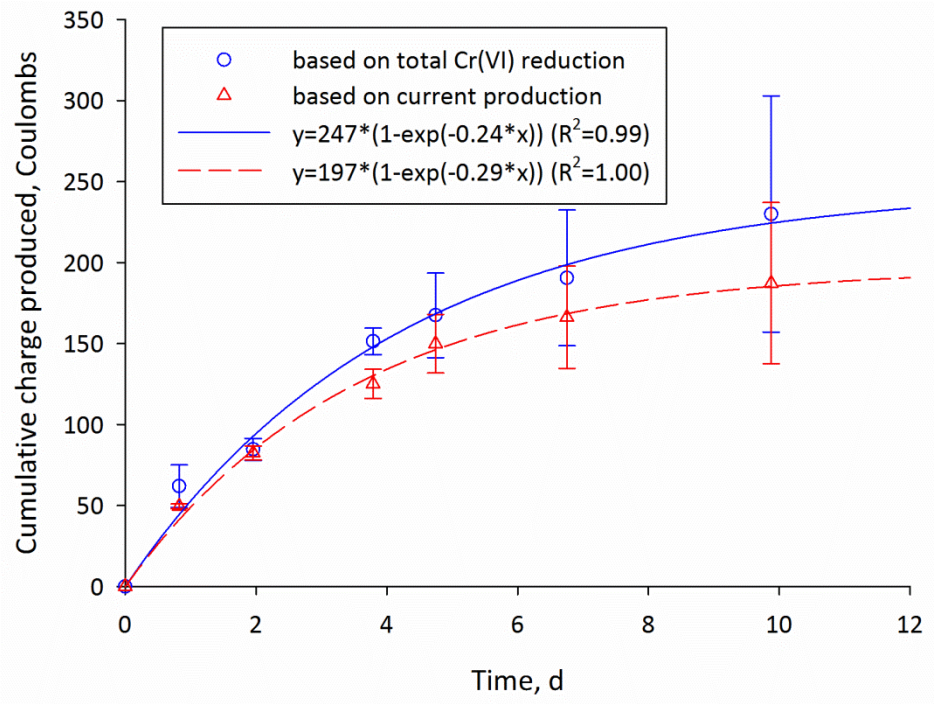

Figure 4-42: Charge produced during the Cr(VI) inhibition test (average values from two reactors are reported; error bars represent min and max of the measurements).

The anodic current inhibition effect was also demonstrated after sequential Cr(VI) additions in a single-chamber MFC. For this purpose, MR-1 was inoculated in a single-chamber reactor containing minimal medium and lactate (30 mM). The reactor additionally had two electrodes, one of them operating as the WE (+300 mV vs. Ag/AgCl) and the other one as the CE for a period of 23 h. Oxygen was not actively removed or added in this instance but MR-1 was allowed to consume the dissolved O_2 present in the medium. As seen in Figure 4-43 and in Figure 4-44, current was increasing for the duration of this period when MR-1 was allowed to form the biofilm on the electrode surface.

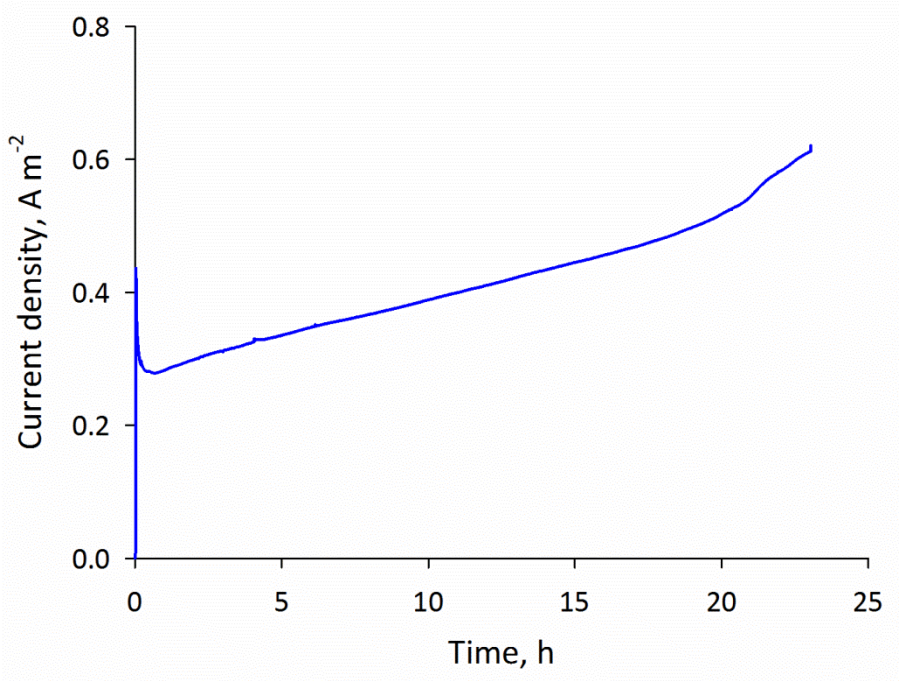

Figure 4-43: Anodic current during start-up in a single chamber reactor containing MR-1.

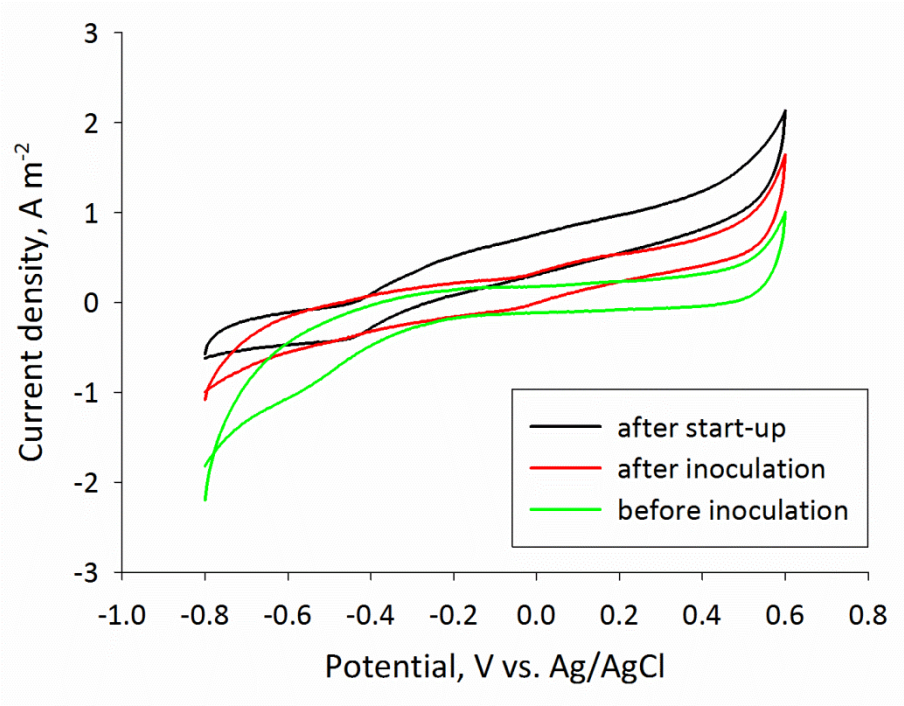

Figure 4-44: CV experiments in the single chamber cell before and after inoculation and after start-up. Scan rate is 5 mV sec^{-1}.

Following this initial 23 h period, the potentiostat was removed from the system, the two electrodes were connected with each other only via a 100 Ω resistor, and current flowing between the two electrodes was allowed to drop below 0.5 μA. Subsequently, 20 mg-Cr(VI) spikes were introduced into the system whilst current and potential of the ex-working electrode were monitored. As seen in Figure 4-45, a maximum initial current of 63 μA was produced after the first Cr(VI) spike, which however dropped after the second and third spikes. After the third spike and 25 h of operation, electron flow between the two electrodes totally collapsed. As seen in Figure 4-45 and Figure 4-46, current collapse was observed with a concurrent increase of the electrode potential up to -25 mV vs. Ag/AgCl. Whilst the electrode potential started decreasing again after the third spike, it remained a lot higher than during the previous two spikes. This effect was more apparent during the fourth spike, where the electrode potential almost remained constant for more than 80 h of operation.

As a result of this current inhibition, only a concentration of 0.8 mg-Cr(VI) L^{-1} (0.2 mg-Cr(VI)) was reduced via the bioelectrochemical pathway by the end of the operation period. Almost all of the Cr(VI) reduced was due to lactate-utilising pathways and the distinctive green Cr(III) precipitates surrounding the cells were observed (Figure 4-47).

Whilst O_2 consumption by heterotrophic metabolism is a main concern in air-cathode single-chamber MFCs, inhibition of the anodic lactate oxidation reaction seems to be the main concern when Cr(VI) is present in the anode. Therefore, more sophisticated remediation designs should be implemented in this case in order to minimise the presence of Cr(VI) in the vicinity of the anode electrode, when the use of a membrane separator is not economic or practical (e.g. groundwater remediation).

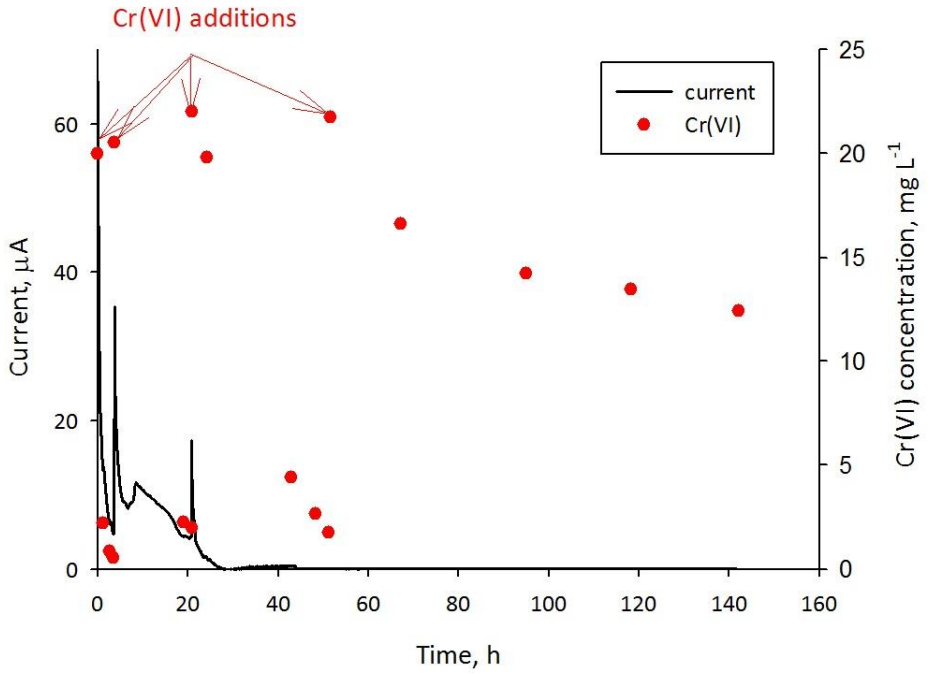

Figure 4-45: Current production and Cr(VI) reduction in the single-chamber cell under a 100 Ohm load.

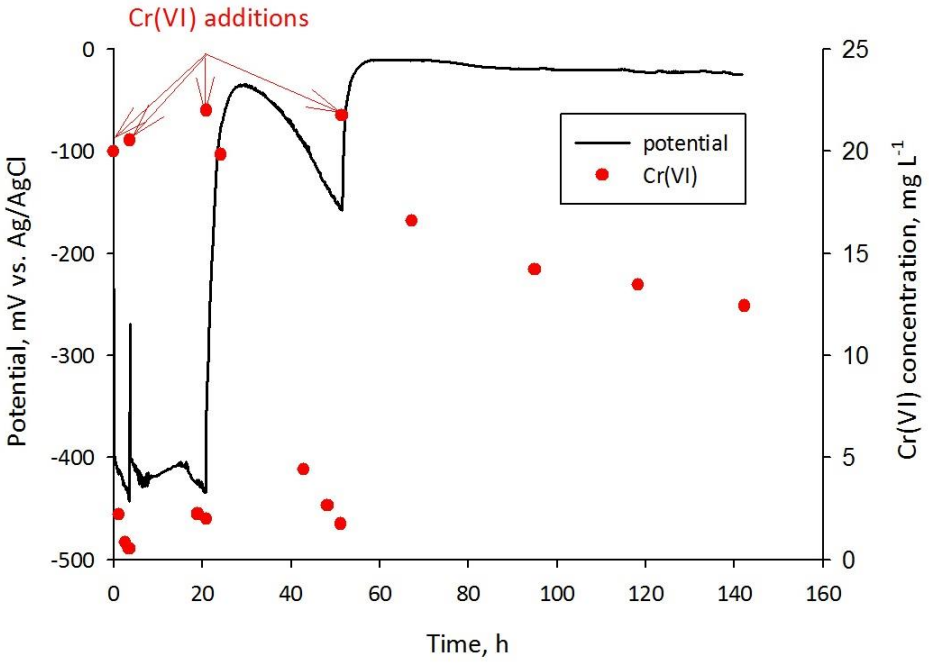

Figure 4-46: Cr(VI) reduction as in Figure 4-45 and potential measured at the ex-working electrode in the single-chamber cell.

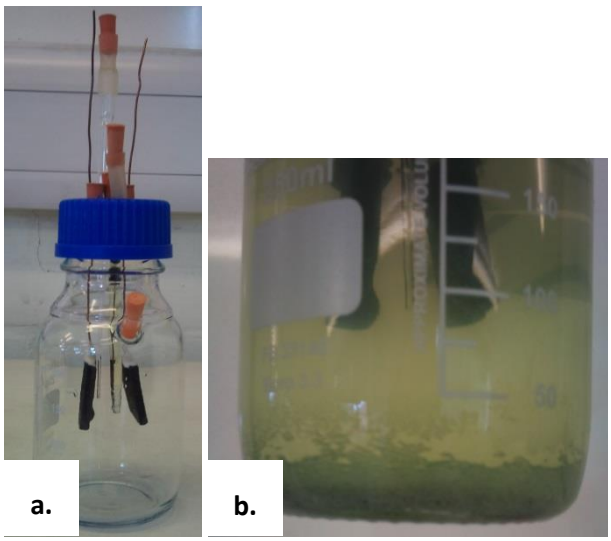

Figure 4-47: a) single chamber reactor assembly (250 mL total reactor volume), b) chromium-biomass precipitation in the reactor.

4.3.3. *Shewanella oneidensis* MR-1 as a cathodophilic biocatalyst

4.3.3.1. Introduction

Despite their significance, studies on the potential use of *Shewanellae* in biocathodes remain limited (Freguia et al., 2010, Hsu et al., 2012), and one reason for this could be the metabolic requirement for organic carbon. This requirement appears to contradict the established view that organic carbon use in the cathode must be minimised, in order to prevent oxygen consumption by heterotrophic metabolism that does not contribute to electrode oxidation (Freguia et al., 2008a, Rabaey et al., 2008). Whilst this view is well founded, it is worth studying the effect of the presence or absence of organic carbon, and particularly of lactate, which is a primary carbon source for *Shewanellae*, when Cr(VI) is used as the final electron acceptor (Viamajala et al., 2003). The presence of lactate may also offer potential benefits for the biocathode, including the production of new bacterial cells and the production and utilisation of useful bioelectrocatalysts (Coursolle et al., 2010, Marsili et al., 2008), but also the chelation of Cr(III) products, which tend to form on the electrode surface and deactivate it (Clark and McCreery, 2002, Hurley and McCreery, 2003). The aim of this study is to investigate the performance of Cr(VI)-reducing biocathodes when *Shewanella oneidensis* MR-1 is used as a catalyst in the presence or absence of lactate.

4.3.3.2. Preliminary experiments with MR-1 in the cathode

4.3.3.2.1. Cr(VI) as the final electron acceptor in the absence of lactate

4.3.3.2.1.1. Experimental plan

In an effort to operate a Cr(VI) reducing biocathode by following the established concept on Cr(VI) biocathodes, which excludes alternative electron donors like lactate from the cathode, MR-1 (OD_{600}=0.8) was inoculated in a potentiostatically controlled reactor that initially contained 1,860 mg L^{-1} (21 mM) lactate in the WE chamber. The WE chamber was maintained anaerobic by continuous N_2 flushing and the electrode was initially poised at +300 mV vs. Ag/AgCl during a start-up period of 5 d. The purpose of this start-up period was to let the culture acclimatise to the anaerobic conditions and to start utilising the electrode for its respiration needs. After this start-up period and when lactate could no longer be detected, the electrode potential was reversed to -500 mV vs. Ag/AgCl and Cr(VI) was spiked into the WE chamber. After 4 d in the presence of Cr(VI), the potential was reversed again to +300 mV vs. Ag/AgCl and lactate was again added into the system (Figure 4-48).

4.3.3.2.1.2. Results and discussion

As can be seen in Figure 4-48, anodic current density remained at 0.08 A m^{-2} during the first 2 d of operation, which gradually dropped as lactate was being consumed. After 5 d of operation, lactate was totally consumed, electrode polarity was reversed to -500 mV vs. Ag/AgCl for the electrode to act as the electron donor and Cr(VI) was spiked at an initial concentration of 6 mg L^{-1}. As seen in Figure 4-48, Cr(VI) reduction was slow (average of 0.3 mg-Cr(VI) reduced per day for a period of 4 d) and did not seem to be related to cathodic current generation. Cathodic current produced was negligible and close to the background current normally observed and extracted from the graphs produced during potentiostatic experiments (5-10 µA or 0.003 to 0.006 A m^{-2}).

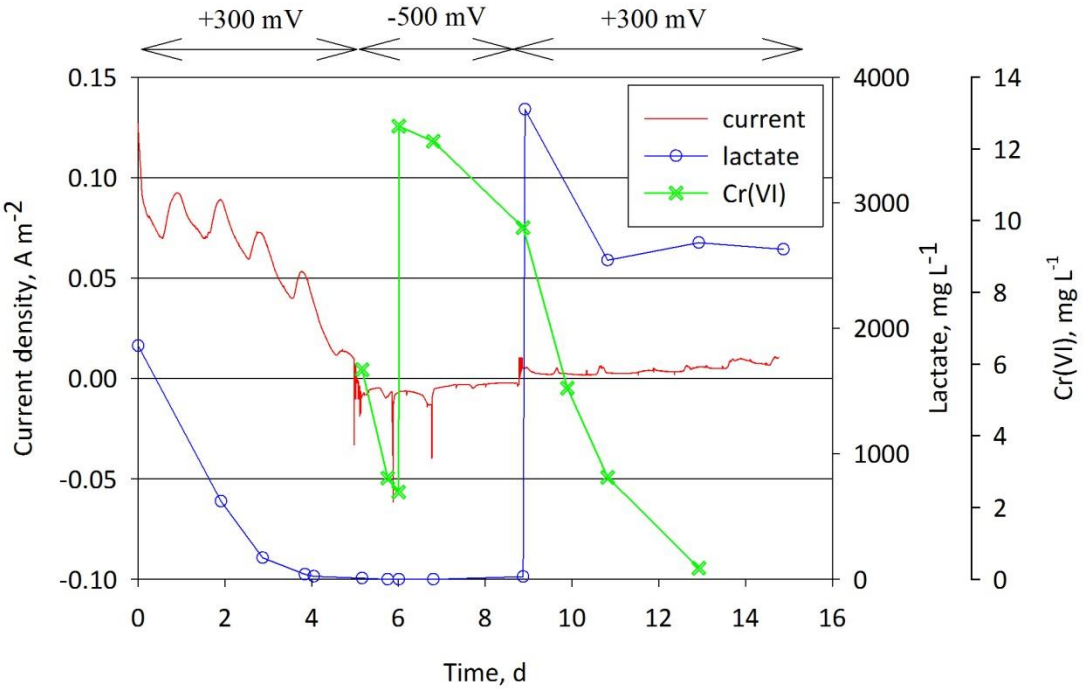

Figure 4-48: Current evolution, lactate and Cr(VI) concentration in a half-cell reactor with MR-1 (polarity reversal from +300 to -500 and back to +300 mV vs. Ag/AgCl as indicated by the arrows); raw current data presented without extraction of background current.

Acetate is produced during lactate oxidation (430 mg L^{-1} acetate was present at the time of the first polarity reversal), however its potential to prevent electrode oxidation should be considered low, as MR-1 is not known to oxidise acetate under anaerobic conditions (Lovley, 1991, Lowe et al., 2003, Lanthier et al., 2008). Cell lysis and carbon stored in the bacterial cells should have mainly provided the energy needed for the Cr(VI) reduction observed during the

time the WE was constantly poised at -500 mV vs. Ag/AgCl (approximately 8 C of charge were transferred to Cr(VI) during that 5 d period).

When the electrode's polarity was again restored to +300 mV on day 9 and lactate was added, Cr(VI) reduction increased (average of 0.7 mg-Cr(VI) reduced per day for the first 2 d), however current was not restored back to its previous levels. A number of reasons could be responsible for this effect, including the shock caused to the system after lactate depletion and also the inability of MR-1 to utilise lactate as an electron donor after exposure to Cr(VI), as already discussed in Section 4.3.2.4. Lower activity levels were also observed in a previous study conducted on *Shewanella oneidensis* by Lanthier et al. (2008), when an MFC system initially operating with lactate, was operated with acetate as the only electron donor for a period of 10 days. When lactate was restored in the system, a lower current production was observed compared to the initial current production with lactate, and that according to the authors, was due to the lower activity of the bacterial cells during the incubation with acetate.

4.3.3.2.2. Reduction of MR-1 redox mediators in the presence of lactate

4.3.3.2.2.1. Experimental plan

As a conclusion from above and also from numerous other similar experiments that were conducted in the lab and gave similar results with no considerable Cr(VI) electroreduction rates, a different strategy was decided to be applied in Cr(VI) reducing biocathodes. In order to study whether MR-1 is capable of oxidising the electrode with Cr(VI) as the final electron acceptor, the electrode deactivation phenomenon had to be minimised. Therefore, the new strategy should involve the use of a metal chelator. Lactate was selected for this purpose, as also discussed in Section 4.2.1.1, due to its natural abundance, its low toxicity and also its use in numerous studies with Cr(VI) reduction by *Shewanella*.

For this reason, MR-1 was initially tested for its ability to oxidise the electrode by utilising its own electroactive compounds as the terminal electron acceptors, in the presence of lactate. In order to study this phenomenon, the following experiment was conducted. After initial growth in Luria-Bertani-50 mM phosphate buffer medium (pH 7.0), MR-1 was harvested by centrifugation (5,000 x g, 4 ^{o}C, 20 min), rinsed twice with 100 mM NaCl-50 mM PBS solution (pH 7.0), and resuspended in 2 × 100 mL of the minimal medium, which was amended to contain 30 mM Na-Lactate. The MR-1 containing flasks were then incubated in an orbital shaker incubator (25 ^{o}C, 200 rpm) for 24 h. After 24 h, the flasks' content was centrifuged and then the supernatant was filter-sterilised (0.2 μM pore size) and added into the WE chamber

of a potentiostatically controlled cell for cyclic voltammetry and chronoamperometry analysis. Also, MR-1 was harvested and resuspended in a flask with 200 mL of fresh minimal medium. Both media were flushed with N_2 for 30 minutes before the start and continuously afterwards and stirring was applied at 250 rpm only during the chronoamperometry experiment and between the cyclic voltammetry experiments, as already described in Chapter 3.

4.3.3.2.2.2. Results and discussion

The chronoamperometry experiment at -500 mV vs. Ag/AgCl (Figure 4-49) showed initial current production in the absence of oxygen, which reached 0.16 A m^{-2} during the first 30 minutes of operation and remained considerably stable for 30 minutes afterwards. This initial current shows that soluble compounds released in the medium by MR-1 remained in the oxidised form by the end of the agitation period, during which oxygen was constantly supplied. When MR-1 (1 mL) was added, an increased current production was observed, which reached 0.3 A m^{-2} within the next hour and remained almost stable for 1 hour afterwards. This increased current production upon MR-1 addition indicated the bio-catalysed reduction of soluble compounds released in the medium by MR-1 during flask growth, and probably also of membrane-associated electroactive compounds added in the medium with MR-1. It should be noted that this bio-electro-catalysis observed was independent of the presence of 4 mM lactate in the solution, clearly showing that MR-1 can utilise the electrode as an electron donor to reduce these compounds even when lactate is present. Finally, current started decreasing by the end of the operation period, indicating complete reduction of the electron acceptor.

The cyclic voltammogram before MR-1 addition (Figure 4-50) revealed a clear cathodic current, which became more apparent at electrode potentials lower than -200 mV vs. Ag/AgCl and which had an increasing rate that reached its maximum at -430 mV vs. Ag/AgCl. After MR-1 addition and reduction of the potential electron acceptors present in solution, the cyclic voltammograms clearly revealed two formal potentials, at -20 mV vs. Ag/AgCl and at -430 mV vs. Ag/AgCl. Whilst the later can be directly attributed to the production of riboflavin and mediated electron transfer, the former, which also had a broad potential window, could be attributed to membrane-related proteins responsible for direct electron transfer.

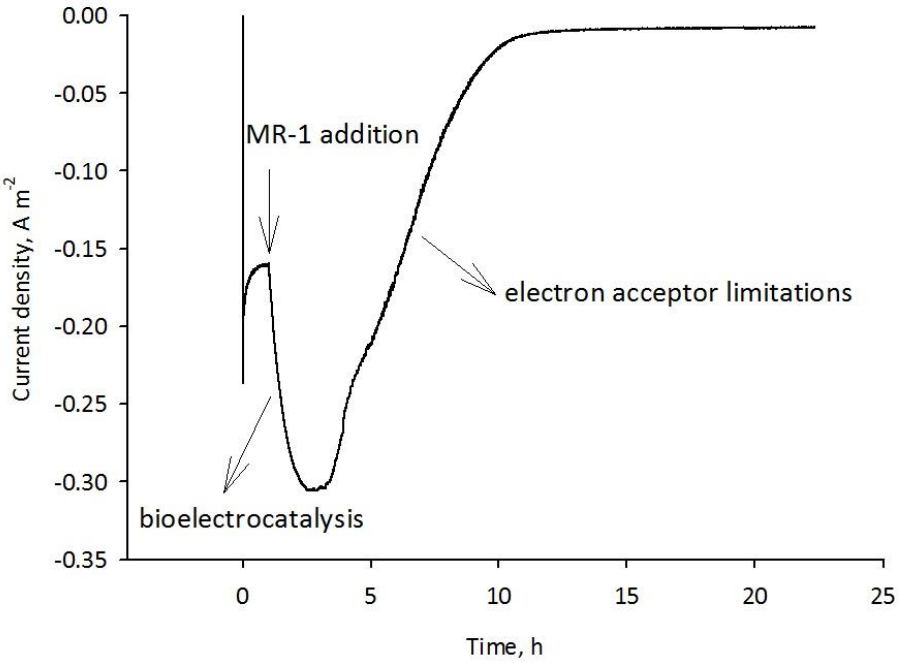

Figure 4-49: Chronoamperometry profile (-500 mV vs. Ag/AgCl) of the flask's filtered supernatant- effect of MR-1 addition.

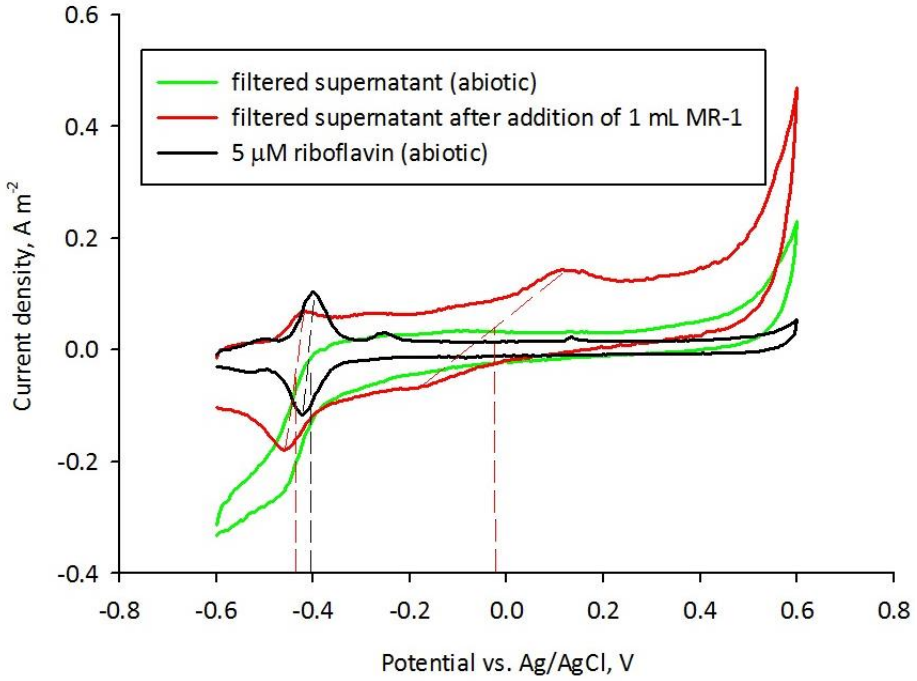

Figure 4-50: Cyclic voltammograms of the filtered supernatant before and after MR-1 addition and chronoamperometry experiment. Dashed lines indicate the midpoint potentials of redox active species present in the solution; midpoint potentials close to -0.4 V vs. Ag/AgCl indicate the presence of flavins. Scan rate is 5 mV sec^{-1}.

4.3.3.3. Cr(VI) reduction in potentiostatically poised cells

4.3.3.3.1. Experimental plan

Potentiostatically controlled chronoamperometry experiments were conducted in two phases: in the start-up phase (phase 1), which has already been discussed in Section 4.3.2.3, MR-1 was tested for its ability to produce anodic current, and in the main phase (phase 2) Cr(VI) was spiked and the reactor was allowed to produce cathodic current for a 4 h period. As discussed earlier, the WE was maintained at either -500 mV vs. Ag/AgCl (notated as $C(-El)^{ph1}$) or +300 mV vs. Ag/AgCl (notated as $C(+El)^{ph1}$) during phase 1, for 14-18 h after introduction of the MR-1 cell culture into the chamber. Generally, WE chambers operated in the presence of lactate, under aerated conditions and without riboflavin or Cr(VI) during phase 1, except if indicated by annotation.

Phase 2 of most experiments was conducted in the same reactors as phase 1. An exception was made for the reactors notated as $(-Lac)^{ph2}$ or $(-Shew)^{ph2}$, where phase 2 was lacking the lactate-containing supernatant of phase 1, or the MR-1 cells respectively. To separate the supernatant from MR-1, the respective WE chamber contents from phase 1 were centrifuged at 5,000 × g for 20 min and at 4^{o}C. In the reactors notated as $(-Lac)^{ph2}$ this centrifugation step was followed by rinsing of the MR-1 pellet twice with PBS solution to remove lactate and metabolites produced in phase 1. The MR-1 pellet was then resuspended in a clean reactor using the original electrodes from phase 1. In the reactors notated as $(-Shew)^{ph2}$ the phase 1 supernatant was additionally filtered through 0.2 μm filters to remove the MR-1 cells, in order to determine whether metabolites released in the medium could have a catalytic effect during Cr(VI) reduction. In the $C(+El)^{ph1}(-Lac+RF(1))^{ph2}$ cell, 1 μM riboflavin was added into the WE chamber before the beginning of phase 2 in order to investigate whether MR-1 could still express electrochemical activity in the absence of lactate but in the presence of its redox mediator.

All WE chambers operated under continuous N_2 flushing during phase 2, and all except that of $C(+El\text{-}Air+Cr(VI))^{ph1}$, were flushed with N_2 for one extra hour between phases 1 and 2, whilst current was allowed to stabilise to background levels at -500 mV vs. Ag/AgCl. This current-stabilisation step was omitted in $C(+El\text{-}Air+Cr(VI))^{ph1}$, however, as Cr(VI) was present from phase 1 in this reactor. Background current was normally less than 7 mA m^{-2}, and the value recorded was deducted from the experimental results.

Table 4-4: Experimental conditions applied in potentiostatically controlled experiments with MR-1.

Cell #	Phase 1	Phase 2 (-500 mV, Cr(VI) and N_2)
C(+El)^{ph1}	30 mM lactate, air, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (20 mM lactate)
C(+El)^{ph1}(-Lac)^{ph2}	30 mM lactate, air, 14 h, +300 mV (common phase 1 for reactors C(+El)^{ph1}(-Lac)^{ph2} and C(+El)^{ph1}(-Shew)^{ph2})	MR-1 from phase 1 resuspended in another reactor with the same electrode (no lactate)
C(+El)^{ph1}(-Shew)^{ph2}		supernatant of phase 1 filtered (0.2 μm) and resuspended in another reactor (17 mM lactate)
C(-El)^{ph1}	30 mM lactate, air, 14 h, -500 mV	MR-1 in the same reactor as in phase 1 (25 mM lactate)
C(-El)^{ph1}(-Lac)^{ph2}	30 mM lactate, air, 14 h, -500 mV (common phase 1 for reactors C(-El)^{ph1}(-Lac)^{ph2} and C(-El)^{ph1}(-Shew)^{ph2})	MR-1 from phase 1 resuspended in another reactor with the same electrode (no lactate)
C(-El)^{ph1}(-Shew)^{ph2}		supernatant of phase 1 filtered (0.2 μm) and resuspended in another reactor (23 mM lactate)
C(+El)^{ph1}(-Lac+RF(1))^{ph2}	30 mM lactate, air, 14 h, +300 mV	MR-1 from phase 1 resuspended in another reactor with the same electrode and 1 μM riboflavin (no lactate)
C(+El-Air)^{ph1}	30 mM lactate, N_2, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (28 mM lactate)
C(+El-Air)^{ph1}(+RF(1))^{ph2}	30 mM lactate, N_2, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (29 mM lactate) with 1 μM riboflavin added before phase 2
C(+El-Air+RF(1))^{ph1}	30 mM lactate, 1 μM riboflavin, N_2, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (28 mM lactate)
C(+El-Air+Cr(VI))^{ph1}	30 mM lactate, Cr(VI), N_2, 18 h, +300 mV	MR-1 in the same reactor as in phase 1 (25 mM lactate)
C(+El-Air-Lac)^{ph1}	N_2, 14 h (no lactate), +300 mV	MR-1 in the same reactor as in phase 1 (no lactate)

4.3.3.3.2. Results and discussion

As seen earlier in Section 4.3.2.3, oxygen enhanced anodic current production during phase 1, probably due to the higher energy obtained by MR-1 during complete lactate oxidation under aerated conditions. A thicker biofilm that could be visually observed in this instance (Figure 4-51) and a presumably higher production of redox mediators, which exhibited (anodic) current onsets at potentials similar to the midpoint potentials of flavins, were also possible reasons for this enhanced performance. Furthermore, MR-1 demonstrated the ability to enhance electroreduction of its own soluble metabolic products even when lactate was present and it is possible that these products could participate in reduction-oxidation cycles with Cr(VI) as the final electron acceptor during phase 2.

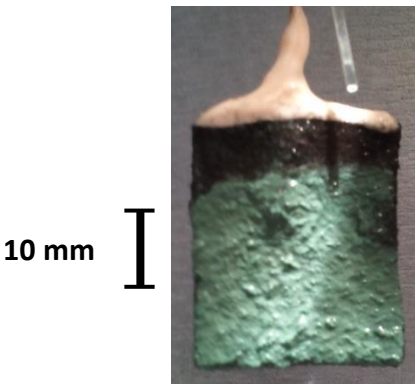

Figure 4-51: MR-1 biofilm on the electrode surface after aerated incubation during phase 1 and treatment with Cr(VI) during phase 2; green Cr(III) precipitates are visible on the biofilm's surface.

When MR-1 was continuously supplied with oxygen in the presence of lactate and a poised electrode at +300 mV during phase 1 ($C(+El)^{ph1}$), dosing with Cr(VI) gave a lower initial current response than in the absence of MR-1 (Figure 4-52a). Current was sustained for longer in $C(+El)^{ph1}$, however, probably due to the bacterial energy-gaining processes and the abiotic redox cycles promoted by electron shuttle mediators. The charge produced in $C(+El)^{ph1}$ by the end of the 4 h period was 5.5 and 1.7 times more than in the abiotic control without lactate and in the abiotic control with 30 mM lactate (Figure 4-52c). Also the current produced in $C(+El)^{ph1}$ at the end of the experiment (52 mA m^{-2}) was higher than that in the abiotic control without lactate (1 mA m^{-2}) and in the abiotic control with 30 mM lactate (9 mA m^{-2}), despite the fact that $C(+El)^{ph1}$ had a considerably lower Cr(VI) concentration (11 mg L^{-1} Cr(VI)) compared to the abiotic control without lactate (19 mg L^{-1} Cr(VI)) and to the abiotic control with 30 mM lactate (17 mg L^{-1} Cr(VI)) (Figure 4-52b). Although cathodic Cr(VI) reduction was

observed in C(+El-Air+Cr(VI))^{ph1}, the charge produced was still lower than that of C(+El)^{ph1} (Figure 4-52c). In this instance, it has previously been shown that even if electron mediators can be produced when the electron acceptor is in soluble form, they are not essential for *Shewanella*'s respiration (von Canstein et al., 2008). Also, the production of redox mediators can be downregulated under Cr(VI) exposure (Chourey et al., 2006) and this could explain the performance of C(+El-Air+Cr(VI))^{ph1}.

C(+El-Air)^{ph1} showed lower cathodic activity during phase 2, probably as a result of the limited electrophilic activity of the MR-1 cells during phase 1. MR-1 obtained less energy from lactate during phase 1 in this instance and thus cells occupying the electrode surface might have become less active. However, process was enhanced upon addition of riboflavin (either before phase 1: C(+El-Air+RF(1))^{ph1} or before phase 2: C(+El-Air)^{ph1}(RF(1))^{ph2}), when 4 mg L^{-1} more Cr(VI) was reduced (Figure 4-53b) and 1.9 times more charge was produced (Figure 4-53c) compared to C(+El-Air)^{ph1}.

This process enhancement seen in C(+El)^{ph1} (aerobic pre-treatment) compared to C(+El-Air)^{ph1} (anaerobic pre-treatment) and C(+El-Air+Cr(VI))^{ph1} (anaerobic pre-treatment with Cr(VI)) indicates that cathodic electrochemical activity can be enhanced when oxygen is supplied during the pre-treatment step, under the conditions described. The charge produced in both C(+El-Air)^{ph1} and C(+El-Air+Cr(VI))^{ph1} during the 4 h test period was similar to that in the abiotic control with 30 mM lactate (Figure 4-52c). However, the current in C(+El-Air)^{ph1} and C(+El-Air+Cr(VI))^{ph1} (32 mA m^{-2} and 40 mA m^{-2}) was approximately 4 times that in abiotic control with 30 mM lactate towards the end of test, despite the lower Cr(VI) concentrations in the MR-1 containing chambers (Figure 4-52).

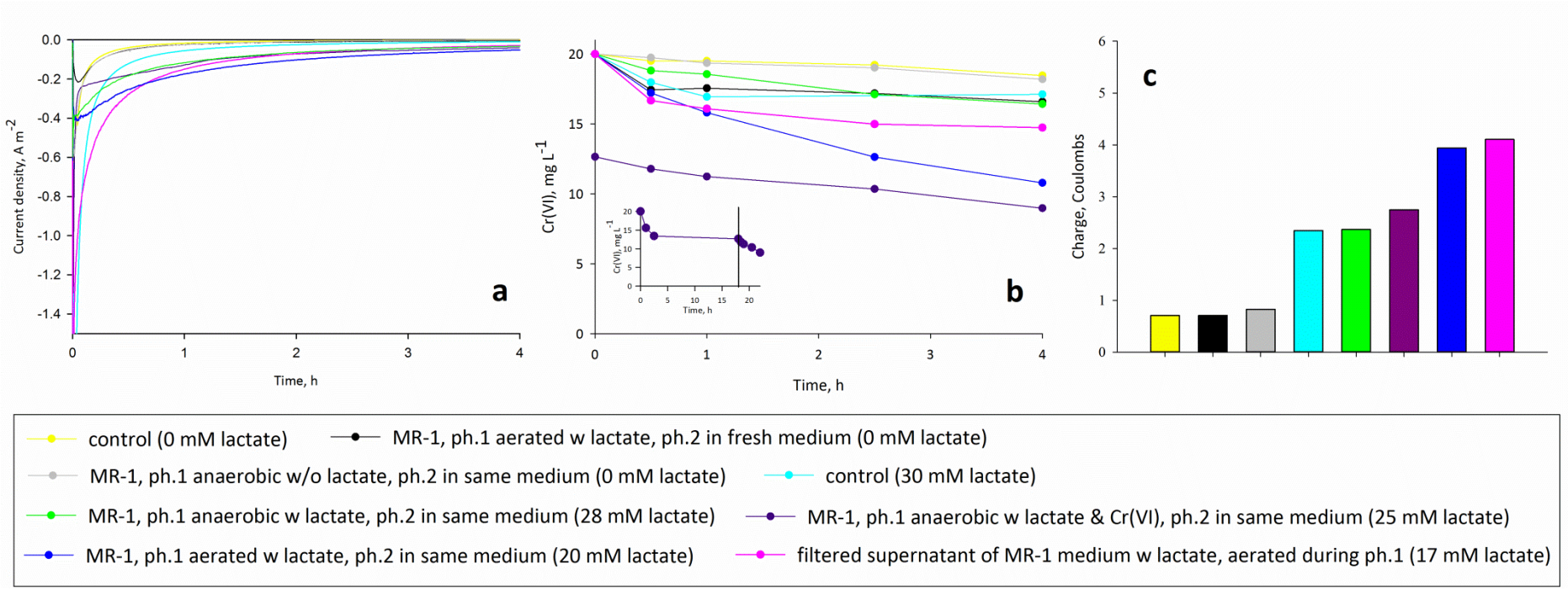

Figure 4-52: Current production (a), Cr(VI) reduction [inset: Cr(VI) reduction by MR-1 throughout both phases 1 and 2] (b) and charge production by the end of the 4 h operation period (c) in mediatorless cathodes (-500 mV); a potential of +300 mV vs. Ag/AgCl was applied during phase 1 in all MR-1 experiments presented in this graph.

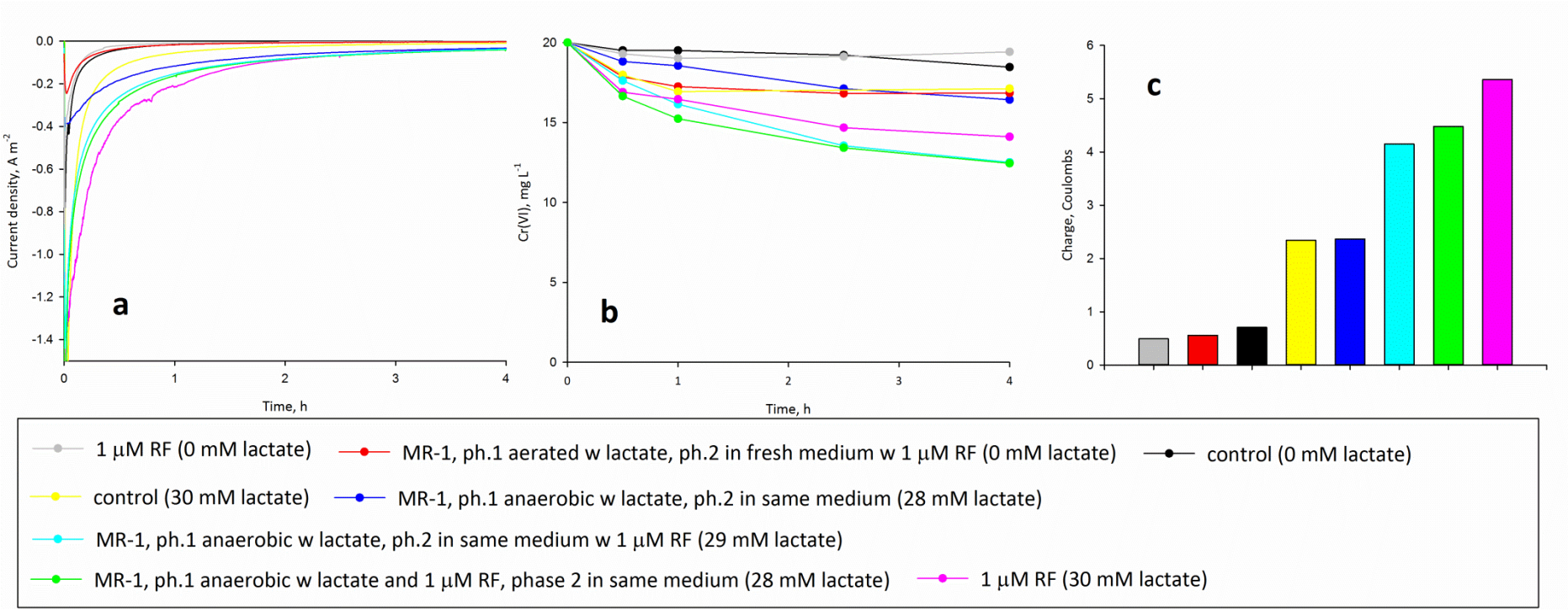

Figure 4-53: Current production (a), Cr(VI) reduction (b) and charge production by the end of the 4 h operation period (c) in cathodes with MR-1 and/or riboflavin (-500 mV); a potential of +300 mV vs. Ag/AgCl was applied during phase 1 in all MR-1 experiments presented in this graph.

Reactor C(+El)^{ph1} produced 15% more charge than that produced by C(-El)^{ph1} (-500 mV vs. Ag/AgCl with air during phase 1) by the end of the 4 h operation period. Also, C(+El)^{ph1}(-Shew)^{ph2} produced 32% more charge than C(-El)^{ph1}(-Shew)^{ph2} (Figure 4-54, Table 4-5). This can be linked to the enhanced anodic current showed in the CV tests after phase 1, in the experiments where both oxygen and the electrode were supplied as the electron acceptors during phase 1, compared to the experiments where the electron acceptor was more limited (C(-El)^{ph1}, C(-El)^{ph1}(-Lac)^{ph2}, C(-El)^{ph1}(-Shew)^{ph2}, C(+El-Air)^{ph1}) (Figure 4-37). In addition, when *Shewanella* was also present in planktonic form and lactate was present (e.g. C(+El)^{ph1} and C(-El)^{ph1} reactors), Cr(VI) was also reduced due to reasons other than current production (e.g. lactate utilisation) (Figure 4-54).

When lactate was absent, limited current and charge were produced regardless of the electrode potential during phase 1. However, 2 times more Cr(VI) was reduced in total when the electrode was also an electron acceptor during phase 1, compared to when only oxygen was the electron acceptor. This could be an indication that more biomass (planktonic and biofilm) was produced in the first instance, where MR-1 could obtain more energy for its metabolic needs. It should also be noted that more lactate was consumed during phase 1 in this instance (12 mM), compared to the second instance (6 mM). On the one hand, this indicates more biomass growth in the C(+El)^{ph1} experiments. On the other hand, this fact shows that more charge was produced in the lactate containing C(+El)^{ph1} experiments even at lower lactate concentrations when lactate was not as strong a chelator, most probably due to a combination of higher redox mediator concentrations and also higher electroactivity of MR-1.

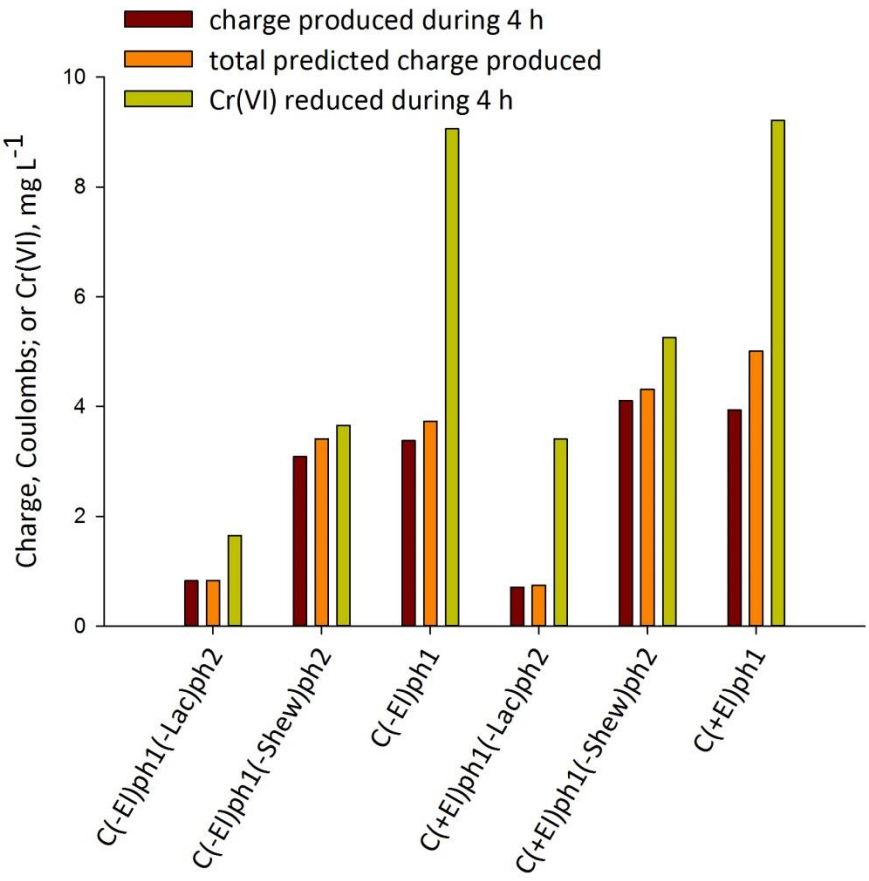

Figure 4-54: The effect of applied potential during start-up: charge produced and Cr(VI) reduced after 4 h, and maximum predicted charge produced calculated from the regression equations presented in Table 4-5.

Table 4-5: Regression parameters for the charge produced (based on current production), after MR-1 incubation at +300 mV or at -500 mV vs. Ag/AgCl. Equations are in the form y=a*(1-exp(-b*x)+c*(1-exp(-d*x), where y is cumulative charge produced (C), x is time (h), "a" and "c" are parameters representing a maximum possible charge produced for x→∞ (C), and "b" and "d" are parameters representing how fast the maximum possible charge produced can be approached (h-1).

Cell #	Equation (y=)	R^2
C(-El)^{ph1}(-Lac)^{ph2}	0.40*(1-exp(-9.88*x))+0.43*(1-exp(-0.86*x))	1.00
C(-El)^{ph1}(-Shew)^{ph2}	0.90*(1-exp(-4.70*x))+2.51*(1-exp(-0.50*x))	1.00
C(-El)^{ph1}	1.15*(1-exp(-4.57*x))+2.58*(1-exp(-0.49*x))	1.00
C(+El)^{ph1}(-Lac)^{ph2}	0.35*(1-exp(-3.94*x))+0.39*(1-exp(-0.65*x))	1.00
C(+El)^{ph1}(-Shew)^{ph2}	1.34*(1-exp(-5.18*x))+2.97*(1-exp(-0.64*x))	1.00
C(+El)^{ph1}	0.96*(1-exp(-1.82*x))+4.05*(1-exp(-0.33*x))	1.00

Figure 4-55 shows the relationship between current produced and Cr(VI) reduced with the electrode as the initial electron donor. This relationship is important because of the Cr(III) inhibition phenomenon explained earlier in Section 4.1, which explains why current is decreased with increasing Cr(VI) reduced. In general, because of this effect, higher current for the same amount of Cr(VI) electrochemically reduced would indicate higher electrocatalytic activity in the WE chamber. However, the lower concentrations of lactate in some instances should also be taken into account. As shown in Figure 4-55, more current continues to be produced for the same amount of Cr(VI) bioelectrochemically reduced, in the case where MR-1 and artificially added riboflavin were both present in the medium. On the other hand, anaerobic incubation of MR-1 without riboflavin had the poorest performance, as it gave lower current values for the same amount of Cr(VI) bioelectrochemically reduced and at relatively high lactate concentrations (28 mM).

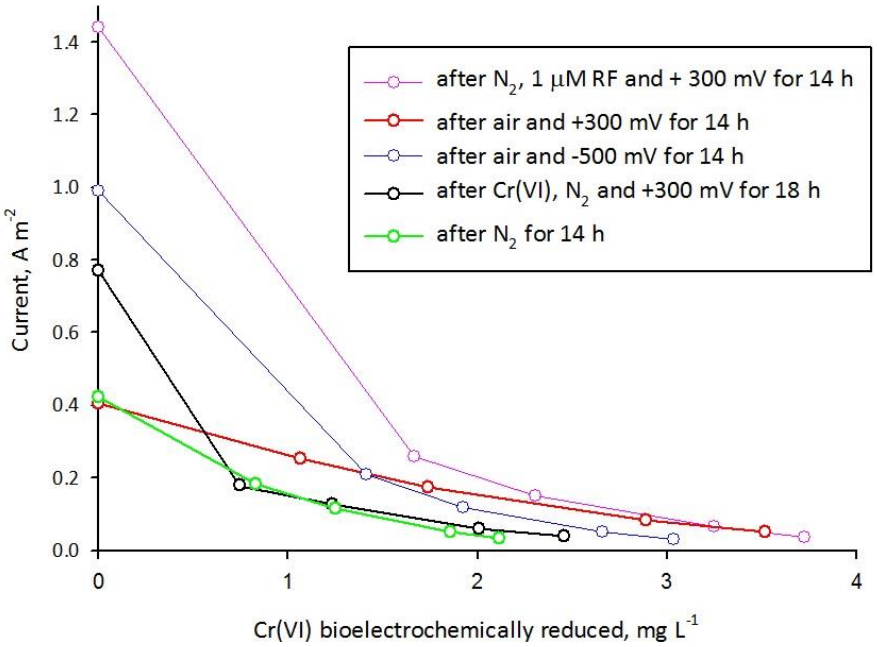

Figure 4-55: Relationship between current produced and Cr(VI) reduced due to the electrode for five different MR-1 incubation conditions; from left to right, points represent 5 different times: 0.0 h, 0.5 h, 1.0 h, 2.5 h and 4.0 h.

In total, regarding the six experiments described above (at -500 mV and +300 mV vs. Ag/AgCl during start-up), 7.3 Coulombs were produced during the 4 h operation period and after pre-treatment at -500 mV, compared to 8.8 Coulombs (+21%) after pre-treatment at + 300 mV. In addition, 14.4 mg-Cr(VI) L^{-1} was reduced during the 4 h operation period and after pre-treatment at -500 mV, compared to 17.9 mg-Cr(VI) L^{-1} (+24%) after pre-treatment at + 300 mV.

The CV experiments at the end of phase 2 provide further evidence that the pre-treatment conditions play an important role in the catalytic activity of MR-1 (Figure 4-56). The CV results showed that MR-1 had an active catalytic role in $C(+El)^{ph1}$, as it provided cathodic current over a broader potential range, not only when compared to abiotic control with 30 mM lactate and 1 µM riboflavin (Figure 4-57), but also to $C(-El)^{ph1}$ and $C(+El\text{-}Air)^{ph1}$ (Figure 4-56). As will also be discussed in Section 4.3.3.4, catalytic activity of the biocatalyst over a broad potential range is important for power-producing applications such as MFCs, where imposed overpotentials will increase the energy losses of the system. As can be seen in Figure 4-58, the abiotic control with riboflavin and lactate gave a clear catalytic current at low applied potentials (<-400 mV), and similar patterns were also observed in the filtered supernatants (0.2 µm filtered supernatant after MR-1 pre-treatment with air for 14 h). This indicates that even in the absence of active cells, compounds released in the medium are responsible for Cr(VI) electroreduction; and is in

agreement with previous studies where compounds released in the medium by Shewanella were found to be able to catalyse oxygen electroreduction (Biffinger et al., 2011, Freguia et al., 2010, Liu et al., 2012), but also in agreement with the findings already discussed in Section 4.2.1.2.

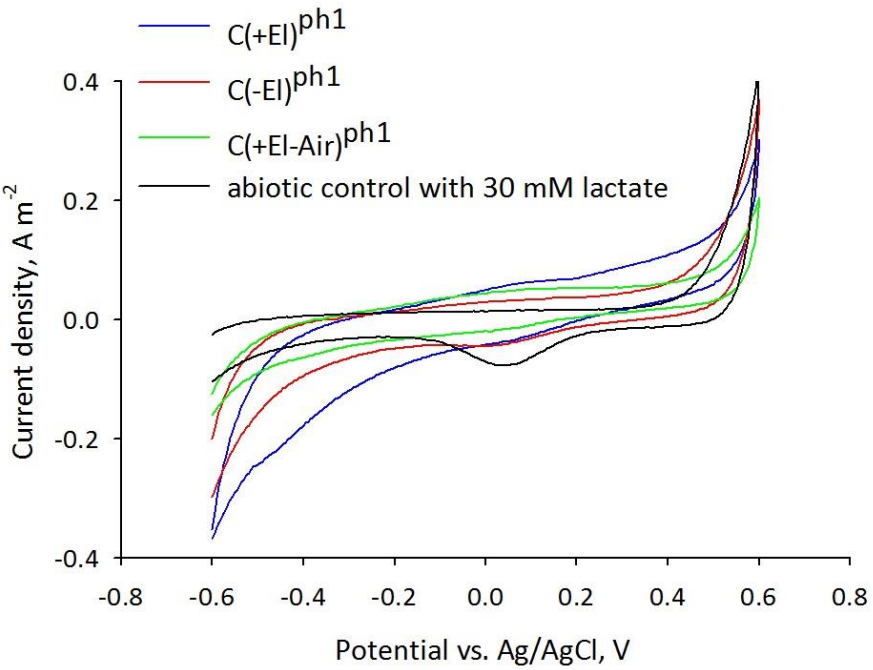

Figure 4-56: Cyclic voltammograms at the end of phase 2. Comparison between $C(+El)^{ph1}$, $C(-El)^{ph1}$, $C(+El\text{-}Air)^{ph1}$ and abiotic control with 30 mM lactate, showing the importance of the pre-treatment conditions. Scan rate is 5 mV sec^{-1}.

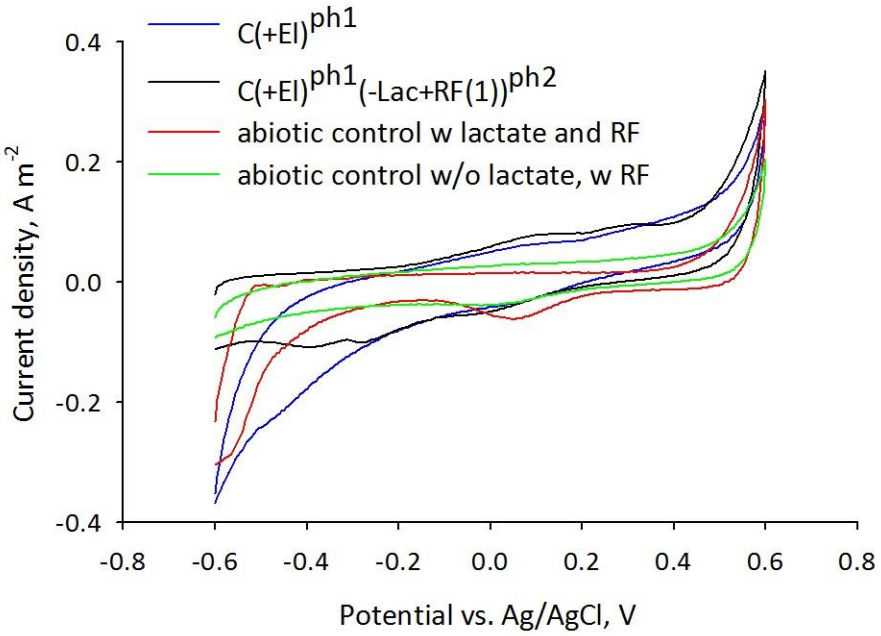

Figure 4-57: Cyclic voltammograms at the end of phase 2. Comparison between $C(+El)^{ph1}$, $C(+El)^{ph1}(-Lac+RF(1))^{ph2}$, abiotic control with 30 mM lactate and 1 μM riboflavin and abiotic control with only 1 μM riboflavin, showing the importance of lactate on the expression of catalytic activity. Scan rate is 5 mV sec^{-1}.

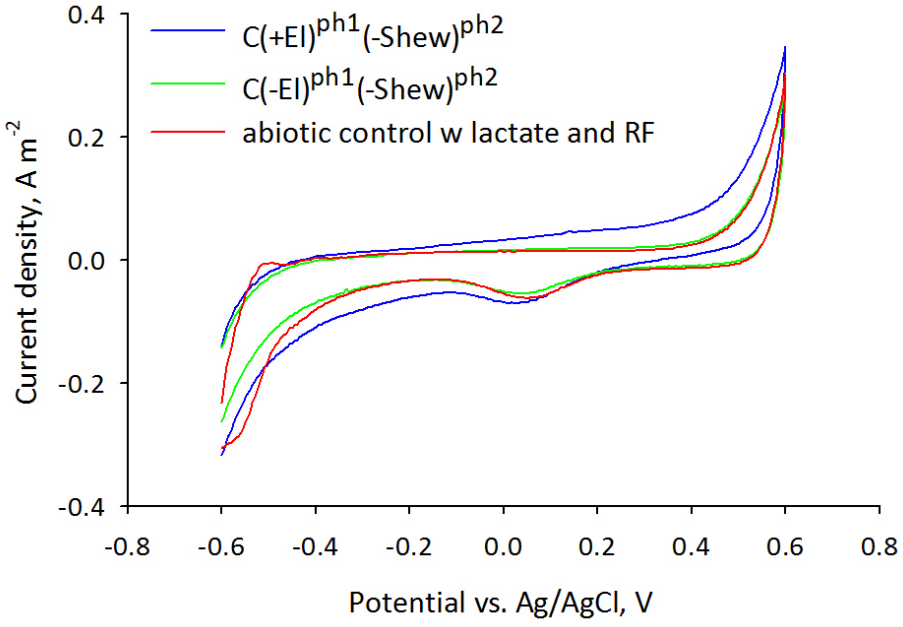

Figure 4-58: Cyclic voltammograms at the end of phase 2. Comparison of the filtered supernatants $C(+El)^{ph1}(-Shew)^{ph2}$ and $C(-El)^{ph1}(-Shew)^{ph2}$ with the abiotic control with 30 mM lactate and 1 μM riboflavin. Scan rate is 5 mV sec^{-1}.

4.3.3.4. Cr(VI) reduction in microbial fuel cells

4.3.3.4.1. Experimental plan

The experiments described in Section 4.3.3.3 showed that an improved catalytic activity of MR-1 could be achieved by manipulation of culture conditions: this demonstration, however, was carried out under energy-consuming potentiostatic control. The efficiency of power-producing devices such as MFCs is restricted by internal resistances and potential losses (Rabaey and Verstraete, 2005) and therefore the conditions applied in the potentiostatically controlled experiments needed to be verified in MFCs. For this purpose, MFCs were tested for their current producing and Cr(VI) reducing efficiency under different operational conditions. As the primary purpose was not to maximise power production, the same simple H-type reactor configuration was used, which had a relatively small ion exchange membrane area and low electrode TASA to volume ratio.

Five MFCs were assembled and tested, each with different cathode conditions. The cathode of the MFC notated as "None" only contained MM and 10 mg L^{-1} Cr(VI), whilst the other four cathodes additionally contained: 30 mM lactate ("+Lac"), 30 mM lactate and 1 µM riboflavin ("+Lac+Rbf"), 30 mM lactate with MR-1 and Cr(VI) since phase 1 ("+Lac+Cr(VI)^{ph1}+Shew"), and 30 mM lactate with MR-1 ("+Lac+Shew").

Anodes in MFCs "None", "+Lac", "+Lac+Rbf" and "+Lac+Cr(VI)^{ph1}+Shew" were inoculated with MR-1 and left overnight with air continuously flushed (160 mL min^{-1}), until the anodic potential under open circuit conditions dropped and stabilised at -470 mV vs. Ag/AgCl. In the cathode N_2 was flushed overnight and then continuously during the experiment. For the MFC experiment "+Lac+Cr(VI)^{ph1}+Shew", MR-1 was added in the cathode and was left overnight with 30 mM lactate and 15 mg-Cr(VI) L^{-1}, until Cr(VI) concentration was approximately 11 mg L^{-1}. After stabilisation, the circuits were closed using 1,000 Ω external resistors and Cr(VI) was spiked (time zero), except for the "+Lac+Cr(VI)^{ph1}+Shew" MFC where Cr(VI) was already available in the cathode. In the bioanodes, air continued to be flushed for the duration of the experiment and lactate was supplied at intervals to ensure electron donor availability and to promote stable anodic potentials.

The MFC experiment "+Lac+Shew" was conducted in two phases. In the start-up phase 1, chamber 1 (Ch1) of +Lac+Shew contained 30 mM lactate and was inoculated with MR-1. MR-1 was then allowed to produce anodic current (1,000 Ω external resistor) for 3 d, whilst air was continuously flushed into both the anode (Ch1) and the abiotic cathode (Ch2) which only

contained MM. After phase 1, Ch2 was inoculated with MR-1 and lactate was added in both chambers to give a 30 mM initial concentration. Also, Ch1 was continuously flushed with N_2 and the system was allowed to equilibrate for 2 h until the current dropped to background levels (<4 μA) (Figure 4-59). At this point Cr(VI) was spiked into Ch1 to give an initial concentration of 10 mg L^{-1}, whilst Ch2 continued to be flushed with air to test whether Cr(VI) has an advantage over oxygen as an electron acceptor when both MR-1 and lactate are present. From the time point when Cr(VI) was inserted into Ch1, current flow reversed, with Ch1 acting as the biocathode and Ch2 as the bioanode (indicating more oxidative conditions in Ch1 than in Ch2). Additional doses of 10 mg L^{-1} Cr(VI) were given at intervals to ensure the presence of Cr(VI) in the cathode at all times.

Drops in potential across the 1,000 Ω resistors and anodic potentials vs. Ag/AgCl RE were recorded every 1 min. Polarisation curves for "+Lac+Shew" were obtained after repeating this experiment up to the first Cr(VI) spiking step and allowing open circuit voltage to evolve after spiking. Then, the electrical circuit was again closed and current values were obtained, with resistances gradually decreasing from 16,300 Ω to 7 Ω. Sufficient time (approximately 4 min) was given between each resistor change in order to achieve a relatively stable current production.

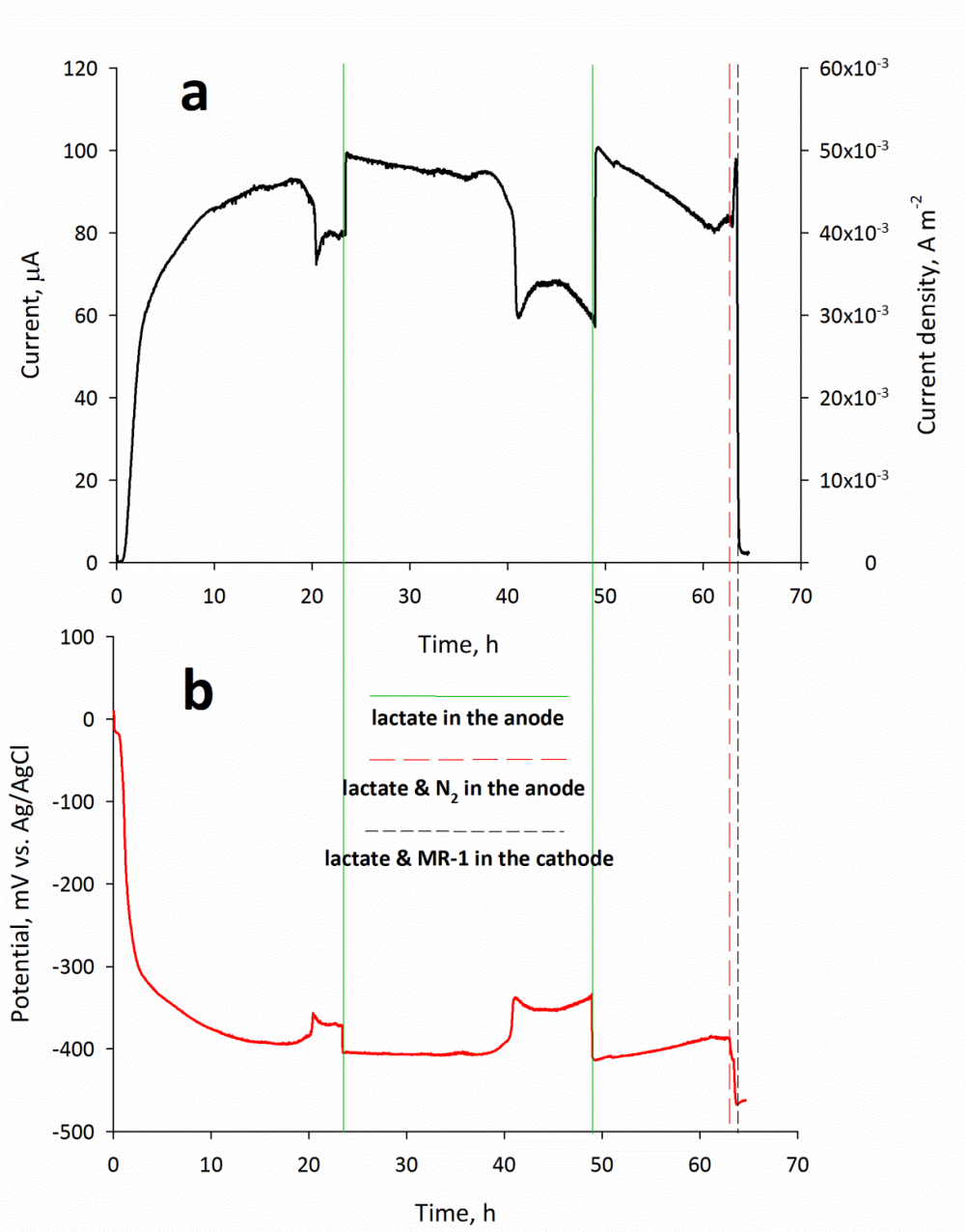

Figure 4-59: Pre-treatment (phase 1) of the MFC "Lac+Shew", where "anode" and "cathode" indicate anode and cathode during phase 1 (reversed for phase 2 with Cr(VI)). a) cell current production ($R_{ext.}$=1,000 Ω) and b) anodic potential of Ch1.

4.3.3.4.2. Results and discussion

All MFC anodes were operated under aerated conditions and had comparable anodic potentials under closed circuit conditions throughout operation: -447±10 mV in "None", -446±17 mV in "+Lac", -450±13 mV in "+Lac+Rbf", -445±10 mV in "+Lac+Cr(VI)^{ph1}+Shew" and -432±28 mV in "+Lac+Shew". Generally, this indicates that the availability of the electron donor

was not limiting the process (See Section 4.3.2.2 for the effect of the absence of lactate on the anodic potential) and therefore the observed differences in performance were mainly due to the limitations in the cathode.

MFC "None" which operated without lactate and MR-1 in the cathode, produced an initial current of 84 μA; the system stopped operating within the first few hours, however, although there was still 9 mg L^{-1} Cr(VI) available (Figure 4-60). This behaviour was similar to that of the potentiostatically controlled cathodes without lactate, as discussed previously. MFC "+Lac" which contained 30 mM lactate in the cathode achieved an initial current of 145 μA, which dropped to background levels (4 μA; 0.3 mg L^{-1} d^{-1} of Cr(VI) reduced assuming 100% cathodic efficiency) by the end of the 48 h operation period, with 43% of Cr(VI) still in solution. MFC "+Lac+Rbf", with an additional 1 μM riboflavin, produced an initial current of 153 μA. Its performance was not significantly better than that of "+Lac", however, as the addition of riboflavin only enhanced the process at low applied potentials (see Section 4.2.1.2) which are difficult to achieve in MFCs. Also, only a negligible increase in current production was observed after a further increase in Cr(VI) concentration to 10 mg L^{-1} in both "+Lac" and "+Lac+Rbf" (Figure 4-60). This indicates the dominating effect of Cr(III) precipitates, which probably formed and deactivated the cathodes.

MFC "+Lac+$Cr(VI)^{ph1}$+Shew", which contained MR-1 and 29 mM lactate at the beginning of phase 2, produced an initial current of 154 μA (Figure 4-60). This initial current however, which included both Faradaic and non-Faradaic current, dropped faster than the controls described above. Cr(VI) reduction during the 48 h operating period was similar to the controls (6.1 mg-Cr(VI) L^{-1}), however this included both electrode (3.7 mg-Cr(VI) L^{-1}) and non-electrode (2.4 mg-Cr(VI) L^{-1}) utilising pathways. As seen earlier in Section 4.3.3.3, anaerobic pre-treatment of MR-1 in the presence or absence of Cr(VI) gave similar current production as the control experiment with lactate. In the MFC case presented here, current production was lower than the control experiments and this could be the result of inactive MR-1 cells occupying the cathode electrode. As discussed earlier, the bacterial cells have limited options for energy gaining in MFCs, since they have limited ability to utilise the produced flavins for electrode oxidation. Therefore, any biofilm occupying the electrode surface in this instance would most probably act as a barrier rather than a transporter of the electrons to Cr(VI).

On the other hand, despite the lower initial value of 65 μA (32.5 mA m^{-2} TASA), current was produced by MFC "+Lac+Shew" (MR-1 biocathode) after every Cr(VI) addition (Figure 4-61). The maximum current produced by "+Lac+Shew" occurred after the first addition and was

comparable to the 46.6 mA m^{-2} observed by Tandukar et al. (2009), who used a mixed culture biocathode with a similar configuration (electrode TASA to working volume ratio: 16 m^2 m^{-3} [vs. 340-9,600 m^2 m^{-3} in other studies (Huang et al., 2010, Huang et al., 2011a, Huang et al., 2011b)], $Cr(VI)_0$: 22 mg L^{-1}, Rext.: 1,000 Ω). Current production in "+Lac+Shew" responded immediately to every Cr(VI) addition even though air was continuously supplied to the anode: this clearly indicates that Cr(VI) has an advantage over oxygen for electroreduction when organic carbon is provided in the biocathode. Even though MR-1 can cause low redox conditions and inhibit oxygen electroreduction within a few hours when lactate is available, this did not seem to be the case for Cr(VI) where MR-1 used both lactate and the electrode as the electron donor for Cr(VI) reduction.

However, when MR-1 (OD_{600}= 0.6) was spiked with a single spike of a high 100 mg-Cr(VI) L^{-1} concentration, current was not sustained as it was for the "+Lac+Shew" MFC (Figure 4-62, Figure 4-63, Table 4-6), and the electrode did not perform differently than the "+Lac" MFC electrode. This clearly indicates that Cr(VI) concentration is important for bioelectrochemical reduction in MFCs and high Cr(VI) concentrations can inhibit this particular Cr(VI) reduction pathway utilised by *Shewanella*.

In total, 3.5 mg Cr(VI) were reduced by the cathode electrode of "+Lac+Shew" by the end of the MFC operation, compared to the maximum Cr(VI) reduction of 1.2 mg Cr(VI) by the "+Lac" MFC and by the "+Lac+Shew" MFC with the one-off 100 mg-Cr(VI) L^{-1} spike (Figure 4-63, Table 4-6). In the absence of an electrode, Cr(VI) reduction was considerably slowed down after the first 3-4 days, as already discussed in Section 4.3.1, regardless of the biomass concentration and the presence or absence of lactate. In the "+Lac+Shew" MFC a similar plateau was observed for the pathways other than electrode oxidation, which accounted for only 1.1 mg Cr(VI) reduced within the last 240 h of operation. In the reactor where a one-off 100 mg-Cr(VI) L^{-1} spike was added, the Cr(VI) reduction routes that did not involve electrode oxidation did not seem to be inhibited as the electrode oxidation route was. This could be an indication of the different Cr(VI) reduction pathways that might be utilised by *Shewanella* cells when the electron donor is in the poised electrode form rather than in the lactate form.

The relatively slow decrease of the Cr(VI) bio-electroreduction rate in "+Lac+Shew" on one hand, along with the rapidly decreasing rate of Cr(VI) reduction due to lactate utilisation and other pathways on the other hand, were the reasons why an increase in cathodic efficiency was observed, from 14% (1st addition) to 64% (5th addition) and to 81% when the cathode was switched to potentiostatic control at -500 mV (6th addition) (Figure 4-64). By the end of

the operation, Cr(VI) bio-electroreduction accounted for 46% of the total Cr(VI) reduction in "+Lac+Shew", clearly showing that MR-1 could utilise both the electrode and lactate as an electron donor.

Whilst it is well established that mixed culture biocatalysts will yield higher current and power densities than pure cultures (Kan et al., 2011), the "+Lac+Shew" MFC produced a maximum of 1.7 mW m^{-2} (Figure 4-65). However, its Cr(VI) reducing performance was considerably higher compared to the performance of the only other study which investigated Cr(VI) reduction in an MFC biocathode with MR-1 as the biocatalyst (Hsu et al., 2012). In contrast to the current study, the biocathodes used in the Hsu et al. experiments were supplied with fumarate as an electron acceptor in a biocathode inoculated with MR-1 as part of a pre-treatment step. When the electron acceptor was switched to Cr(VI), the overall cathodic efficiency in the system was 67%, which decreased to 42% in the last 3 days of a 9-day operation period. According to the authors, this cathodic efficiency decrease was due to the unavoidable Cr(VI) reduction by other electron sources (presumably dead cells and succinate, the metabolic by-product of fumarate), towards the end of the operation period. It is possible that electrode fouling by Cr(III) hydroxides over time was one of the reasons for this metabolic shift when MR-1 started utilising electron donors other than the electrode. In the present study, although the external resistor was 100 times higher (1,000 Ω vs. 10 Ω) compared to that used by Hsu et al. (2012), the electrode in the "+Lac+Shew" cathode reduced considerably higher amounts of Cr(VI) than the one in Hsu et al. (2012), i.e. 1,750 mg m^{-2} in 8 days vs. an estimated 87 mg m^{-2} in 9 days. Considering the total Cr(VI) reduction in the biocathodes, i.e. Cr(VI) reduced by electrical current and other pathways, 5,000 mg m^{-2} of Cr(VI) were reduced in "+Lac+Shew" in 8 days of MFC operation, whereas only 130 mg m^{-2} of Cr(VI) reduction was achieved in 9 days in Hsu et al. (2012). Overall, the abiotic cathode in Hsu et al. (2012) performed equally or better than many Shewanella strains, reducing 139 mg m^{-2} Cr(VI) in 9 days vs. 104 mg m^{-2} in the MR-4 cathode and 130 mg m^{-2} in the MR-1, PV-4 and ANA-3 cathodes. On the contrary, MR-1 in this thesis exhibited a clear enhancement of the process and the biocathode clearly outperformed the abiotic cathode. By adding lactate along with MR-1 pre-treated under the conditions described, the electrocatalytic activity and Cr(VI) reducing ability of the cathode was considerably increased and this is the first study to demonstrate this process enhancement.

Enhanced performance caused by the presence of organic carbon in biocathodes has been previously described in a number of studies, however the reasons for this enhanced behaviour vary from study to study. In an early study, Gregory et al. (2004) had to supply *Geobacter*

sulfurreducens with 2 mM of acetate in order to catalyse the fumarate reduction with the electrode as the electron donor. Jeremiasse et al. (2012) recently reported a faster start-up period in a H_2-producing microbial electrolysis cell biocathode when 1 mM acetate was added. Clark et al. (2012) also found that the presence of acetate in a perchlorate-reducing biocathode containing the heterotroph *Azospira suillum* PS, enhanced cathodic current production by stimulating bacterial oxidation of the redox mediator anthraquinone-2,6-disulphonate from AH_2QDS to AQDS.

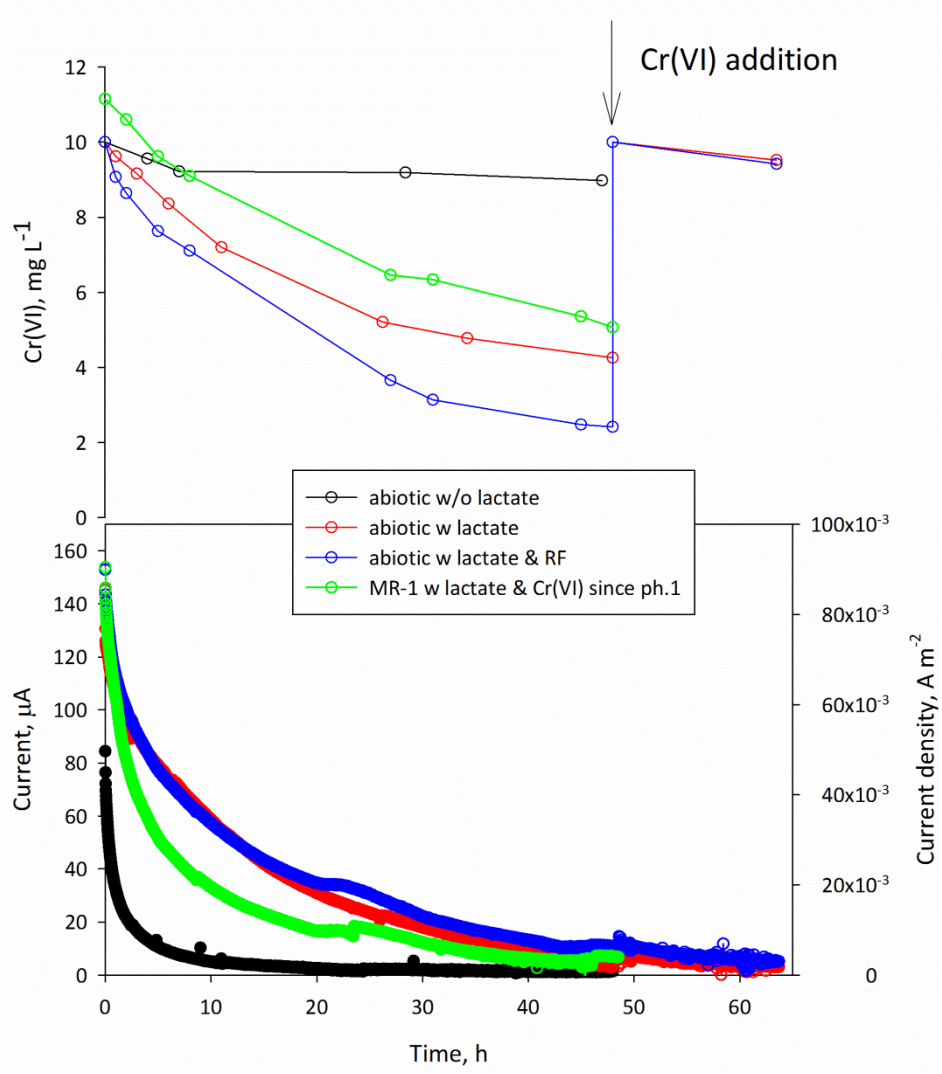

Figure 4-60: Parameters during MFC operation: current production and Cr(VI) concentration in control experiments.

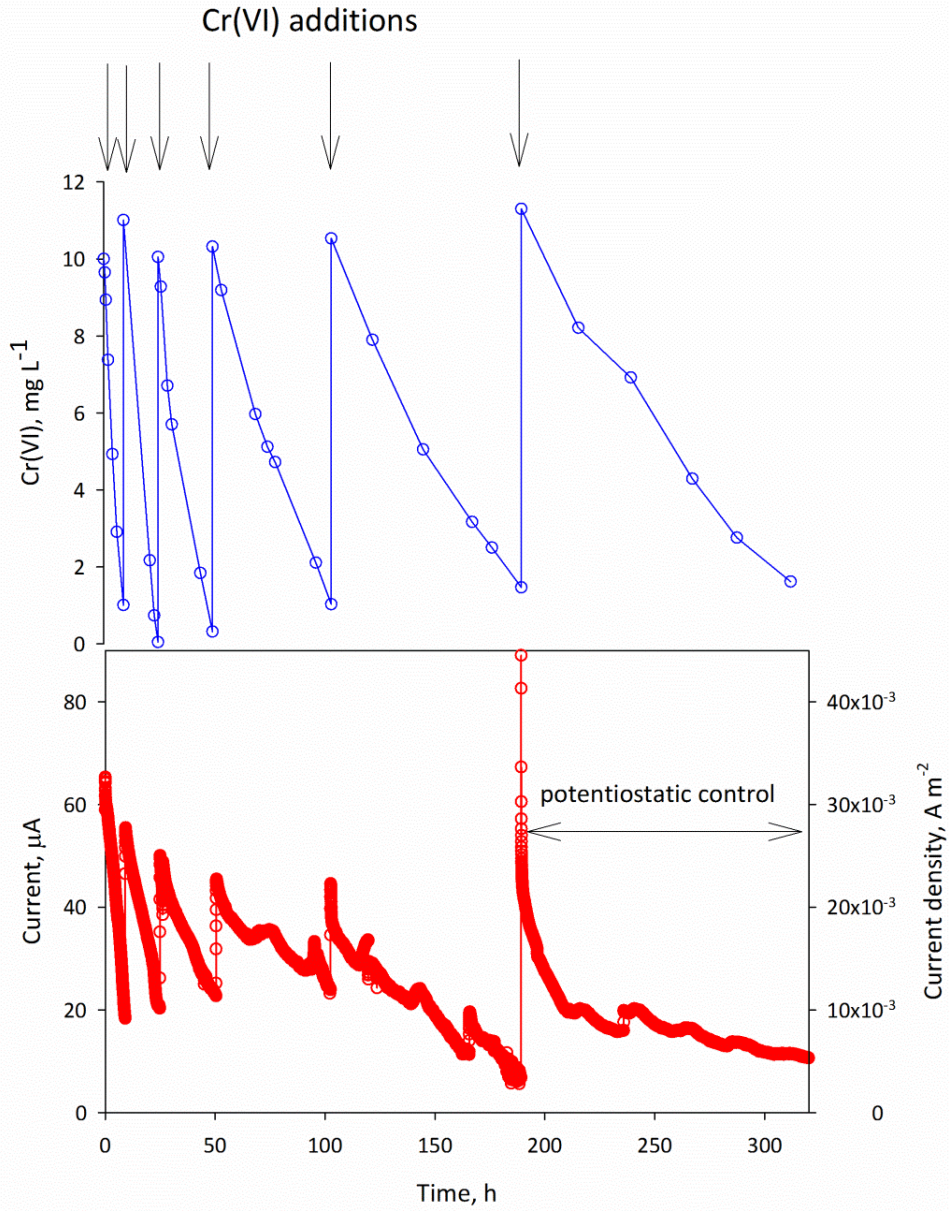

Figure 4-61: Parameters during MFC operation: Cr(VI) concentration and current production when MR-1 was used in the cathode after an aerated phase 1.

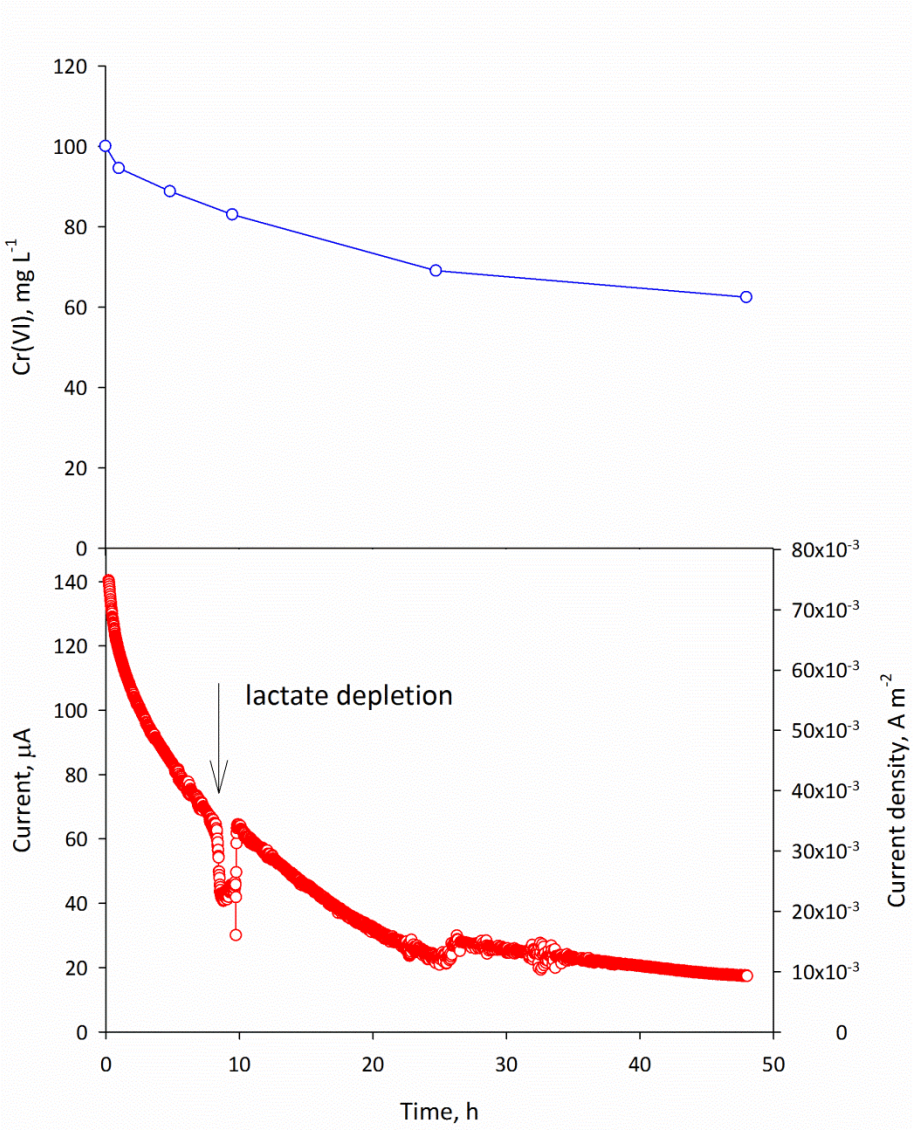

Figure 4-62: Cr(VI) concentration and current production when MR-1 was used in the cathode with 100 mg-Cr(VI) L^{-1}, after an aerated phase 1. At 9 h, lactate depletion in the anode caused the sharp current drop which was recovered after lactate addition.

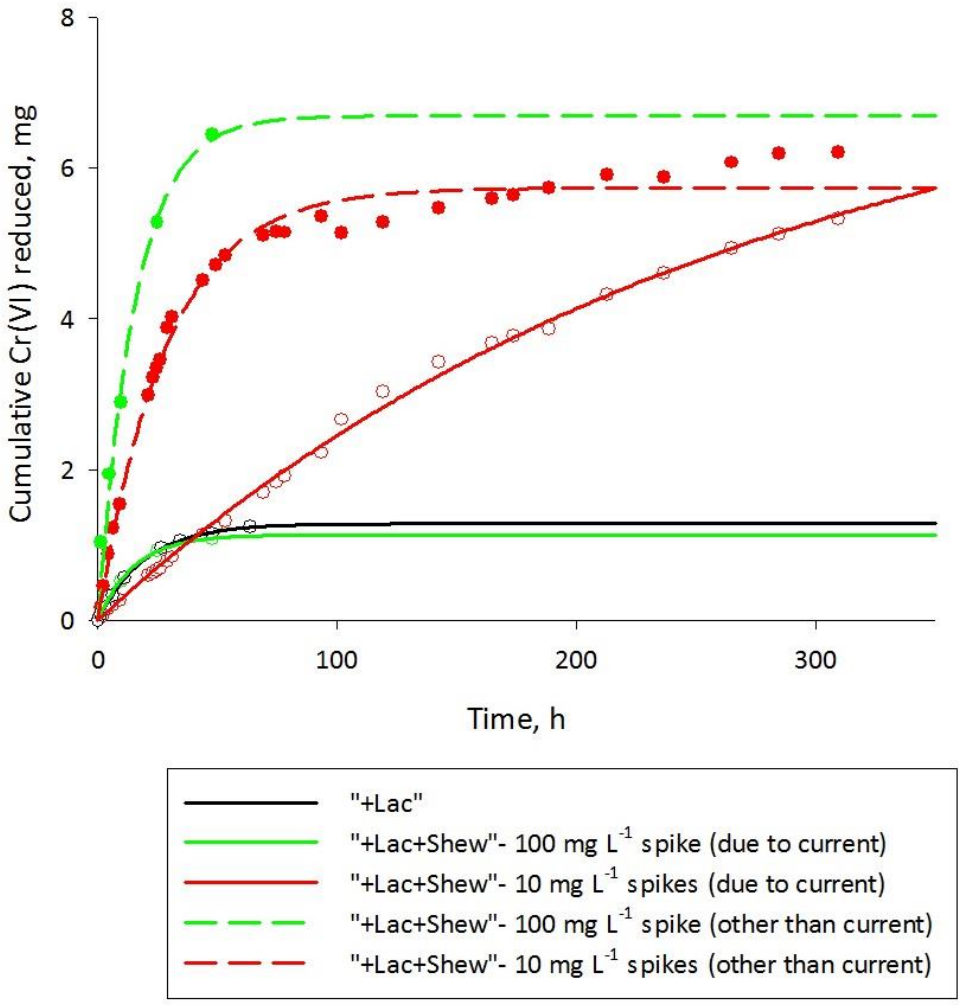

Figure 4-63: Cumulative Cr(VI) reduced in "+Lac+Shew" due to current and other pathways; comparison with a similar reactor that accepted one 100 mg-Cr(VI) L^{-1} spike and with the abiotic control with 30 mM lactate. Regression parameters of the graph fits are presented in Table 4-6.

Table 4-6: Regression parameters for the graphs presented in Figure 4-63 showing cumulative Cr(VI) reduced through time, in different reactors. Equations are in the form y=a*(1-exp(-b*x), where y is cumulative Cr(VI) reduced (mg), x is time (h), "a" is a parameter representing a maximum possible Cr(VI) reduced for x→∞ (mg) and "b" a parameter representing how fast the maximum possible Cr(VI) reduced can be approached (h^{-1}).

Reactor	Equation (y=)	R^2
"+Lac"	1.3*(1-exp(-0.051*x))	1.00
"+Lac+Shew"-100 mg L^{-1} due to current	1.1*(1-exp(-0.066*x))	1.00
"+Lac+Shew"-10 mg L^{-1} due to current	7.8*(1-exp(-0.004*x))	1.00
"+Lac+Shew"-100 mg L^{-1} other than current	6.7*(1-exp(-0.064*x))	0.99
"+Lac+Shew"-10 mg L^{-1} other than current	6.0*(1-exp(-0.037*x))	0.98

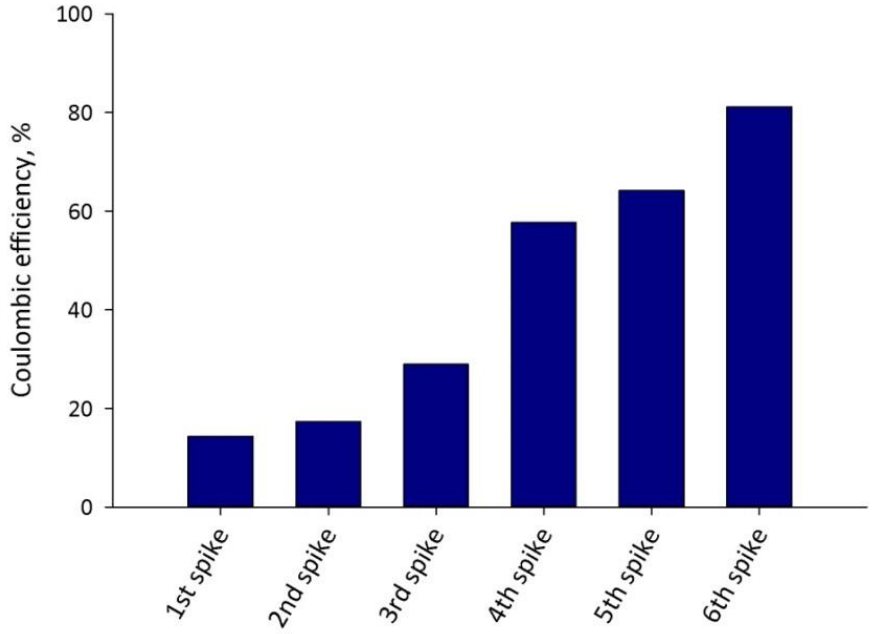

Figure 4-64: Parameters during MFC operation: Coulombic efficiency during MFC operation (spikes 1-5) and during potentiostatic control of the cathode at -500 mV (6th spike) with MR-1 in the cathode after an aerated phase 1.

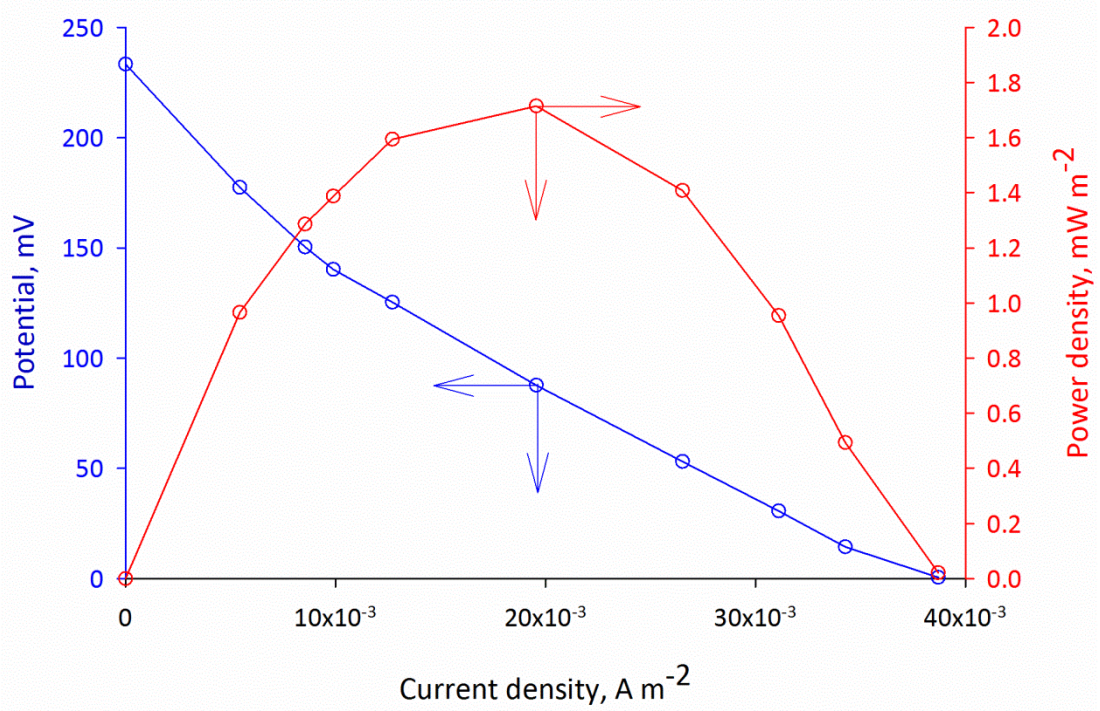

Figure 4-65: Parameters during MFC operation: polarisation curves of MFC after the first Cr(VI) spike with MR-1 in the cathode after an aerated phase 1.

4.3.3.4.3. Conclusions

The presence of lactate in the *Shewanella oneidensis* MR-1 containing biocathode facilitated Cr(VI) reduction with simultaneous current production. Besides delaying the deactivation of the electrode by non-conductive Cr(III) deposits, lactate also allowed MR-1 to produce redox mediators needed for cathodic Cr(VI) reduction. Despite the heterotrophic nature of *Shewanella*, its electrophilic character allowed it to reduce Cr(VI) with both lactate and the electrode as the electron donor. The study also demonstrated that aerated conditions during the pre-treatment phase enhanced both anodic and cathodic current production, whilst redox compounds released in the medium by MR-1 played an important role in this current enhancement.

In summary, current is believed to be enhanced in three ways when lactate was present in the biocathode: (i) Cr(III)-lactate interaction delayed the deactivation of the electrode (Figure 4-66b), (ii) electron shuttle mediators produced during phase 1 mediated electrons from the electrode to Cr(VI) and promoted indirect Cr(VI) reduction (Figure 4-66c), and (iii) the presence of lactate and redox mediators produced during phase 1 enabled MR-1 to be actively involved in the electrode oxidation process and drive direct or indirect Cr(VI) reduction (Figure 4-66d).

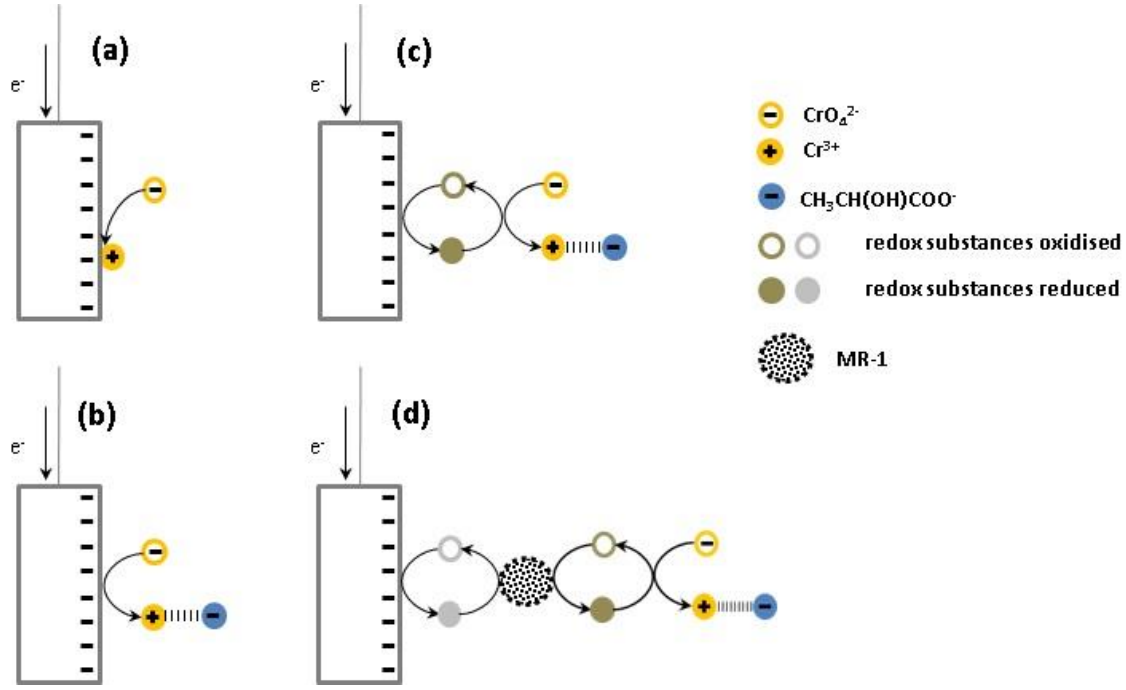

Figure 4-66: Main electron transfer mechanisms involved in Cr(VI) reduction: (a) abiotic; (b) abiotic in the presence of a metal chelator; (c) abiotic in the presence of a metal chelator and a redox mediator; (d) MR-1 mediated (via direct and/or indirect electron transfer from the electrode to MR-1) in the presence of a metal chelator.

4.3.3.4.4. Practical implications

The addition of lactate in a Cr(VI) reducing MFC cathode considerably increased the operating life and the Cr(VI) reducing ability of the electrode, which are of critical importance for the implementation of the MFC technology in Cr(VI) remediation. In the presence of MR-1, the Cr(VI) reducing ability of the electrode was further increased by 3 times compared to the abiotic control, and in total, Cr(VI)-wastewater bioremediation was considerably enhanced by simultaneously providing two separate electron sources to the bacteria; lactate and the poised electrode. As a result, the electrode accounted for almost the same amount of Cr(VI) reduced by MR-1 and lactate alone, indicating that bio-electroreduction and MFC technology could be considered in addition to conventional Cr(VI) bioremediation.

Furthermore, the demonstrated capability of MR-1 to operate both in the anode and the cathode could offer more flexibility in applying the MFC technology for Cr(VI) remediation. In this case, microbes, lactate and redox mediators needed in the cathode could be provided by recirculating anodic effluent to the cathode. The reverse action is also possible to allow COD removal and Cr(VI) polishing of the cathodic effluent: however, further research would be

required to elucidate to what extent and under what conditions (e.g. biomass density in the anode), low levels of Cr(VI) present in the cathodic effluent would affect the established redox conditions in the anode.

4.4. Assessment of the Cr(VI) removal potential of anaerobic, primary and activated sludge

4.4.1. Introduction

Anaerobic, primary and activated sludge contain a large variety of microorganisms and can contain both Cr(VI) reducing and electrophilic microorganisms. As shown earlier, most Cr(VI) bioelectrochemical systems so far reported in the literature have used mixed inocula in both the bioanode and the biocathode. The purpose of this study is to show the ability of such inocula to i) reduce Cr(VI) and ii) produce cathodic current under conditions comparable to the conditions applied previously for MR-1.

4.4.2. Cr(VI) removal in the absence of current

4.4.2.1. Experimental plan

The ability of 3 types of inocula for Cr(VI) reduction in the absence of current was investigated by conducting flask experiments. For this reason, 15 mL of primary (363 mg-TVSS), anaerobic (345 mg-TVSS) and activated (239 mg-TVSS) sludge were inoculated in 200 mL flasks containing 110 mL of the nutrient medium described in Section 3.3. Acetate was selected as the organic electron donor in all occasions, because it is a comparatively easy to degrade organic molecule (it has been used extensively in MFC studies), that can be utilised by a range of bacteria for Cr(VI) reduction (some have already been presented in Table 2-1). Acetate was provided with spikes that increased its concentration at approximately 1,500 mg L^{-1}. Hexavalent chromium was provided at increasing concentrations as shown in Figure 4-67, after complete Cr(VI) reduction was observed. All experiments were conducted in duplicate and over a period of 73 d. N_2 was supplied before start and after each acetate or chromium spike to maintain anoxic conditions in the flasks. Experiments were conducted in an orbital shaking incubator operating at 200 rpm and 25 ^{o}C.

4.4.2.2. Results and discussion

As shown in Figure 4-67, anaerobic sludge seemed more capable of dealing with Cr(VI) contamination, as it was able to reduce Cr(VI) concentrations up to 120 mg Cr(VI) L^{-1} within the

time provided. In addition, as seen in Figure 4-68, anaerobic sludge reduced a total of 274 mg (g VSS)$^{-1}$, whilst activated and primary sludge reduce 139 mg (g VSS)$^{-1}$ and 80 mg (g VSS)$^{-1}$ respectively. As a comparison, *Clostridium* sp. SS1, isolated from activated sludge, was found able to tolerate Cr(VI) concentrations up to 50 mg L^{-1}, and it could reduce up to 72 mg-Cr(VI) (g of biomass dry weight)$^{-1}$ when incubated at pH 7, 30^{o}C and aerated conditions (Nguema and Luo, 2012). On the other hand, Middleton et al. (2003) who tested *Shewanella oneidensis* MR-1 under anaerobic conditions, found that the strain had a Cr(VI)-reducing capacity of up to 3,500 mg-Cr(VI) (g protein)$^{-1}$ when it was accepting Cr(VI) concentrations of approximately 4 mg-Cr(VI) L^{-1}; however, the Cr(VI)-reducing capacity dropped to only 780 mg-Cr(VI) (g protein)$^{-1}$ when MR-1 received single spikes of up to 26 mg-Cr(VI) L^{-1}.

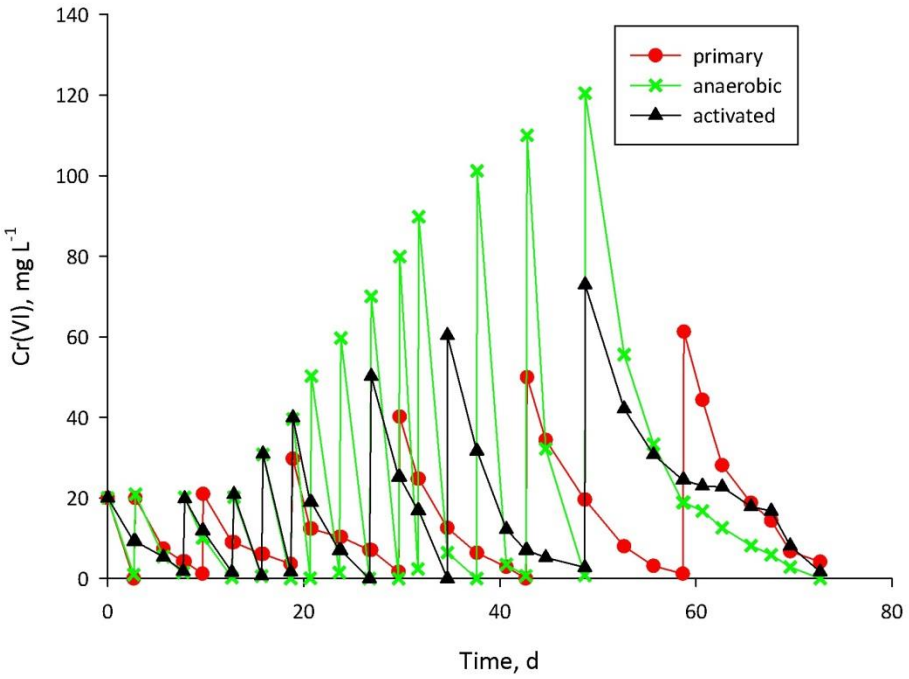

Figure 4-67: Cr(VI) reduction by primary, anaerobic and activated sludge in the presence of acetate (average values are reported).

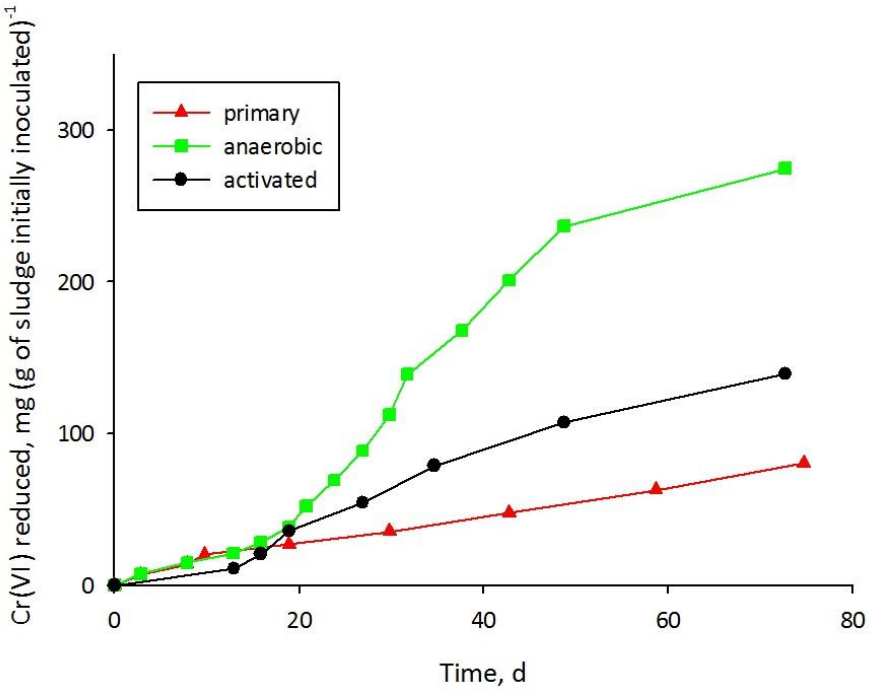

Figure 4-68: Total Cr(VI) reduced by primary, anaerobic and activated sludge in the presence of acetate (average values are reported).

In a parallel set of experiments, flasks were only supplied with inorganic carbon ($NaHCO_3$) to test whether these 3 types of inocula could remove Cr(VI) without the addition of an external electron donor. Whilst acetate consumption is the main reason for the Cr(VI) reduction observed previously, the organic carbon molecules released from the cell lysis should be able to provide a source of electrons in this case. As can be seen in Figure 4-69, a rapid reduction was observed within the first 3 d of operation, followed by a very slow reduction rate (110 d for a 20 mg Cr(VI) L^{-1} reduction). However, as soon as acetate was provided in the flasks on the 113th day, Cr(VI) reduction rates recovered (Figure 4-69), indicating both the importance of the organic source for Cr(VI) reduction and the ability of mixed cultures to recover from long periods of starvation.

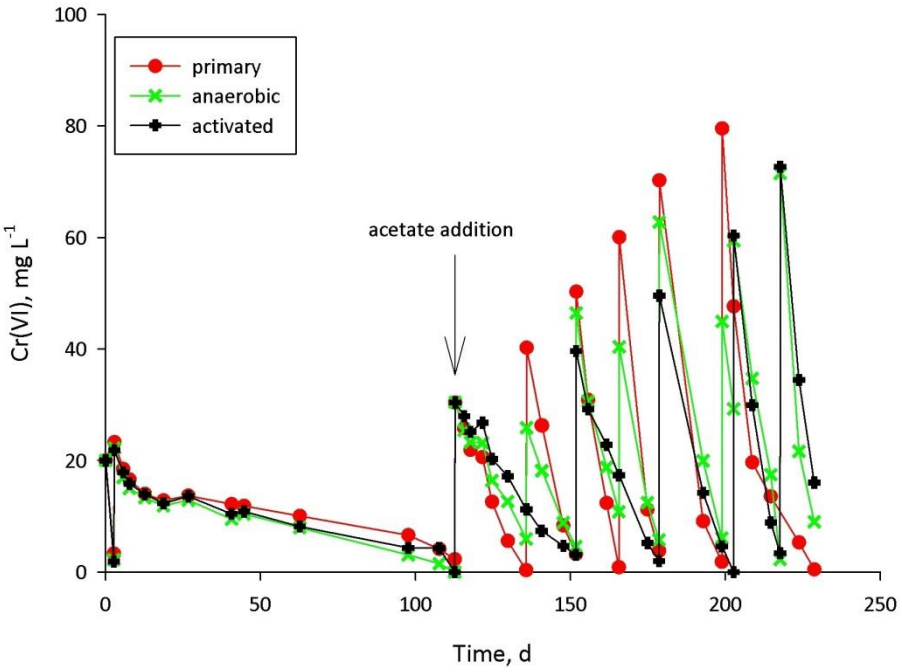

Figure 4-69: Cr(VI) reduction by primary, anaerobic and activated sludge after 113 days of starvation (average values are reported).

The results from these experiments indicate that sludge from wastewater treatment and especially anaerobic sludge, could be used in Cr(VI) reducing MFCs. However, Cr(VI) reduction was demonstrated in the presence of acetate in this instance, and the absence of an external organic electron donor might be a barrier for autotrophic cathodes, even in the case where relatively large electrode surface areas are used. In addition, acetate is a comparatively low-cost and easy to handle biodegradable carbon source, which might have advantages for Cr(VI) reduction over an autotrophic MFC cathode.

4.4.3. Cr(VI) removal in bioelectrochemical systems

4.4.3.1. Experimental plan

Following the flask experiments described above, which showed a more efficient Cr(VI) reduction by anaerobic sludge, bioelectrochemical cells were tested with anaerobic sludge as the microbial biocatalyst. For this purpose, two of the reactors described in Section 4.2.2 that were operated for a period of more than 4 months with anaerobic sludge as the anodophilic inoculum, were used in this study to evaluate whether the use of such a rich inoculum could give results comparable to those of MR-1.

Both reactors underwent phase 1 and phase 2, where the working electrodes were poised at +300 mV and -500 mV vs. Ag/AgCl respectively, similarly to the operating conditions described earlier for MR-1. One of the two reactors operated in the presence of 30 mM lactate during phase 1, whilst the other of the two reactors was fed with 8 mM initial acetate concentration for a period of 180 h, until anodic current was not dropping considerably. N_2 was continuously sparged in the reactors for the duration of the experiments to maintain anaerobic conditions during both phases 1 and 2. Also, because the bioanodes previously operated at alkaline pH conditions (pH 8), the pH in the half-cell reactors had to be neutralised before start. Similarly to the MR-1 potentiostatically controlled experiments, the two reactors were spiked with 20 mg-Cr(VI) L^{-1} after cathodic current dropped to background levels.

4.4.3.2. Results and discussion

As can be seen in Figure 4-70, anodic current was produced in the lactate containing reactor soon after the experiment started. Figure 4-71 shows the cyclic voltammogram produced at the end of phase 1, where anaerobic sludge was producing anodic current in the presence of 27 mM lactate. As can be seen, anodic current was produced at potentials over the value of approximately -380 mV vs. Ag/AgCl, with at least one redox couple present around that potential.

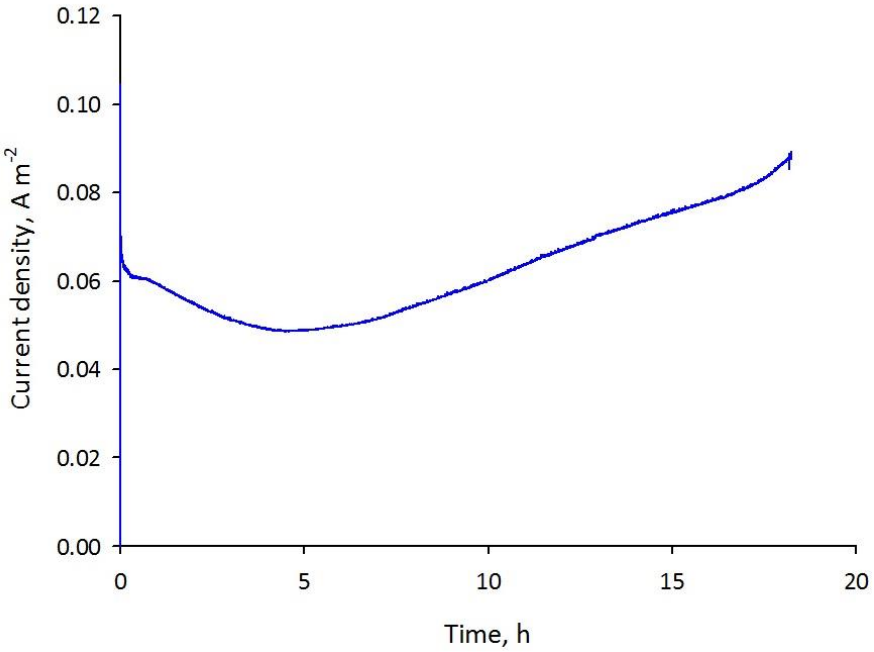

Figure 4-70: Chronoamperometry profile of anaerobic sludge in the presence of lactate, before spiking with Cr(VI) (+300 mV vs. Ag/AgCl).

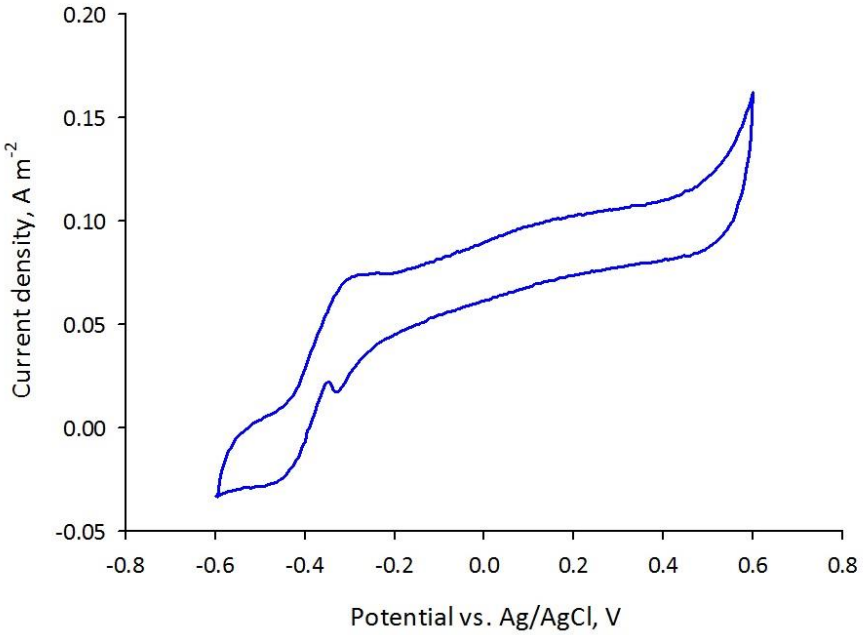

Figure 4-71: Cyclic voltammogram of anaerobic sludge after phase 1 in the presence of 27 mM lactate. Scan rate is 5 mV sec^{-1}.

When the potential was reversed, current produced was at similar levels with the current produced by MR-1 in the presence of 28 mM lactate, after anaerobic pre-treatment (Figure 4-72). Electrochemical Cr(VI) reduction by the end of the 4 h operating period was 2.5 mg-Cr(VI) L^{-1} (Figure 4-73), approximately 90% of the total amount expected to be electrochemically reduced (calculated according to both the maximum expected charge- inset of Figure 4-72- and according to the maximum expected Cr(VI) electrochemically reduced- inset of Figure 4-73). In addition, cyclic voltammograms produced by the end of the 4 h (anaerobic) operation period (Figure 4-74) showed that cathodic current was not produced in this instance at a broad potential range, as was the case when MR-1 was pre-treated in the presence of oxygen. Finally, anodic current production was totally diminished from the cyclic voltammogram of anaerobic sludge after exposure to 20 mg-Cr(VI) L^{-1}, as opposed to the (limited after Cr(VI) addition) anodic current showed in the cyclic voltammogram of MR-1.

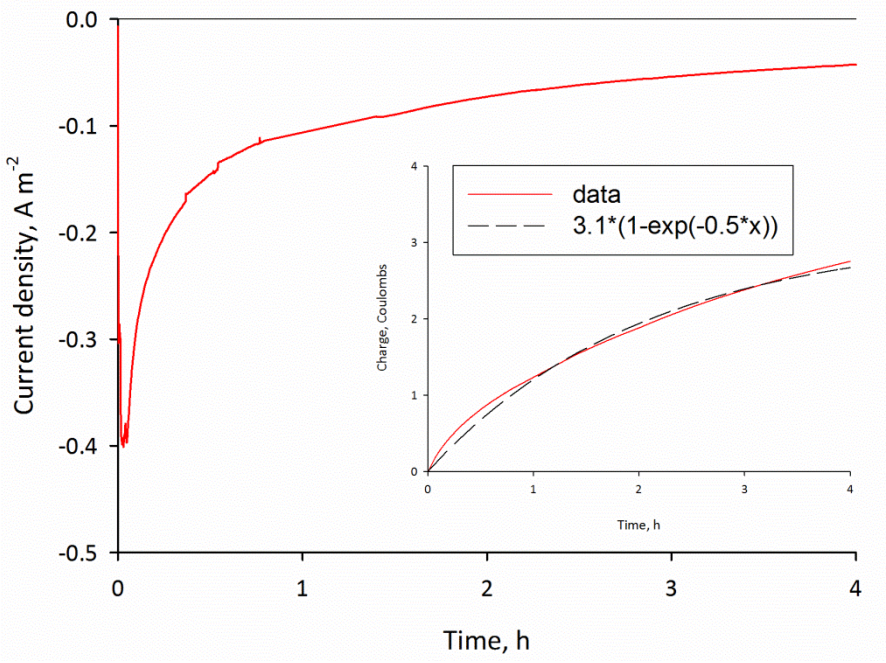

Figure 4-72: Chronoamperometry profile of anaerobic sludge in the presence of 27 mM lactate and 20 mg-Cr(VI) L^{-1} at start (-500 mV vs. Ag/AgCl); 0.004 A m^{-2} was extracted as background current. Inset: charge produced during the 4 h experimental period and curve fit (R^2=0.99).

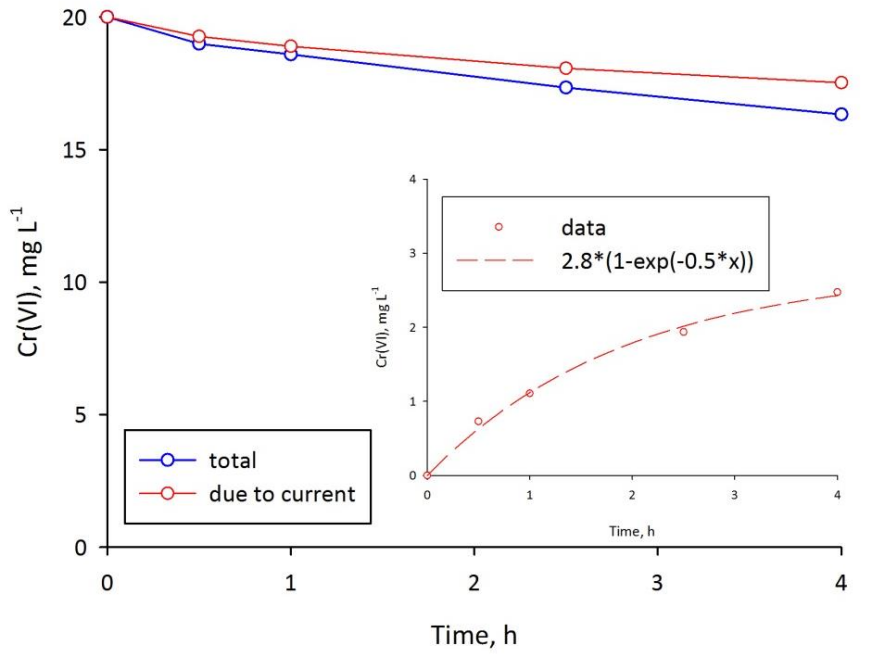

Figure 4-73: Cr(VI) concentration profiles during reduction by anaerobic sludge in the presence of 27 mM lactate at start (-500 mV vs. Ag/AgCl). Inset: bioelectrochemically reduced Cr(VI) concentration during the 4 h experimental period and curve fit (R^2=0.99).

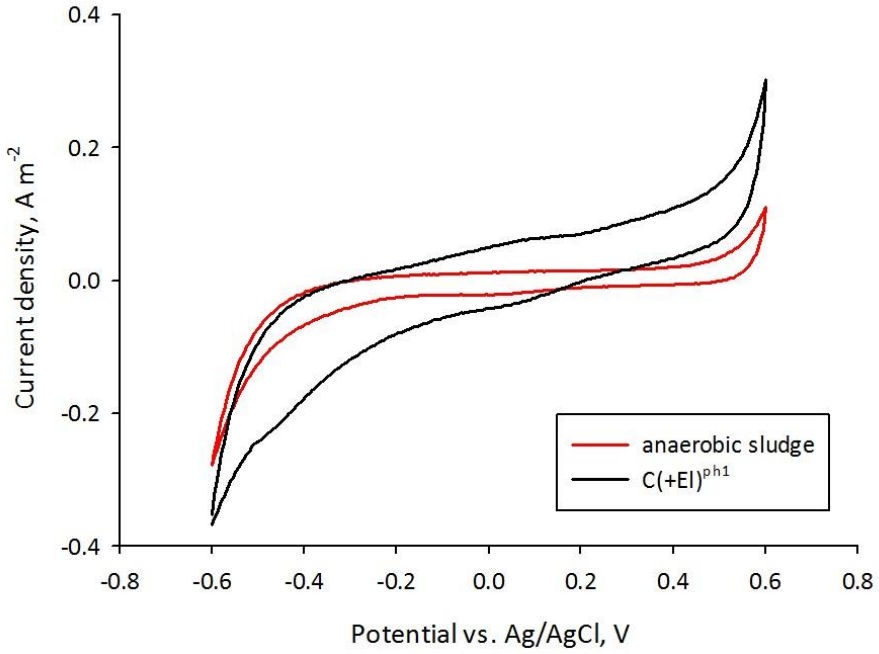

Figure 4-74: Comparison of the MR-1 ($C(+El)^{ph1}$) and anaerobic sludge CVs 4 h after spiking with Cr(VI). Scan rate is 5 mV sec^{-1}.

In the reactor where acetate was present at an initial concentration of 8 mM, current dropped to background levels by the end of the 180 h phase 1 operation period. By the end of phase 1, acetate concentration remained at approximately 1.5 mM and was not dropping considerably. Because replacing the nutrient medium would also remove any bacterial products produced during the whole operation period, it was decided to operate phase 2 under not-strictly autotrophic conditions but in the presence of 1.5 mM acetate. Current followed a decreasing trend as in the previous experiment with lactate, however current drop was slower than previously (Figure 4-76). Bacterial metabolic products could be responsible for this slower current drop, however the initial current produced was still one order of magnitude lower than when the reactor operated with lactate. The reactor operated for 65 h during phase 2 in this instance and current was allowed to completely drop to background levels. Cr(VI) reduced electrochemically by the end of the 65 h phase 2 operation period (Figure 4-77) was no more than 1.8 mg L^{-1}, 20% of the total Cr(VI) reduced (0.8 mM acetate was still left at the end of phase 2). In conclusion, no considerable reactor performance enhancement was observed when anaerobic sludge was utilised as the biocatalyst, compared to the systems where MR-1 was present.

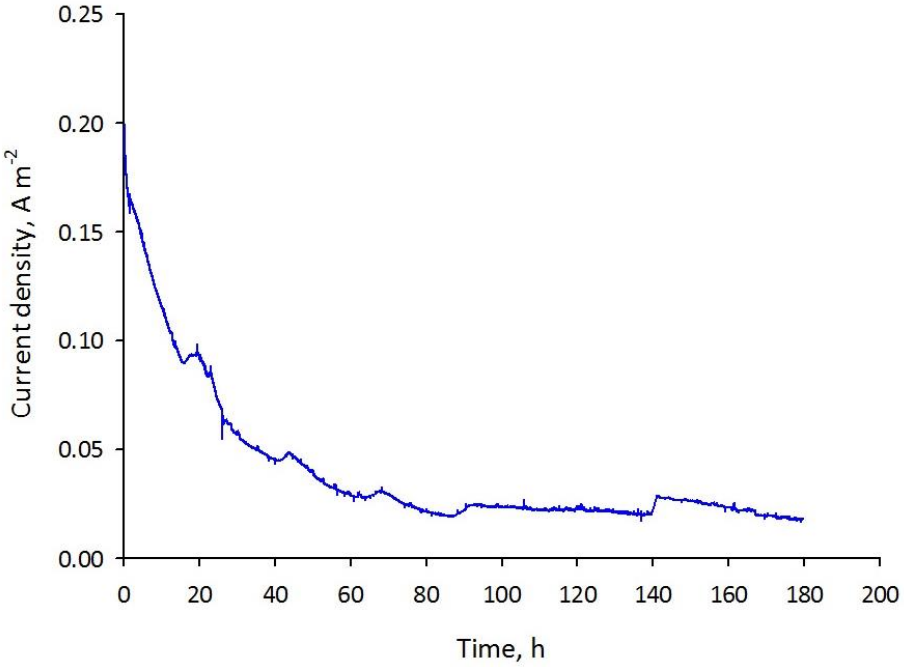

Figure 4-75: Chronoamperometry profile of anaerobic sludge with decreasing acetate concentrations before spiking with Cr(VI) (+300 mV vs. Ag/AgCl).

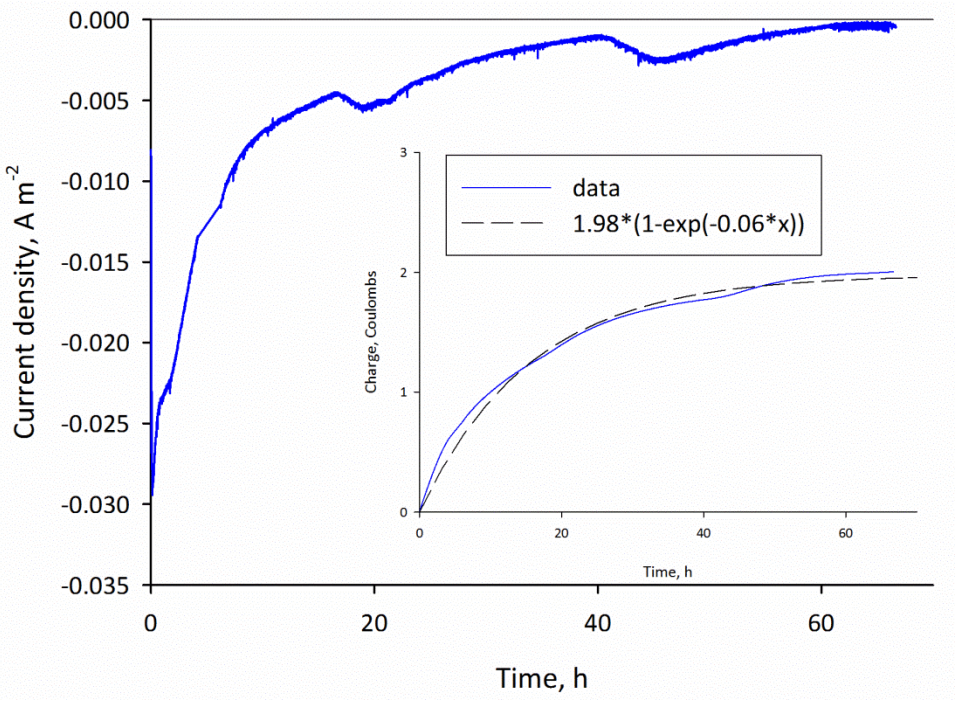

Figure 4-76: Chronoamperometry profile of anaerobic sludge after acetate depletion and Cr(VI) spike (-500 mV vs. Ag/AgCl); 0.004 A m^{-2} was extracted as background current. Inset: charge produced during the 70 h experimental period and curve fit (R^2=0.99).

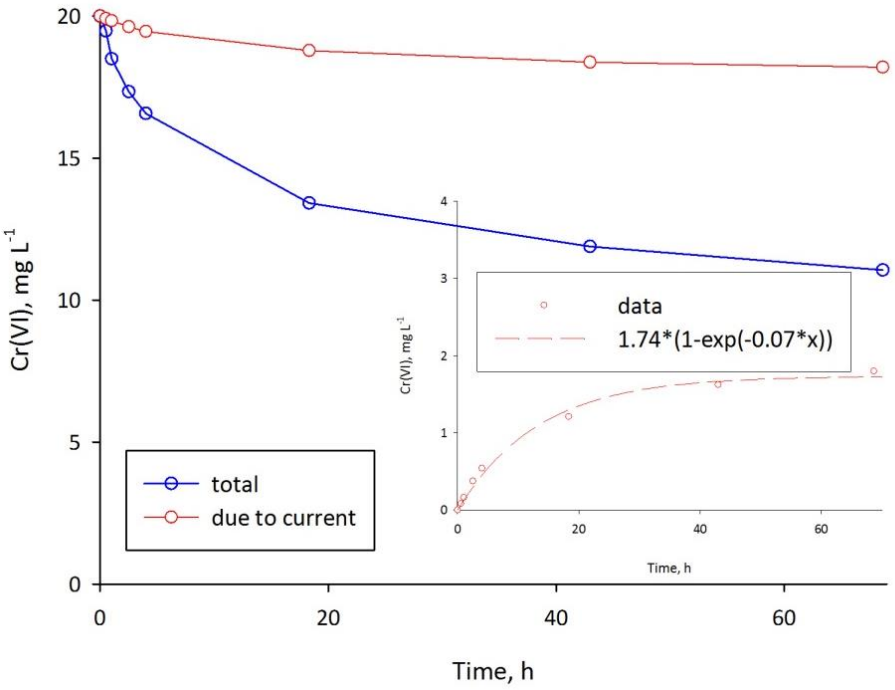

Figure 4-77: Cr(VI) concentration profiles during reduction by anaerobic sludge after acetate depletion (-500 mV vs. Ag/AgCl). Inset: bioelectrochemically reduced Cr(VI) concentration during the 70 h experimental period and curve fit (R^2=0.99).

Chapter 5 Conclusions, future perspectives, and suggestions for future work

5.1. Conclusions

The electron transfer mechanisms present in Cr(VI) reducing biocathodes had not been investigated thoroughly in previous studies, leaving an important gap in understanding of such systems. One important reason for this is that the cathode electrode deactivation problem was not adequately addressed; consequently, researchers had to choose between using large electrode surface areas or letting their system collapse under the formation of inhibitory products. Whilst the first option will be impractical for large scale real applications, the second option can lead to false conclusions regarding the mechanisms, the limitations and the applicability of Cr(VI) reducing biocathodes.

In the present study it has been demonstrated how organic acids can enhance the system performance. The addition of lactate (a natural compound used extensively in Cr(VI) remediation studies) in a Cr(VI) reducing MFC cathode considerably increased the operating life and the Cr(VI) reducing ability of the electrode, which are of critical importance for the implementation of the MFC technology in Cr(VI) remediation. Charge produced by Cr(VI)-reducing current within the 72 h operation period provided, increased with increasing lactate concentrations (up to 200 mM lactate), and was up to approximately 8 times the charge produced without lactate addition (8.2 C vs. 1.0 C). In addition, power produced in abiotic, Cr(VI) reducing MFCs with 200 mM lactate in the cathode was at similar levels as that produced elsewhere in biotic Cr(VI) reducing MFCs (up to 8.8 mW m^{-2}). Considerable performance was observed even with alkaline pH MFC cathodes (up to 21.4 mW m^{-2} at pH 8) and this is important taking into consideration the alkaline pH of chromite ore processing wastewaters (typically between 7.5 to 12.5, depending on the site).

Riboflavin, a redox mediator naturally produced by bacteria, was also shown to have an active role in electrochemical Cr(VI) remediation. In the presence of 30 mM lactate and 5 μM riboflavin, the electrode (-500 mV vs. Ag/AgCl) reduced more than 3 times the amount of Cr(VI) reduced by the control with 30 mM lactate alone (10 mg Cr(VI) L^{-1} vs. 3 mg Cr(VI) L^{-1}), within the 4 h operation period provided. However, the presence of riboflavin (1 μM) did not have any effect in the absence of lactate and this is because at the low concentrations provided (up to 5 μM), riboflavin alone could not prevent the electrode deactivation

phenomenon. Also, riboflavin did not have a considerable effect when it was supplied in an abiotic cathode MFC because its midpoint potential is located at very low potential ranges not easily reached in the MFC cathodes (approximately -0.4 V vs. Ag/AgCl).

When *Shewanella oneidensis* MR-1 was supplied in potentiostatically poised Cr(VI) biocathodes (-500 mV vs. Ag/AgCl) in the presence of lactate, the cathode exhibited an enhanced current production and Cr(VI) reduction, and 3 times more Cr(VI) was totally reduced within the 4 h operation period (9 mg Cr(VI) L^{-1} vs. 3 mg Cr(VI) L^{-1} in the abiotic cathode with 30 mM lactate). However, the MR-1 pre-treatment conditions were found to be important. When supplied with either riboflavin or air during the pre-treatment phase, both anodic and cathodic currents were enhanced, showing the interrelated pathways for both the electrode oxidation and the electrode reduction. Up to 88% more electrons were bioelectrochemically reducing Cr(VI) when 1 μM riboflavin was provided along with MR-1 during phase 1, compared to MR-1 alone (anaerobic conditions during phase 1 in both cases). Conversely, MR-1 pre-treatment under anaerobic conditions showed no considerable performance enhancement compared to the abiotic control with lactate, and this was probably because of the limited energy gained by MR-1 during the pre-treatment step. In addition, no considerable performance enhancement, compared to that of MR-1, was observed when a mixed anodophilic population was used to produce cathodic current by electrochemically reducing Cr(VI).

When *Shewanella oneidensis* MR-1 was supplied in MFC biocathodes in the presence of 30 mM lactate, the Cr(VI) reducing ability of the cathode was further increased by 3 times compared to the abiotic control with lactate (3.5 mg vs. 1.2 mg). In total, Cr(VI)-wastewater bioremediation was considerably enhanced by simultaneously providing two separate electron sources to the bacteria; lactate and the poised electrode. As it was shown, MR-1 exhibited Cr(VI) reduction pathways that behaved differently, with the electrode-oxidising ones being slower but with a longer effect than the lactate-oxidising ones. As a result, the electrode accounted for almost the same amount of Cr(VI) reduced by MR-1 and lactate alone by the end of the 312 h operation period (5.5 mg vs. 6.2 mg), indicating that bio-electroreduction and MFC technology could be considered in addition to conventional Cr(VI) bioremediation.

Finally, the demonstrated capability of MR-1 to operate both in the anode and the cathode could offer more flexibility in applying the MFC technology for Cr(VI) remediation. In this case microbes, lactate and redox mediators needed in the cathode could be provided by recirculating the anodic effluent to the cathode. The reverse action is also possible to allow

COD removal and Cr(VI) polishing of the cathodic effluent; however, as was already shown in this study, Cr(VI) can inhibit anodic current production and extra care should be taken to avoid this phenomenon that will finally lead to a system collapse. To what extent and under what conditions (e.g. biomass density in the anode), low levels of Cr(VI) present in the cathodic effluent will affect the established redox conditions in the anode, should be thoroughly evaluated.

5.2. Future perspectives and suggestions for future work

The work was designed and conducted to provide the tools for effective Cr(VI) bioremediation. The work has merged the established bioremediation methodology with the bioelectrochemical systems technology and an improvement of the Cr(VI) remediation practices has been achieved to minimise the damage that Cr(VI) causes to the environment. A more clear understanding of the mechanisms behind Cr(VI) bioremediation using BES has been reached, and this is important to bring the BES technology a step closer to real applications. In particular, the study on *Shewanella*, which is an environmentally significant bacterium, has produced results that can be taken as directions for designing environmental applications related to the remediation of wastewater and polluted groundwater. Overall, the work can provide guidance to environmental scientists working on water remediation, to electrochemists researching the metals' reduction and recovery and the electron transfer mechanisms, and to biologists studying the bacterial physiology.

The following suggestions could be pursued as part of future work on Cr(VI) reducing biocathodes:

- Chelating agents can interact with Cr(VI)-reduction products, however the exact speciation and charge of the products remains unclear. An analysis of the electrode surface and of the Cr(III) species that are present with and without a metal chelator is expected to shed more light on the mechanisms and therefore the limitations of such systems designed for Cr(VI) remediation.
- Organic acids can be excreted by bacteria, which might be present in mixed bacteria biocathodes and future research should also focus on whether the chelation effect is also playing an important role in mixed culture Cr(VI) biocathodes. Additionally, it should be studied whether there is the potential for enhancing the performance discussed herein by utilising other microbial biocatalysts in addition to the lactate or other chelate agent containing cathodes. Also, it is important to investigate whether

and how other chelate agents with environmental presence and/or importance like EDTA and oxalate, can enhance Cr(VI) electroremediation.

- Regarding the electron transfer mechanisms, the active role of electroactive species other than riboflavin (e.g. cytochromes) needs to be further examined. From a microbiological point of view, it is worth examining which mechanisms and bacterial energy gaining cycles are involved in each case, in *Shewanella* and in other important microbes. This will then give the tools to environmental engineering for applying this knowledge to Cr(VI) remediation.
- From a practical point of view, continuous and larger scale systems should be evaluated before any application. Here, the challenge will be to find microorganisms that are tolerant to Cr(VI), can reduce Cr(VI) and are also able to oxidise conductive solid surfaces relying solely on the biofilm's direct electron transport mechanisms.

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APPENDIX A **Journal publication**

Credits

Enhanced performance of hexavalent chromium reducing cathodes in the presence of *Shewanella oneidensis* MR-1 and lactate

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Biocathodes for the reduction of the highly toxic hexavalent chromium (Cr(VI)) were investigated using *Shewanella oneidensis* MR-1 (MR-1) as a bio-catalyst and performance was assessed in terms of current production and Cr(VI) reduction. Potentiostatically controlled experiments (-500 mV vs. Ag/AgCl) showed that a mediatorless MR-1 biocathode started up under aerated conditions in the presence of lactate, received 5.5 and 1.7 times more electrons for Cr(VI) reduction over a 4-hour operating period than controls without lactate and with lactate but without MR-1, respectively. Cr(VI) reduction was also enhanced, with a decrease in concentration over the 4-h operating period of 9 mg/L Cr(VI), compared to only 1 and 3 mg/L respectively in the controls. Riboflavin, an electron shuttle mediator naturally produced by MR-1, was also found to have a positive impact in potentiostatically controlled cathodes. Additionally, a microbial fuel cell (MFC) with MR-1 and lactate present in both anode and cathode produced a maximum current density of 32.5 mA/m^2 (1,000 Ω external

load) after receiving a 10 mg/L Cr(VI) addition in the cathode, and cathodic efficiency increased steadily over an 8-day operation period with successive Cr(VI) additions. In conclusion, effective and continuous Cr(VI) reduction with associated current production were achieved when MR-1 and lactate were both present in the biocathodes.

INTRODUCTION

Hexavalent chromium (Cr(VI)) is a highly toxic, mutagenic and carcinogenic substance that is present in the effluent streams of a wide range of industrial processes, including electroplating, leather tanning and wood preserving[1]. It is highly soluble and, because of its long history of use and often its disposal after inappropriate or no treatment, it has become one of the most abundant inorganic contaminants in groundwater. Ideally Cr(VI) should be removed from groundwater in the natural environment, and because of the inherent dangers to health the stringent guideline limit of 50 μg/L has been issued for total chromium concentration in drinking water[1,2]. Removal options include ion-exchange, adsorption and electrodialysis[3]; however in many of these applications chromium keeps its toxic hexavalent state. Reduction of Cr(VI) to the considerably less toxic trivalent form Cr(III) and its subsequent precipitation at neutral pH could be considered a more effective remediation strategy[4].

A possible method for Cr(VI) reduction using a microbial fuel cell (MFC) has recently been proposed, where Cr(VI) was used as an oxidant in the cathode to generate an electrical energy output[5,6]. At low pH values where H^+ is abundant, cathodic Cr(VI) reduction has been demonstrated at relatively fast rates and without the use of a catalyst[5,6]. In the neutral pH region, however, the reaction kinetics are slower due to low H^+ availability:

$$CrO_4^{2-} + 8H^+ + 3e^- = Cr^{3+} + 4H_2O \quad (1)$$

At neutral pH, Cr(VI) reduction takes place under lower cathode potentials and is also severely inhibited by Cr(III) oxyhydroxide monolayers which form progressively on the cathode surface[7]. It has been shown, however, that efficiency can be enhanced by utilizing Cr(VI)-reducing bacterial biofilms as biocatalysts (biocathodes)[8-11]. In this case, the bacteria used must be able to obtain a metabolic gain from the electron transfer process, and prospective candidates for this task are electrophilic bacteria that have been extensively tested in MFC anodes[12]. Amongst these, *Shewanellae* have a well-studied electron transfer mechanism[13,14] and have received much research attention due to their metal and electrode reducing abilities, as well as their respiratory diversity and adaptability[15]. Studying their behavior at both the anode and cathode in bioelectrochemical systems is therefore important for understanding the electron transfer mechanisms utilized and optimizing the processes involved[16].

Despite their significance, studies on the potential use of Shewanellae in biocathodes remain limited[16,17], and one reason for this could be the metabolic requirement for organic carbon. This requirement appears to contradict the established view that organic carbon use in the cathode must be minimized, in order to prevent oxygen consumption by heterotrophic metabolism that does not contribute to electrode oxidation[18,19]. While this view is well founded, it is worth studying the effect of the presence or absence of organic carbon, and particularly of lactate which is a primary carbon source for *Shewanellae*, when Cr(VI) is used as the final electron acceptor[20]. The presence of lactate may also offer potential benefits for the biocathode, including the production of new bacterial cells and the production and utilization of bioelectrocatalysts like flavins, which enhance communication between the bacterial cell and the electrode[21,22]. In addition, lactate can act as a Cr(III) chelator and thus prevent the formation of nonconductive Cr(III) products on the electrode surface[7,23].

The aim of this study was to investigate the performance of Cr(VI)-reducing biocathodes when *Shewanella oneidensis* MR-1 (MR-1) is used as a catalyst in the presence or absence of lactate. Both MR-1 biocathodes and the abiotic counterparts were investigated, and results are expressed in terms of the production of an electrical current and of Cr(VI) reduction. Finally the potential role of riboflavin, an electron shuttle produced by MR-1 in the presence of lactate[24], is also discussed in terms of its efficiency in Cr(VI)-reducing cathodes.

EXPERIMENTAL SECTION

MR-1 cultivation. A starter culture of *Shewanella oneidensis* MR-1 (NCIMB 14063) was grown aerobically on plates of tryptone soya agar (pH 7.3, 30 ^{o}C). A colony was then transferred into 200 mL of Luria-Bertani-50 mM phosphate buffer medium (pH 7.0) and incubated in an orbital shaking incubator at 30 ^{o}C until the required optical densities at 600 nm (OD_{600}) were reached: these were 0.2 for potentiostatically controlled biocathode experiments, 0.3 for MFC biocathodes and 0.5 for MFC bioanodes. Cells were harvested by centrifugation (5,000 x g, 4 ^{o}C, 20 min) with the pellet rinsed twice using 100 mM NaCl-50 mM phosphate buffer saline (PBS) solution (pH 7.0) and resuspended in 200 mL of a minimal medium (MM) (pH 7.0) containing (mM): NaCl (70), KH_2PO_4 (21), K_2HPO_4 (29), $MgCl_2$ (1), NH_4Cl (28), $CaCl_2$ (0.7), before being transferred to the experimental chambers. No vitamins or amino acids were supplemented in the MM but 0.1 % v/v of a trace elements mixture[25] was added.

Chemicals. Na-DL-Lactate (L1375, Sigma Aldrich) and Na-Acetate (S2889, Sigma Aldrich) were dosed in the required volumes to a final 30 mM concentration. The source of chromium was oven-dried potassium dichromate ($K_2Cr_2O_7$) made up as a 4 g/L Cr(VI) stock solution. This solution was added to give an initial concentration of 20 mg/L in potentiostatically controlled cathodes and 10 mg/L in MFC cathodes. Except

where the addition of 1 μM riboflavin (R4500, Sigma Aldrich) is mentioned, all experiments were conducted without supplementation of artificial redox mediators. All additions and sampling were carried out under sterile conditions.

Reactor set-up. Dual-chamber electrochemical reactors were made by connecting two 250 mL borosilicate bottles, each with a sidearm tube. A proton exchange membrane (Nafion 117, Ion Power, Inc.) was fixed between the two sidearms using underwater epoxy adhesive, giving an exposed membrane surface area of 4 cm^2. The membrane was pre-treated as described by Kim et al.[26].

A graphite felt electrode (SIGRATHERM®, SGL Carbon Ltd.) with a 20 cm^2 total apparent surface area (TASA) was fitted in each chamber, and connected through 3 mm ∅ graphite rods to a 1 mm copper wire used as the external circuit cable. The wire was first covered with conductive silver epoxy (ITW, Chemtronics®) and then attached to the felt-rod electrode by inserting it through a hole drilled in the graphite rod. To avoid interference from deposits of Cr(III) oxides and hydroxides, new electrodes were used for each experiment after cleaned as described by Chaudhuri and Lovley[27].

After assembly the reactors were autoclaved at 121 ^oC for 15 min. 3 M NaCl Ag/AgCl reference electrodes (RE) (RE-5B, BASi, UK) that had been sterilised using absolute ethanol and allowed to dry in air, were inserted in the working electrode (WE) chambers of potentiostatically controlled cells for controlling, and in the anodes of MFCs for monitoring of the electrodes' potential.

When used as MFCs, both chambers were filled with 200 mL of MM, inoculated when indicated. When used as potentiostatically controlled cells the WE chamber was filled with 200 mL of MM, inoculated when indicated, and the counter electrode (CE) chamber with 200 mL of PBS.

All experiments were conducted at room temperature (22±3 ^{o}C) and the reactors were covered with aluminium foil to exclude light. When N_2 or air flushing was required, the gas was first passed through a 0.2 μm filter before entering the reactor. N_2 was introduced from a compressed N_2 cylinder and air (160 mL/min) was supplied by an aquarium pump.

Fuel cell operation. *Potentiostatically controlled cells.* Potentiostatically controlled chronoamperometry experiments were conducted in two phases: in the start-up phase (phase 1) MR-1 was tested for its ability to produce anodic current and in the main phase (phase 2) Cr(VI) was spiked and the reactor was allowed to produce cathodic current for a 4-h period. In phase 1 the WE was maintained at either -500 mV (notated as $C(-El)^{ph1}$) or +300 mV (notated as $C(+El)^{ph1}$) (all potentials reported are vs. Ag/AgCl) for 14-18 h after introduction of the MR-1 cell culture into the chamber. WE chambers operated in the presence of lactate, under aerated conditions and without riboflavin or Cr(VI) during phase 1, except if indicated by notation. Phase 2 of most experiments was conducted in the same reactors as phase 1. An exception was made for the reactors notated as $(-Lac)^{ph2}$ or $(-Shew)^{ph2}$, where phase 2 was lacking the lactate-containing supernatant of phase 1, or the MR-1 cells respectively. To separate the supernatant from MR-1, the respective WE chamber contents from phase 1 were centrifuged at 5,000 × g for 20 min. In the reactors notated as $(-Lac)^{ph2}$ this centrifugation step was followed by rinsing of the MR-1 pellet twice with PBS solution to remove lactate and metabolites produced in phase 1. The MR-1 pellet was then resuspended in a clean reactor using the original electrodes from phase 1. In the reactors notated as $(-Shew)^{ph2}$ the phase 1 supernatant was additionally filtered through 0.2 μm filters to remove the MR-1 cells, in order to determine whether metabolites released in the medium could have a catalytic effect during Cr(VI) reduction. In the $C(+El)^{ph1}$(-

Lac+Rbf(1))^{ph2} cell, 1 μM riboflavin was added into the WE chamber before the beginning of phase 2 in order to investigate whether MR-1 could still express electrochemical activity in the absence of lactate but in the presence of its redox mediator. All operations were carried out by applying appropriate sterilization techniques to prevent contamination of the pure culture and the abiotic reactors. A description of the start-up phase, a summary of the experimental conditions but also details on chronoamperometry (CA) and cyclic voltammetry (CV) experiments can be found in Supporting Information (SI).

In addition to the MR-1 experiments, seven abiotic control experiments were conducted with the WE chambers containing: only MM solution (AC), MM with 30 mM lactate (AC+Lac), MM with 30 mM lactate and 0.25, 1 or 5 μM riboflavin (AC+Lac+Rbf(0.25), AC+Lac+Rbf(1) or AC+Lac+Rbf(5) respectively), MM with 1 μM riboflavin (AC+Rbf(1)), and MM with 30 mM acetate (AC+Acet).

Microbial fuel cells. Four MFCs were assembled and tested, each with different cathode conditions. The cathode of the MFC notated as "None" only contained MM and 10 mg/L Cr(VI), while the other three cathodes additionally contained: 30 mM lactate ("+Lac"), 30 mM lactate and 1 μM riboflavin ("+Lac+Rbf"), and 30 mM lactate with MR-1 ("+Lac+Shew"). Details on the MFC start-up can be found in SI.

Drops in potential across the 1,000 Ω resistors and anodic potentials vs. Ag/AgCl RE were recorded every 1 min using a datalogger (DT 505, DataTaker®). Polarization curves for "+Lac+Shew" were obtained by repeating this experiment up to the first Cr(VI) spiking step and allowing open circuit voltage to evolve after spiking. Current values were then obtained by closing the circuit using external resistors, with resistances gradually decreasing from 16,300 Ω to 7 Ω. Sufficient time was allowed between each resistor change in order to achieve a relatively stable current production.

Analytical methods and calculations. For analysis of Cr(VI) and acids the chamber contents were sampled and then centrifuged at 20,800 × g for 3 min. The concentration of dissolved Cr(VI) was determined according to Standard Method 3500-Cr B[28]. Lactic and acetic acids were measured using Ion Chromatography (882 Compact IC Plus, Metrohm) with an ion-exclusion column (Metrosep Organic Acids, 250/7.8 mm, Metrohm). A 0.5 mM H_2SO_4 solution was used as eluent and flow rate was set at 0.6 mL/min.

In MFC experiments, current was calculated from the voltage drop across the external resistor according to Ohm's law. Current density, power density and charge produced were calculated according to Logan et al.[29], while cathodic efficiency was calculated based on the fraction of electrons transferred to Cr(VI) from the cathode. Formulas are presented in SI.

RESULTS AND DISCUSSION

Potentiostatically controlled cells. ***Electrode fouling.*** Initially, cathodic current produced by the potentiostatically controlled cells was measured under abiotic conditions and in the absence of lactate. In the abiotic cathode AC, current showed a high initial response but dropped rapidly (Figure 1a), leaving 19 mg/L Cr(VI) still in solution at the end of the 4-h operation period. Two consecutive runs were then performed in AC, using the same electrode which was cleaned with ultra-pure water between successive Cr(VI) additions (spikings), but replacing the catholyte each time. It can be seen in Figure 1a that the initial current response dropped sharply from 820×10^{-3} in the 1st run to less than 100×10^{-3} A/m^2 in the 2nd and 3rd runs. A recovery of the initial current response was observed in the 4th run, however, after the electrode was cleaned using sequential alkaline, acid and ultra-pure water washing[27]. The decrease in the current response observed in this set of experiments and the recovery after electrode

cleaning indicated the formation and accumulation of inhibitory substances on the electrode surface. This inhibitory effect has also been observed when Cr(VI) was tested as a corrosion inhibitor on carbon, copper and platinum electrodes[7,23]. According to these studies, the reason for this inhibitory effect was the formation of non-conductive Cr(III) oxyhydroxide monolayers on the electrode surface at neutral and near neutral pH, which prevent further reduction of Cr(VI).

Process enhancement in the presence of lactate and riboflavin. By supplying 30 mM of lactate in the control experiment AC+Lac, the initial current peak increased when compared to that of AC (Figure 1b). However, the fall in current to background levels in AC+Lac was also quick and 17 mg/L Cr(VI) were still left in solution at the end of the 4-h operation period (Figure 2a). The higher initial current response in AC+Lac, compared to that in AC, can be partially explained by the ability of Cr(III) to form insoluble chelates with organic ligands[30,31], which may delay or even prevent the deactivation of the electrode's active sites, depending on the initial Cr(VI) and ligand concentration. While electrode deactivation was more apparent in the absence of lactate, increasing lactate concentrations in the cathode generally prolonged current production and Cr(VI) reduction in abiotic cathode MFCs (SI, MFC experiments for studying the effect of lactate in abiotic cathodes and Figure S1). A possible mechanism for this enhancement could involve more effective neutralisation of the metal's positive electrical charges with higher lactate concentrations and a more effective reduction of its affinity to the negatively charged cathode. Lactate-Cr(III) chelates can precipitate and potentially be removed from the solution at neutral pH, however separation with other organic ligands like malate and citrate will not be possible as they form soluble Cr(III)-complexes[30,31]. Acetate can also form insoluble Cr(III) complexes[31], but when

acetate was supplied instead of lactate (AC+Acet), cathodic performance showed no difference from that of AC (Figures 1b, 2a and 3a).

Filtered supernatant ($C(+El)^{ph1}(-Shew)^{ph2}$ and $C(-El)^{ph1}(-Shew)^{ph2}$) and MM containing lactate and riboflavin (AC+Lac+Rbf(0.25,1,5)) showed enhanced performance (Figures 1-3) as a result of the presence of redox mediators. Riboflavin concentration was also important, with up to 10.1 mg/L Cr(VI) reduced and 10.7 Coulombs produced when 5 μM riboflavin was supplied. In the absence of lactate (AC+Rbf(1)), however, the catalytic activity of riboflavin could not be expressed due to the rapid fouling of the electrode by Cr(III) hydroxides like $Cr(OH)^{2+}$, $Cr(OH)_2^{+}$ and $Cr(OH)_3$ present at near neutral pH. Although riboflavin has also been demonstrated to be capable of forming chelates with metal cations[22], the concentrations of riboflavin used (up to 5 μM) would be sufficient for the chelation of less than 0.26 mg/L (5 μM) of chromium ion assuming a 1:1 molar chelation ratio.

CV experiments at the end of the 4-h experimental period showed that AC+Lac+Rbf(1) gave a clear catalytic current at low applied potentials (<-400 mV) with similar patterns also observed in the filtered supernatants $C(+El)^{ph1}(-Shew)^{ph2}$ and $C(-El)^{ph1}(-Shew)^{ph2}$ (Figure 4a). This indicates that even in the absence of active cells, compounds released in the medium are responsible for Cr(VI) electroreduction; and is in agreement with previous studies where compounds released in the medium by *Shewanella* were found to be able to catalyse oxygen electroreduction[17,32,33].

Cathodic current was minimal in all potentiostatically controlled cells which did not contain lactate, although MR-1 and/or riboflavin were present in some cases (Figure 4b). By comparing the CV graphs for $C(+El)^{ph1}$, $C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}$, AC+Lac+Rbf(1) and AC+Rbf(1), it is clear that the cathodic catalytic current produced in $C(+El)^{ph1}$ and AC+Lac+Rbf(1) could be suppressed in the absence of lactate

(C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}, AC+Rbf(1)). Cathodic current was also suppressed by the end of the operation period in the abiotic experiment with 30 mM lactate but without MR-1 or riboflavin (AC+Lac) (Figure 4c). The above results indicate that the effective expression of cathodic current under the conditions described, required the presence of both lactate and redox mediators.

Effect of MR-1 pre-treatment conditions on biocathode performance. Oxygen enhanced anodic current production during phase 1 (SI, Figure S2a), probably due to the higher energy obtained by MR-1 during complete lactate oxidation under aerated conditions[34,35]. A thicker biofilm that could be visually observed in this instance and a presumably higher production of redox mediators which exhibited (anodic) current onsets at potentials similar to the midpoint potentials of flavins[22] (Figure S3), are also possible reasons for this enhanced performance. Furthermore, MR-1 demonstrated the ability to enhance electroreduction of its own soluble metabolic products even when lactate was present (flask experiment description and Figures S4 and S5) and it is possible that these products could later participate in reduction-oxidation cycles with Cr(VI) as the final electron acceptor. From a metabolic point of view this possibility is remarkable, considering the interconnected reduction pathways for Cr(VI) and flavins[21,36].

When MR-1 was continuously supplied with oxygen in the presence of lactate and a poised electrode at +300 mV during phase 1 (C(+El)^{ph1}), dosing with Cr(VI) gave a lower initial current response than in the absence of MR-1 (Figure 1b,c). Current was sustained for a longer time in C(+El)^{ph1}, however, probably due to the bacterial energy-gaining processes and the abiotic redox cycles promoted by electron shuttle mediators. The charge produced in C(+El)^{ph1} by the end of the 4-h period was 5.5 and 1.7 times more than in AC (no lactate) and AC+Lac (30 mM lactate) (Figure 3a). Also the current

produced in C(+El)^{ph1} at the end of the experiment (52 × 10^{-3} A/m^2) was higher than that in AC (1 × 10^{-3} A/m^2) and AC+Lac (9 × 10^{-3} A/m^2), despite the fact that C(+El)^{ph1} had a considerably lower Cr(VI) concentration (11 mg/L Cr(VI)) compared to AC (19 mg/L Cr(VI)) and AC+Lac (17 mg/L Cr(VI)) (Figure 2).

C(+El-Air)^{ph1} showed lower cathodic activity during phase 2, probably as a result of the limited electrophilic activity of the MR-1 cells during phase 1. MR-1 obtained less energy from lactate during phase 1 in this instance and thus cells occupying the electrode surface might have become less active. However, process was enhanced upon addition of riboflavin (C(+El-Air+Rbf(1))^{ph1}), when 4 mg/L more Cr(VI) was reduced (Figure 2b) and 1.9 times more charge was produced (Figure 3a) compared to C(+El-Air)^{ph1}. Although cathodic Cr(VI) reduction was observed in C(+El-Air+Cr(VI))^{ph1}, the charge produced was still lower than that of C(+El)^{ph1} and C(-El)^{ph1} (Figure 3a). This process enhancement seen in C(+El)^{ph1} and C(-El)^{ph1} (aerobic pre-treatment) compared to C(+El-Air)^{ph1} (anaerobic pre-treatment) and C(+El-Air+Cr(VI))^{ph1} (anaerobic pre-treatment with Cr(VI)) indicates that cathodic electrochemical activity can be enhanced when oxygen is supplied during the pre-treatment step. The charge produced in both C(+El-Air)^{ph1} and C(+El-Air+Cr(VI))^{ph1} during the 4-h test period was similar to that in the abiotic AC+Lac (Figure 3a). The current in C(+El-Air)^{ph1} and C(+El-Air+Cr(VI))^{ph1} (32 × 10^{-3} A/m^2 and 40 × 10^{-3} A/m^2) was around 4 times that in AC+Lac towards the end of test, however, despite the lower Cr(VI) concentrations in the MR-1 containing chambers (Figure 2).

C(+El)^{ph1} produced 15 % more charge than that produced by C(-El)^{ph1} by the end of the 4-h operation period and C(+El)^{ph1}(-Shew)^{ph2} produced 32 % more charge than C(-El)^{ph1}(-Shew)^{ph2} (Figure 3a). This can be linked to the enhanced anodic current showed in the CV tests after phase 1, in the experiments where both oxygen and the electrode

were supplied as the electron acceptors during phase 1, compared to the experiments where the electron acceptor was more limited (C(-El)^{ph1}, C(-El)^{ph1}(-Lac)^{ph2}, C(-El)^{ph1}(-Shew)^{ph2}, C(+El-Air)^{ph1}) (SI, Figure S3). The CV experiments provide further evidence that the pre-treatment conditions play an important role in the catalytic activity of MR-1 (Figure 4c). The CV results showed that MR-1 had an active catalytic role in C(+El)^{ph1}, as it provided cathodic current over a broader potential range, not only when compared to abiotic AC+Lac+Rbf(1) (Figure 4b), but also to C(-El)^{ph1} and C(+El-Air)^{ph1} (Figure 4c). Catalytic activity of the biocatalyst over a broad potential range is important for power-producing applications such as MFCs, where imposed overpotentials will increase the energy losses of the system.

Microbial fuel cells. The experiments described above showed that an improved catalytic activity of MR-1 could be achieved by manipulation of culture conditions: this demonstration, however, was carried out under energy-consuming potentiostatic control. The efficiency of power-producing devices such as MFCs is restricted by internal resistances and potential losses[37] and therefore the conditions applied in the potentiostatically controlled experiments needed to be verified in MFCs. As the primary purpose in this instance was to compare the different conditions, the same simple reactor configuration was used, which had a relatively small ion exchange membrane area and low electrode TASA to volume ratio. In addition, all MFC anodes were operated under aerated conditions and had comparable anodic potentials under closed circuit conditions throughout operation: -447±10 mV in "None", -446±17 mV in "+Lac", -450±13 mV in "+Lac+Rbf" and -432±28 mV in "+Lac+Shew".

MFC "None" which operated without lactate and MR-1 in the cathode produced an initial current of 84 μA; the system stopped operating within the first few hours, however, although there was still 9 mg/L Cr(VI) available (Figure 5a). This behaviour

was similar to that of the potentiostatically controlled cathodes without lactate, as already discussed previously. MFC "+Lac" which contained 30 mM lactate in the cathode achieved an initial current of 145 μA, which dropped to background levels (4 μA; around 0.3 mg/L/day of Cr(VI) reduced assuming 100 % cathodic efficiency) by the end of the 48h operation period, with 43 % of Cr(VI) still in solution. MFC "+Lac+Rbf", with an additional 1 μM riboflavin, produced an initial current of 153 μA. Its performance was not significantly better than that of "+Lac", however, as the addition of riboflavin only enhanced the process at low applied potentials (Figure 4a) which are difficult to achieve in MFCs. Also, only a negligible increase in current production was observed after a further increase in Cr(VI) concentration to 10 mg/L in both "+Lac" and "+Lac+Rbf" (Figure 5a). This indicates the dominating effect of Cr(III) precipitates, which finally managed to form and deactivate the cathodes.

On the other hand, despite the lower initial value of 65 μA (32.5 mA/m^2 TASA), current was produced by MFC "+Lac+Shew" (MR-1 biocathode) after every Cr(VI) addition (Figure 5b). The maximum current produced by "+Lac+Shew" occurred after the first addition and was comparable to the 46.6 mA/m^2 observed by Tandukar et al.[11], who used a mixed culture biocathode with a similar configuration (electrode TASA to working volume ratio: 16 m^2/m^3 [vs. 340-9,600 m^2/m^3 in other studies[8-10]], $Cr(VI)_0$: 22 mg/L, $R_{ext.}$: 1,000 Ω). Current production in "+Lac+Shew" responded immediately to every Cr(VI) addition even though air was continuously supplied to the anode: this clearly indicates that Cr(VI) has an advantage over oxygen for electroreduction when organic carbon is provided in the biocathode. In total, 3.5 mg Cr(VI) were reduced by the cathode electrode of "+Lac+Shew" by the end of the MFC operation, compared to the maximum Cr(VI) reduction of around 1.2 mg Cr(VI) by "+Lac" (Figure 5c). In the absence of an electrode, Cr(VI) reduction was considerably slowed down after the first

3-4 days, regardless of the biomass concentration and the presence or absence of lactate (Figure 5d; for Cr(VI) reducing flask experiments description, see SI). In the "+Lac+Shew" MFC a similar plateau was observed for the pathways other than electrode oxidation, which accounted for only 1.1 mg Cr(VI) reduced within the last 240 h. The relatively slow decrease of the Cr(VI) bio-electroreduction rate in "+Lac+Shew" on one hand, along with the rapidly decreasing rate of Cr(VI) reduction due to lactate utilization and other pathways on the other hand, were the reasons why an increase in cathodic efficiency was observed, from 14 % (1st addition) to 64 % (5th addition) and to 81 % when the cathode was switched to potentiostatic control at -500 mV (6th addition) (Figure 5e). By the end of operation, Cr(VI) bio-electroreduction accounted for around 46 % of the total Cr(VI) reduction in "+Lac+Shew", clearly showing that MR-1 could utilise both the electrode and lactate as an electron donor.

While it is well established that mixed culture biocatalysts will yield higher current and power densities than pure cultures[38], the "+Lac+Shew" MFC produced a maximum of 172 nW/cm^2 (Figure 5f). However, its Cr(VI) reducing performance was considerably higher compared to the performance of the only other study which investigated Cr(VI) reduction in an MFC biocathode with MR-1 as the biocatalyst[16]. In contrast to the current study, the biocathodes used in these experiments were supplied with fumarate as an electron acceptor in a biocathode inoculated with MR-1 as part of a pre-treatment step. When the electron acceptor was switched to Cr(VI), the overall cathodic efficiency in the system was around 67 %, which decreased to around 42 % in the last 3 days of a 9-day operation period. According to the authors, this cathodic efficiency decrease was due to the unavoidable Cr(VI) reduction by other electron sources (presumably dead cells and succinate, the metabolic by-product of fumarate), towards the end of the operation period. It is possible that electrode fouling by Cr(III)

hydroxides over time was one of the reasons for this metabolic shift when MR-1 started utilising electron donors other than the electrode. In the present study, although the external resistor was 100 times higher (1,000 Ω vs. 10 Ω) compared to that used by Hsu et al.[16], the electrode in the "+Lac+Shew" cathode reduced considerably higher amount of Cr(VI) than the one in Hsu et al.[16], i.e. 1,750 mg/m^2 in 8 days vs. an estimated 87 mg/m^2 in 9 days. Considering the total Cr(VI) reduction in the biocathodes, i.e. Cr(VI) reduced by electrical current and other pathways, around 5,000 mg/m^2 of Cr(VI) were reduced in "+Lac+Shew" in 8 days of MFC operation, whereas only around 130 mg/m^2 of Cr(VI) reduction was achieved in 9 days in Hsu et al.[16]. Overall, the abiotic cathode in Hsu et al.[16] performed equally or better than many *Shewanella* strains, reducing around 139 mg/m^2 Cr(VI) in 9 days vs. 104 mg/m^2 in the MR-4 cathode and 130 mg/m^2 in the MR-1, PV-4 and ANA-3 cathodes. On the contrary, MR-1 in our study exhibited a clear enhancement of the process and the biocathode clearly outperformed the abiotic cathode. By adding lactate along with MR-1 pre-treated under the conditions described, the electrocatalytic activity and Cr(VI) reducing ability of the cathode were considerably increased and this is the first study to demonstrate this process enhancement.

Freguia et al.[17] have reported that stored carbon within *Shewanella* cells was sufficient to inhibit cathodic oxygen electroreduction, despite the fact that no organic carbon was provided during the experiment. This observation is in line with the rapid current decrease seen in the C(-El)^{ph1} experiments during phase 1, even in the absence of lactate (SI, Figure S2b). Whilst MR-1 can cause low redox conditions and inhibit oxygen electroreduction within a few hours when lactate is available, this did not seem to be the case for Cr(VI). MR-1 used both lactate and the electrode as the electron donor for Cr(VI) reduction, and cathodic efficiency increased over time.

Current is believed to be enhanced in three ways when lactate was present in the biocathode: (i) Cr(III)-lactate interaction delayed the deactivation of the electrode, (ii) electron shuttle mediators produced during phase 1 mediated electrons from the electrode to Cr(VI) and promoted indirect Cr(VI) reduction, and (iii) the presence of lactate and redox mediators produced during phase 1 enabled MR-1 to be actively involved in the electrode oxidation process and drive direct or indirect Cr(VI) reduction.

Practical implications. The addition of lactate in a Cr(VI) reducing MFC cathode considerably increased the operating life and the Cr(VI) reducing ability of the electrode, which are of critical importance for the implementation of the MFC technology in Cr(VI) remediation. In the presence of MR-1, the Cr(VI) reducing ability of the electrode was further increased by around 3 times compared to the abiotic control, and in total, Cr(VI)-wastewater bioremediation was considerably enhanced by simultaneously providing two separate electron sources to the bacteria; lactate and the poised electrode. As a result, the electrode accounted for almost the same amount of Cr(VI) reduced by MR-1 and lactate alone, indicating that bio-electroreduction and MFC technology could be considered in addition to conventional Cr(VI) bioremediation.

Furthermore, the demonstrated capability of MR-1 to operate both in the anode and the cathode could offer more flexibility in applying the MFC technology for Cr(VI) remediation. In this occasion, microbes, lactate and redox mediators needed in the cathode could be provided by recirculating anodic effluent to the cathode. The reverse action is also possible to allow COD removal and Cr(VI) polishing of the cathodic effluent: however, further research would be required to elucidate to what extent and under what conditions (e.g. biomass density in the anode), low levels of Cr(VI) present in the cathodic effluent affect the established redox conditions in the anode.

ASSOCIATED CONTENT

Supporting Information

Description of chronoamperometry (CA) and cyclic voltammetry (CV) experiments, description of the start-up phase in potentiostatically controlled experiments, Tables S1-S2, MFC start-up description, calculations, MFC experiments for studying the effect of lactate in abiotic cathodes, Figures S1-S5, electrochemical activity of MR-1 during phase 1 of potentiostatically controlled cells, flask experiment description and Cr(VI) reducing flask experiments description. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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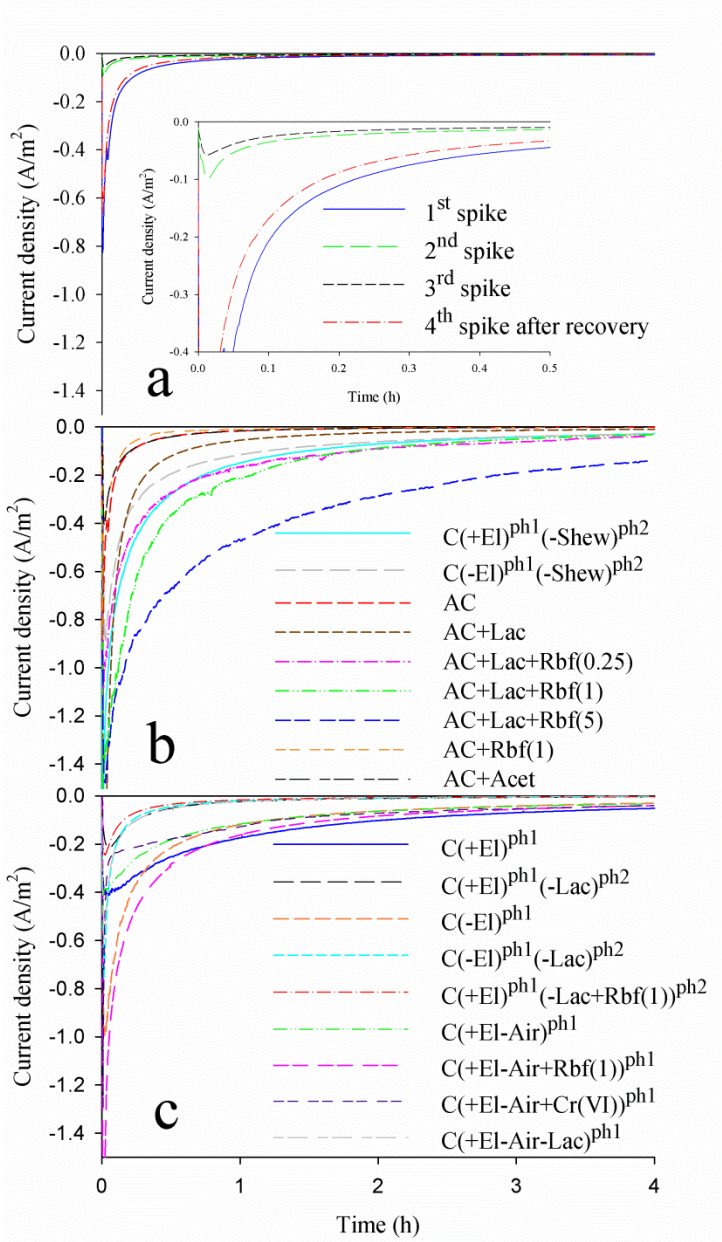


Figure 1. Chronoamperometry profiles at -500 mV for the potentiostatically controlled cathodes. (a) AC experiments (inset: zoom in for the first 0.5 h); (b) experiments without MR-1 cells; (c) experiments with MR-1 cells.

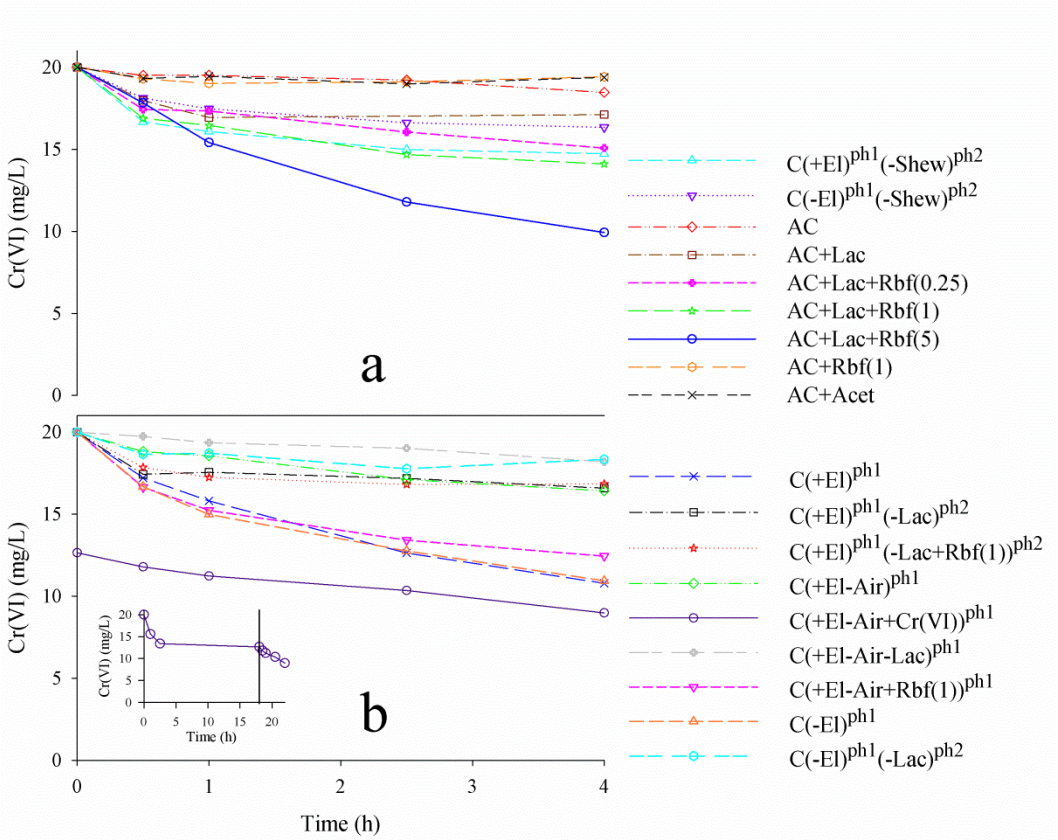


Figure 2. Soluble Cr(VI) concentration in the potentiostatically controlled cathodes. (a) experiments without MR-1 cells; (b) experiments with MR-1 cells (inset: C(+El-Air+Cr(VI))^{ph1} throughout both phases 1 and 2, with phase 2 start indicated by the vertical solid line).

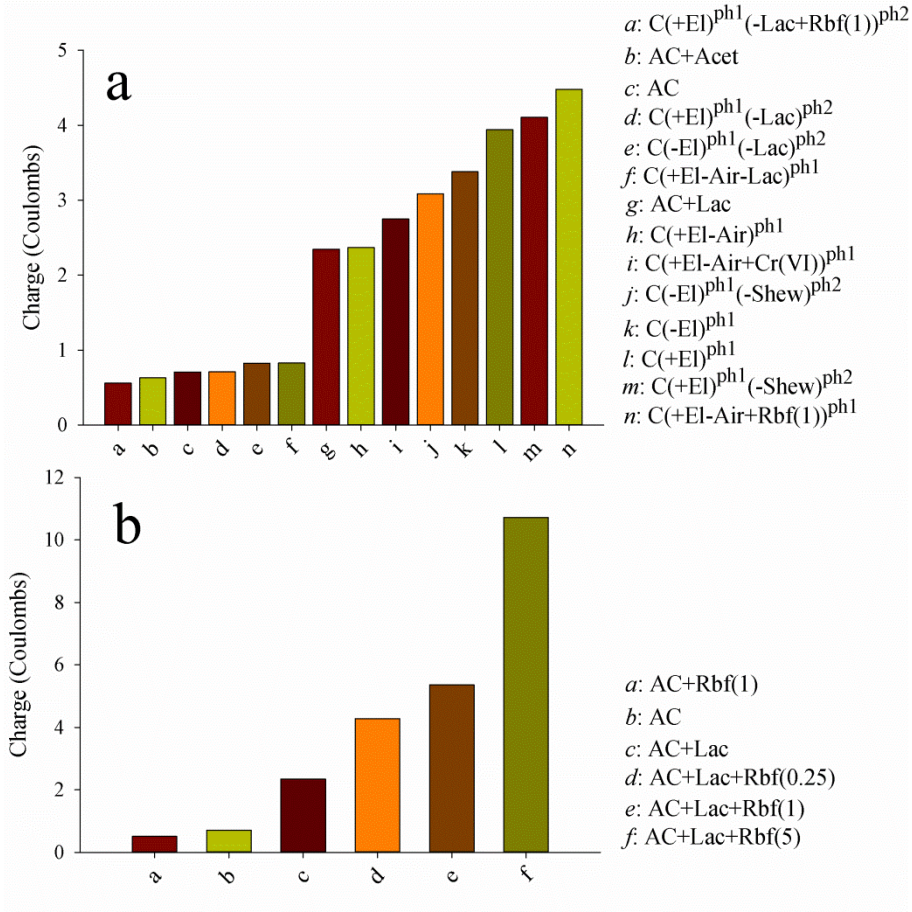


Figure 3. Charge produced after the 4-h operation period. (a) comparison of MR-1 with abiotic controls; (b) comparison of abiotic experiments with riboflavin (AC+Lac+Rbf(0.25,1,5) and AC+Rbf(1)), with AC and AC+Lac.

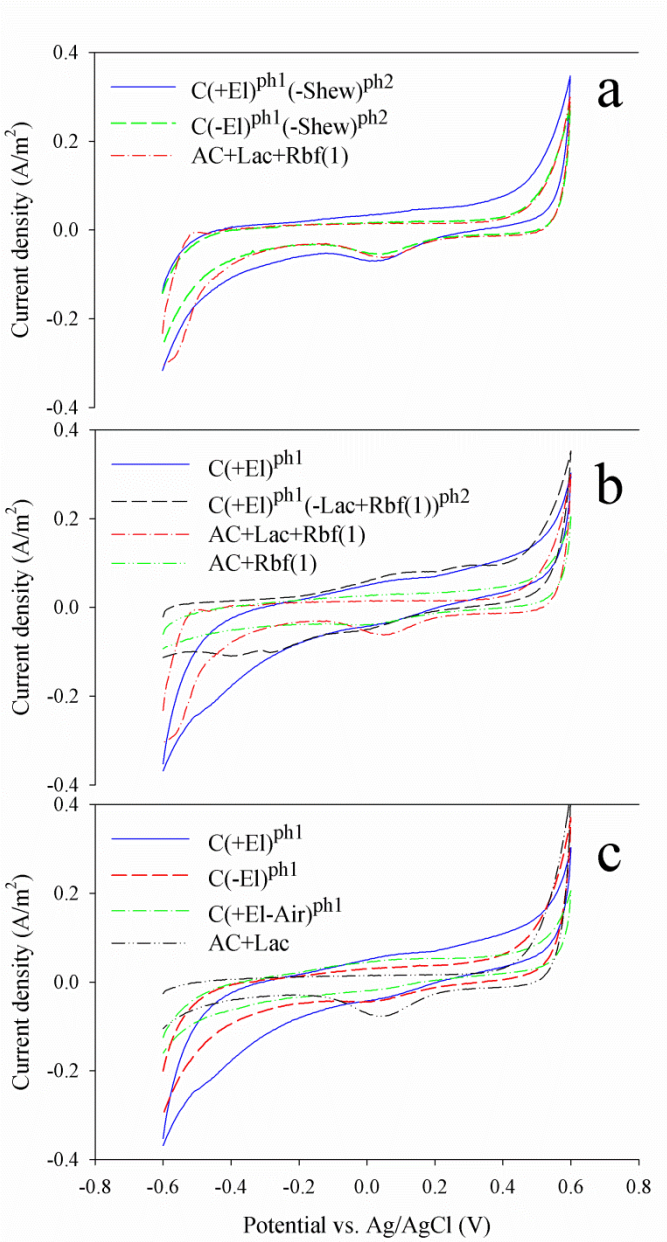


Figure 4. Cyclic voltammograms at the end of phase 2. Scan rate is 5 mV/sec. (a) comparison of the filtered supernatants C(+El)^{ph1}(-Shew)^{ph2} and C(-El)^{ph1}(-Shew)^{ph2} with AC+Lac+Rbf(1); (b) comparison between C(+El)^{ph1}, C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}, AC+Lac+Rbf(1) and AC+Rbf(1), showing the importance of lactate on the expression of catalytic activity; (c) comparison between C(+El)^{ph1}, C(-El)^{ph1}, C(+El-Air)^{ph1} and AC+Lac, showing the importance of pre-treatment conditions.

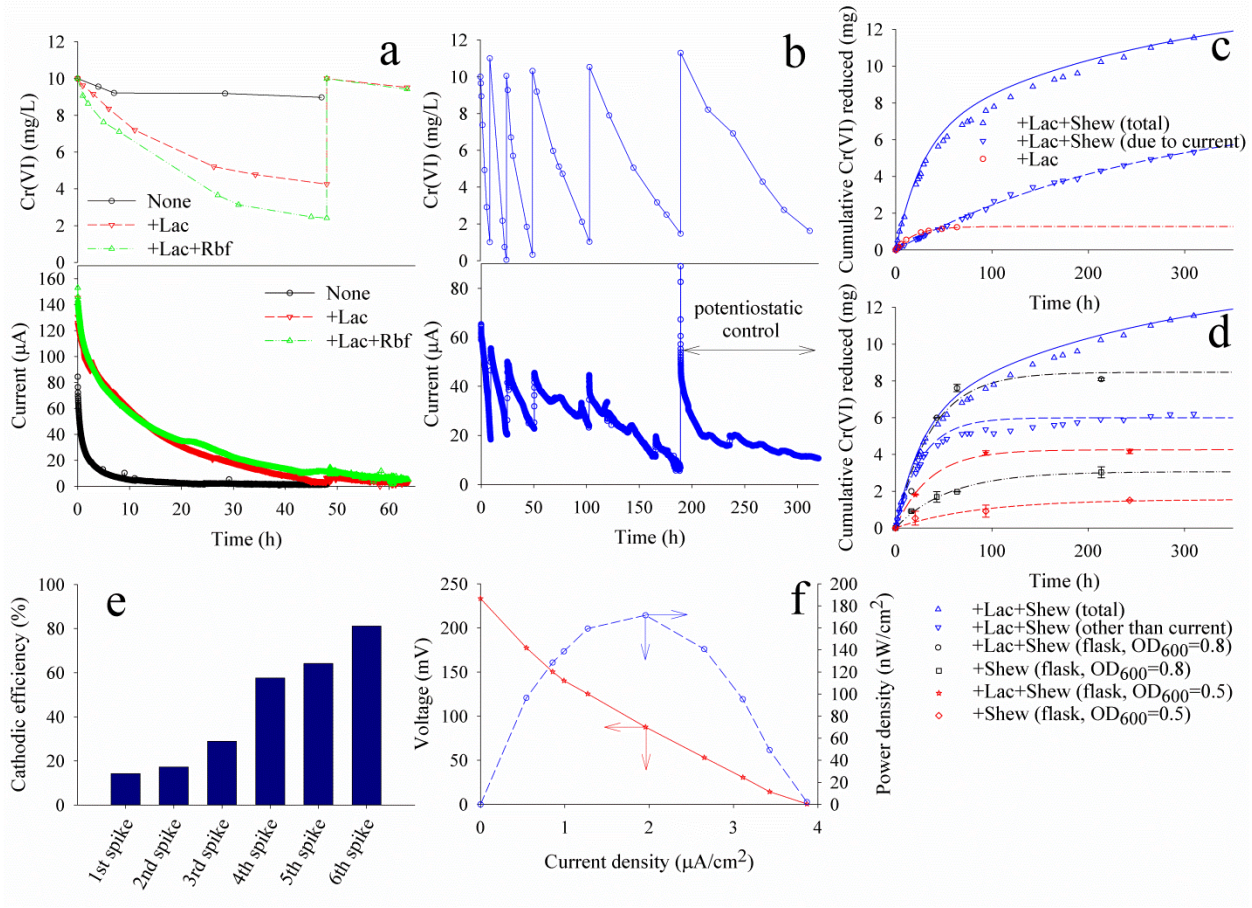
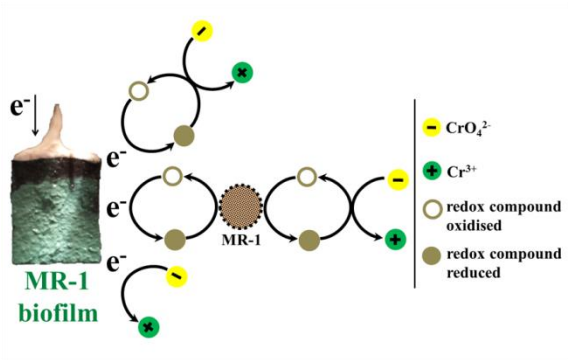


Figure 5. Parameters during MFC operation. (a) current production and Cr(VI) concentration in MFCs with abiotic cathodes; (b) current production and Cr(VI) concentration in the biocathode MFC; (c) comparison of the Cr(VI) reduced in the abiotic lactate MFC with that in the biocathode MFC due to current (equations of the graphs are given in SI, Table S2); (d) comparison of the Cr(VI) reduced in the flasks with that in the biocathode MFC due to pathways other than electrode oxidation (planktonic MR-1 in the "Lac+Shew" MFC had OD_{600}= 0.5 at time 0; equations of the graphs are given in SI, Table S2; error bars represent the min and max of the measurements); (e) cathodic efficiency of the biocathode MFC during MFC operation (spikes 1-5) and during potentiostatic control of the cathode at -500 mV (6^{th} spike); (f) polarization curves of the biocathode MFC after the first Cr(VI) spike.



Abstract art.

Supporting information

Enhanced performance of hexavalent chromium reducing cathodes in the presence of *Shewanella oneidensis* MR-1 and lactate

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Contents:

Description of chronoamperometry (CA) and cyclic voltammetry (CV) experiments

For CA and CV experiments, the cells operated in a three-electrode configuration with a potentiostat (PG580, Uniscan Instruments Ltd., UK) controlling the potential difference between the WE and the RE. The potentiostat was interfaced to a computer and UiEchem™ software was used to record the current every 4 seconds in CA and every 1 second in CV experiments. CV analysis was conducted for each set of experiments, to test the electrocatalytic activities of the WE chambers. CVs were performed from -600 mV to +600 mV (all potentials reported are vs. Ag/AgCl), with a scanning rate of 5 mV/sec under quiescent, N_2-flushed conditions. CV experiments were repeated until consistent results were obtained (normally after 2 to 5 times). The WE chambers were stirred at 250 rpm during CA and between CV experiments, and both chambers were stirred during MFC operation.

Description of the start-up phase in potentiostatically controlled experiments

A number of operational conditions were tested during phase 1, in which MR-1 was allowed to produce the metabolites needed for electron transfer and to form a biofilm. At the end of phase 1, CV tests were conducted and then the potentiostat potential was set at -500 mV so that the electrode could act as the electron donor for Cr(VI) reduction. All WE chambers except that in C(+El-Air+Cr(VI))^{ph1} were flushed with N_2 for one extra hour between phases 1 and 2, and current was allowed to stabilise to background levels at -500 mV. This was normally less than 7×10^{-3} A/m^2, and the value recorded was deducted from the result. This stabilisation step was omitted in C(+El-Air+Cr(VI))^{ph1}, however, as Cr(VI) was present from phase 1 in this reactor.

Table S1. Experimental conditions applied in potentiostatically controlled experiments

Cell #	Phase 1	Phase 2 (-500 mV, Cr(VI) and N_2)
$C(+El)^{ph1}$	30 mM lactate, air, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (20 mM lactate)
$C(+El)^{ph1}(-Lac)^{ph2}$	30 mM lactate, air, 14 h, +300 mV (common phase 1 for reactors $C(+El)^{ph1}(-Lac)^{ph2}$ and $C(+El)^{ph1}(-Shew)^{ph2}$)	MR-1 from phase 1 resuspended in another reactor with the same electrode (no lactate)
$C(+El)^{ph1}(-Shew)^{ph2}$		supernatant of phase 1 filtered (0.2 μm) and resuspended in another reactor (17 mM lactate)
$C(-El)^{ph1}$	30 mM lactate, air, 14 h, -500 mV	MR-1 in the same reactor as in phase 1 (25 mM lactate)
$C(-El)^{ph1}(-Lac)^{ph2}$	30 mM lactate, air, 14 h, -500 mV (common phase 1 for reactors $C(-El)^{ph1}(-Lac)^{ph2}$ and $C(-El)^{ph1}(-Shew)^{ph2}$)	MR-1 from phase 1 resuspended in another reactor with the same electrode (no lactate)
$C(-El)^{ph1}(-Shew)^{ph2}$		supernatant of phase 1 filtered (0.2 μm) and resuspended in another reactor (23 mM lactate)
$C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}$	30 mM lactate, air, 14 h, +300 mV	MR-1 from phase 1 resuspended in another reactor with the same electrode and 1 μM riboflavin (no lactate)
$C(+El\text{-}Air)^{ph1}$	30 mM lactate, N_2, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (28 mM lactate)
$C(+El\text{-}Air+Rbf(1))^{ph1}$	30 mM lactate, 1 μM riboflavin, N_2, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (28 mM lactate)
$C(+El\text{-}Air+Cr(VI))^{ph1}$	30 mM lactate, Cr(VI), N_2, 18 h, +300 mV	MR-1 in the same reactor as in phase 1 (25 mM lactate)
$C(+El\text{-}Air\text{-}Lac)^{ph1}$	N_2, 14 h (no lactate), +300 mV	MR-1 in the same reactor as in phase 1 (no lactate)
AC	n/a	MM only (abiotic)
AC+Lac	n/a	MM + 30 mM lactate (abiotic)
AC+Lac+Rbf(0.25, 1 or 5)	n/a	MM + 30 mM lactate + 0.25, 1 or 5 μM riboflavin (abiotic)
AC+Rbf(1)	n/a	MM + 1 μM riboflavin (abiotic)

AC+Acet	n/a	MM + 30 mM acetate (abiotic)

MFC start-up description

Anodes in MFCs "None", "+Lac" and "+Lac+Rbf" were inoculated with MR-1 and left overnight with air continuously flushed (160 mL/min), until the anodic potential under open circuit conditions dropped and stabilised at around -470 mV. In the cathode N_2 was flushed overnight and then continuously during the experiment. After stabilisation, the circuits were closed using 1,000 Ω external resistors and Cr(VI) was spiked (time zero). In the bioanodes, air continued to be flushed for the duration of the experiment and lactate was supplied at intervals to ensure electron donor availability and to promote stable anodic potentials.

The MFC experiment "+Lac+Shew" was conducted in two phases. In the start-up phase 1, chamber 1 (Ch1) of +Lac+Shew contained 30 mM lactate and was inoculated with MR-1. MR-1 was then allowed to produce anodic current (1,000 Ω external resistor) for 3 d, while air was continuously flushed into both the anode (Ch1) and the abiotic cathode (Ch2) which only contained MM. After phase 1, Ch2 was inoculated with MR-1 and lactate was added in both chambers to give a 30 mM initial concentration. Ch1 was then continuously flushed with N_2 and the system was allowed to equilibrate for 2 h until the current dropped to background levels (<4 μA). At this point Cr(VI) was spiked into Ch1 to give an initial concentration of 10 mg/L, while Ch2 continued to be flushed with air to test whether Cr(VI) has an advantage over oxygen as an electron acceptor when both MR-1 and lactate are present. From then on, current flow was reversed, with Ch1 acting as the biocathode and Ch2 as the bioanode. Additional doses of 10 mg/L Cr(VI) were given at intervals to ensure the presence of Cr(VI) in the cathode at all times.

Calculations

Current (A) and current density (A/m^2) were calculated according to (1) and (2), respectively:

$$I=E_{cell}/R_{ext} \quad (1)$$

$$I_{cat}=I/A_{cat} \quad (2)$$

where I: current (A); E_{cell}: voltage drop across R_{ext} (V), recorded at a fixed time interval; R_{ext}: external resistor (Ω); I_{cat}: current density (A/m^2); A_{cat}: total apparent surface area of the cathode (m^2).

Power (W) and power density (W/m^2) were calculated according to (3) and (4), respectively:

$$P=E_{cell}^2/R_{ext} \quad (3)$$

$$P_{cat}=E_{cell}^2/(R_{ext}A_{cat}) \quad (4)$$

The charge transferred from the cathode electrode was calculated based on current measurements at fixed time intervals, as:

$$C_1=\Sigma I\Delta t \quad (5)$$

where C_1: the proportion of charge (Coulombs) transferred to Cr(VI) by the electrode; I: current (A) recorded at a fixed time interval Δt; Δt: time interval (s).

The total amount of charge transferred to Cr(VI), assuming complete reduction of Cr(VI) to Cr(III), was calculated as follows:

$$C_2=nFv\Delta[Cr(VI)]/M \quad (6)$$

where C_2: total charge (Coulombs) transferred to Cr(VI) for complete Cr(VI) to Cr(III) reduction; n: number of moles of electrons transferred per mole of Cr(VI) reduced to Cr(III), 3 moles/mol of Cr(VI); F: Faraday constant, 96,485.3 Coulombs/mol of electrons; v: catholyte volume (L); $\Delta[Cr(VI)]$: Cr(VI) concentration reduction at a given time interval (g/L); M: atomic weight of Cr, 52 g/mol of Cr.

Cathodic efficiency (CE, %) was calculated as below:

$$CE=C_1/C_2\times100\% \quad (7)$$

The cumulative mass of Cr(VI) (g) reduced due to current was calculated according to the cumulative electrical charge produced, as below:

$$\Sigma Cr(VI)=M\Sigma I\Delta t/(nF) \quad (8)$$

MFC experiments for studying the effect of lactate in abiotic cathodes

To study the effect of lactate in Cr(VI) reducing abiotic cathodes, 4 lactate concentrations were studied, i.e. 0, 2, 20 and 200 mM. The same reactor design described in the "Experimental Section" was used and all experiments were ran in triplicates. Anode effluent (15 mL) from an MFC initially inoculated with anaerobic sludge, was used to inoculate each of the 3 MFC anodes which were connected with the cathodes through 1,000 Ω external loads. Then, the MFC reactors were let operate with aerated cathodes until current stabilised at around 120 ± 4 μA for more than 3 d. The catholyte was then replaced with an anaerobic phosphate buffer (50 mM, pH 7) which also contained Na-DL-Lactate at the concentrations mentioned (0, 2, 20, 200 mM). To keep the solution's ionic strength at similar levels, NaCl was added to give a final Na^+ concentration of 200 mM in all cathodes. The experimental period started by adding 10 mg-Cr(VI)/L in the cathodes and connecting the two chambers with the external load of 1,000 Ω. N_2 was continuously flushed in both chambers and all experiments were ran at room temperature (22±3 ^oC). To ensure that the anode was not considerably affecting the cathode's performance, acetate was supplied in the anodes before the start of each experiment to increase acetate concentration at around 40 mM. In addition, the anodic potential and pH were monitored; the anodic potential remained at -542 mV ± 20 mV vs. Ag/AgCl under closed circuit conditions, while anodic pH increased during start-up and remained at around 7.9 ± 0.2 throughout operation in all experiments. Results are presented in Figure S1.

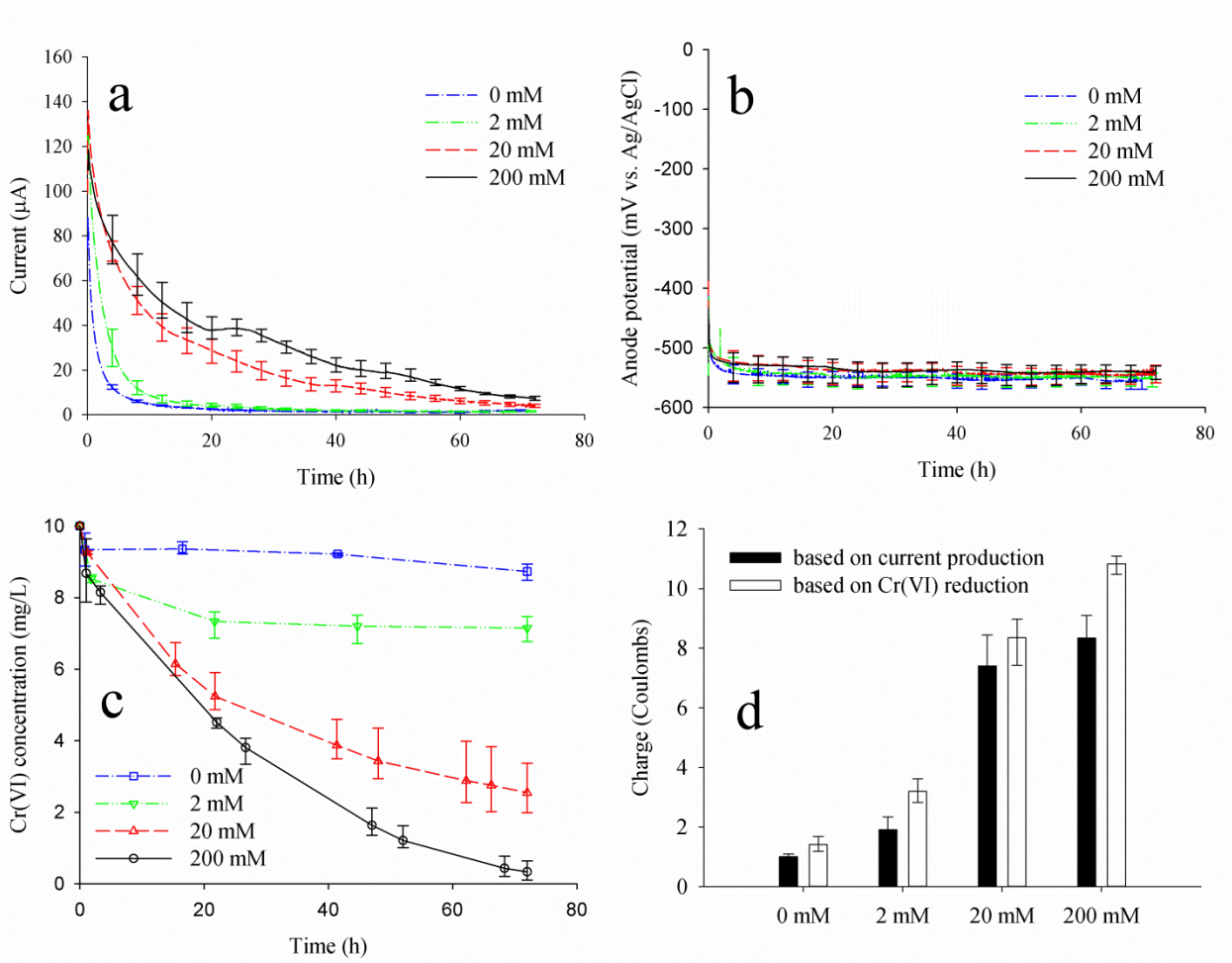

Figure S1. The effect of different lactate concentrations present in the cathode at pH 7. Values reported are average of triplicate experiments and error bars represent the min and max of the measurements from the three reactors running in parallel. (a) current evolution ($R_{ext.}$= 1,000 Ω) (error bars added every 4 h); (b) anode potentials (error bars added every 4 h); (c) Cr(VI) remaining in solution; (d) charge produced by the end of the 72 h operation period (charge calculated based on both current production and Cr(VI) reduction).

Electrochemical activity of MR-1 during phase 1 of potentiostatically controlled cells.

When MR-1 was kept under anaerobic conditions without any external redox mediator addition ($C(+El\text{-}Air)^{ph1}$), lactate consumption caused a current increase during the first 3 h which then stabilised at a level of 40×10^{-3} A/m^2 (Figure S2a). Air flushing ($C(+El)^{ph1}$ reactors with air) had a positive effect, with anodic current production constantly increasing and reaching 270×10^{-3} A/m^2 by the end of phase 1. Enhancement of anodic current by redox mediators was confirmed when a 7-fold higher value (compared to that of $C(+El\text{-}Air)^{ph1}$) was produced and remained stable when 1 μM of riboflavin was added under anaerobic conditions ($C(+El\text{-}Air+Rbf(1))^{ph1}$). On the other hand, anodic current remained close to background levels (around 20×10^{-3} A/m^2) when lactate was not supplied to MR-1 ($C(+El\text{-}Air\text{-}Lac)^{ph1}$). The presence of 20 mg/L Cr(VI) ($C(+El\text{-}Air+Cr(VI))^{ph1}$) totally inhibited anodic current production, and this was reflected in the high open circuit potential observed after phase 1 (+150 mV vs. -350 mV in $C(+El\text{-}Air)^{ph1}$, -400 mV in $C(+El)^{ph1}$ and -430 mV in $C(+El\text{-}Air+Rbf(1))^{ph1}$ under N_2 flushing anaerobic conditions). When MR-1 was aerated and the electrode was poised at -500 mV ($C(-El)^{ph1}$, $C(-El)^{ph1}(-Lac)^{ph2}$, $C(-El)^{ph1}(-Shew)^{ph2}$), oxygen electroreduction was inhibited, as a result of the heterotrophic oxygen consumption (Figure S2b).

CV experiments at the end of phase 1 showed the onset of anodic current at -0.45 to -0.40 mV in the presence of MR-1 (Figure S3). Also, poising the electrode at +300 mV under aerated conditions during phase 1 ($C(+El)^{ph1}$, $C(+El)^{ph1}(-Lac)^{ph2}$, $C(+El)^{ph1}(-Shew)^{ph2}$, $C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}$) gave CV graphs with higher anodic current than when the electrode was poised at -500 mV under aeration ($C(-El)^{ph1}$, $C(-El)^{ph1}(-Lac)^{ph2}$, $C(-El)^{ph1}(-Shew)^{ph2}$) or when phase 1 was kept anaerobic ($C(+El\text{-}Air)^{ph1}$) (Figure S3b).

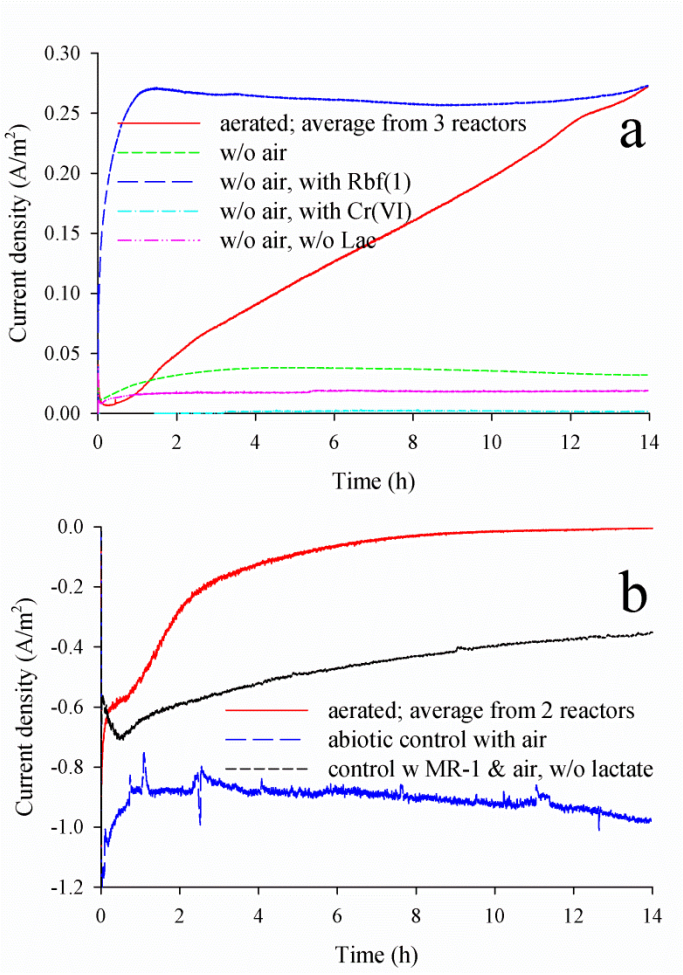

Figure S2. Chronoamperometry profiles at +300 mV (a) and at -500 mV (b) during phase 1 of the potentiostatically controlled experiments.

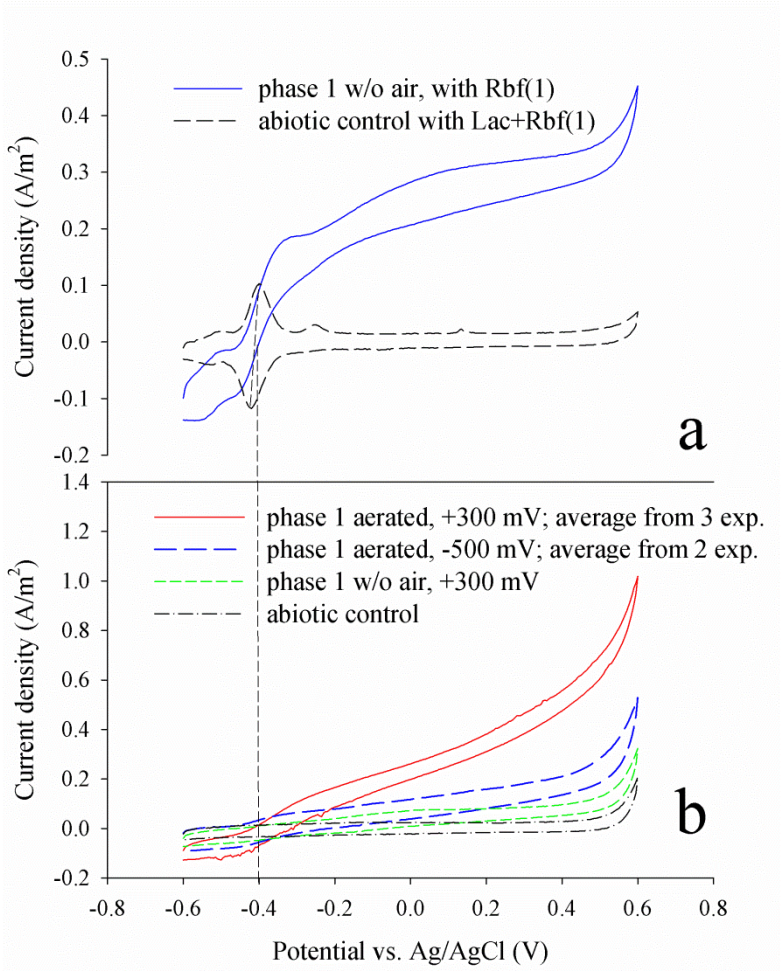

Figure S3. Cyclic voltammograms at the end of phase 1 of the potentiostatically controlled experiments. Scan rate is 5 mV/sec. (a) experiments with riboflavin addition; (b) mediatorless experiments with MR-1 under different pre-treatment conditions.

Flask experiment description

After initial growth in Luria-Bertani-50 mM phosphate buffer medium (pH 7.0), MR-1 was harvested by centrifugation (5,000 x g, 4 ^{o}C, 20 min), rinsed twice with 100 mM NaCl-50 mM phosphate buffer saline (PBS) solution (pH 7.0) and resuspended in 2 × 100 mL of the minimal medium which was amended to contain 30 mM Na-Lactate. The MR-1 containing flasks were then incubated in an orbital shaker incubator (25 ^{o}C, 200 rpm) for 24 h. After 24 h, the flasks' content was centrifuged and then the supernatant was filter-sterilized (0.2 μM pore size) and added into the working electrode chamber of a potentiostatically controlled cell for cyclic voltammetry and chronoamperometry analysis. Also, MR-1 was harvested and resuspended in 200 mL of fresh minimal medium. Both media were flushed with N_2 for 30 minutes before start and continuously afterwards and stirring was applied at 250 rpm only during the chronoamperometry experiment.

Chronoamperometry experiment at -500 mV vs. Ag/AgCl (Figure S4) showed initial current production in the absence of oxygen, which reached around 160 × 10^{-3} A/m^2 during the first 30 minutes of operation and remained considerably stable for 30 minutes afterwards. This initial current shows that soluble compounds released in the medium by MR-1 remained in the oxidized form by the end of the agitation period, during which oxygen was constantly supplied. When MR-1 (1 mL) was added, an increased current production was observed which reached 300 × 10^{-3} A/m^2 within the next hour and remained almost stable for 1 hour afterwards. This increased current production upon MR-1 addition indicated the bio-catalysed reduction of soluble compounds released in the medium by MR-1 during flask growth, and probably also of membrane-associated electroactive compounds added in the medium with MR-1. It should be noted that this

bio-electro-catalysis observed was independent of the presence of 4 mM lactate in the solution, clearly showing that MR-1 can utilise the electrode as an electron donor to reduce these compounds even when lactate is present. Finally current started decreasing by the end of the operation period, indicating complete reduction of the electron acceptor.

The cyclic voltammogram before MR-1 addition (Figure S5) revealed a clear cathodic current which became more apparent at electrode potentials lower than -200 mV vs. Ag/AgCl and which had an increasing rate which reached its maximum at -430 mV vs. Ag/AgCl. After MR-1 addition and reduction of the potential electron acceptors present in solution, the cyclic voltammograms clearly revealed two formal potentials, at -20 mV vs. Ag/AgCl and at -430 mV vs. Ag/AgCl. While the later can be directly attributed to the production of riboflavin and mediated electron transfer, the former, which also had a broad potential window, could be attributed to membrane-related proteins responsible for direct electron transfer.

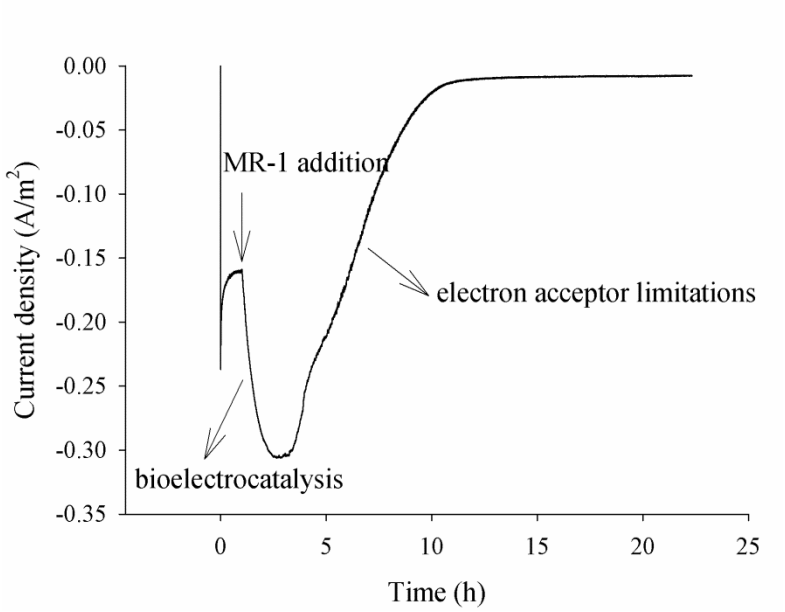

Figure S4. Chronoamperometry profile (-500 mV vs. Ag/AgCl) of the flask's filtered supernatant- effect of MR-1 addition.

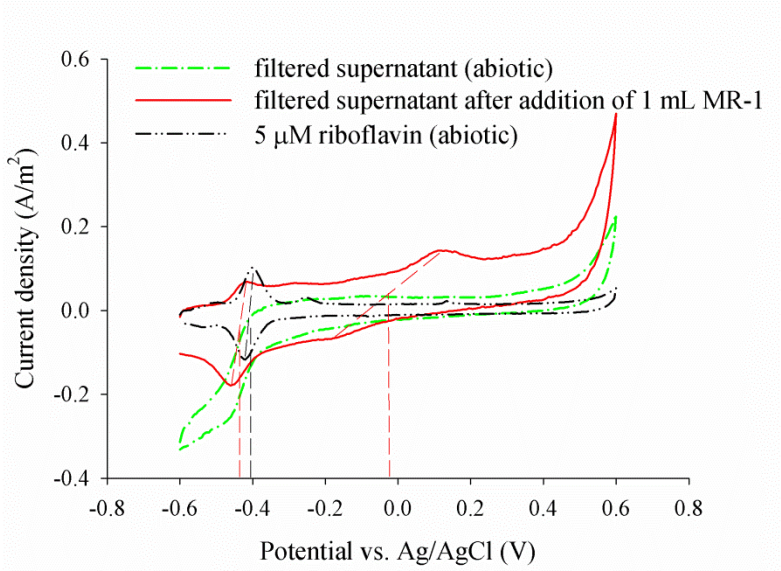

Figure S5. Cyclic voltammograms of the filtered supernatant before and after MR-1 addition and chronoamperometry experiment. Scan rate is 5 mV/sec.

Table S2. Regression parameters of the graphs presented in Figures 5c,d

Figure	Cell #	Equation	R^2
5c	+Lac	1.278*(1-exp(-0.051*x))	0.999
5c	+Lac+Shew (due to current)	7.847*(1-exp(-0.004*x))	0.998
5c,d	+Lac+Shew (total)	6*(1-exp(-0.037*x))+7.847*(1-exp(-0.004*x))	0.987
5d	+Lac+Shew (other than current)	6*(1-exp(-0.037*x))	0.975
5d	+Lac+Shew (flask, OD_{600}=0.8)	8.476*(1-exp(-0.027*x))	0.956
5d	+Shew (flask, OD_{600}=0.8)	3.064*(1-exp(-0.018*x))	0.991
5d	+Lac+Shew (flask, OD_{600}=0.5)	4.254*(1-exp(-0.028*x))	0.997
5d	+Shew (flask, OD_{600}=0.5)	1.557*(1-exp(-0.012*x))	0.929

Cr(VI) reducing flask experiments description

To investigate the ability of MR-1 for lactate-utilizing Cr(VI) reduction, four sets of flask experiments were conducted, each one in duplicate. Two of these sets (one with 30 mM lactate and one without lactate) contained the biomass of MR-1 present in planktonic form in "+Lac+Shew" right before the first Cr(VI) addition (OD_{600}= 0.5 in the 200 mL cathode) and another two sets (one with 60 mM lactate and one without lactate) contained higher biomass concentration (OD_{600}= 0.8). For these experiments, MR-1 was initially grown aerobically in 250 mL Erlenmeyer flasks with LB medium (pH 7.4) for 24 h, centrifuged and washed twice in a 100 mM NaCl- 50 mM phosphate buffer solution (pH 7.0). After washing, MR-1 was resuspended in the flasks to achieve the desired biomass concentration using the minimal medium described in the experimental section. The content of each flask was amended to contain adequate concentrations of lactate at all times and Cr(VI) was supplied each time after depletion, using the same stock solution described in the experimental section. The content of each flask was flushed with filter-sterilised oxygen-free N_2 for 20 min before the first Cr(VI) addition and for 5 min after each Cr(VI) addition. After each Cr(VI) addition, flasks were closed using rubber stoppers and incubated at 22±1 ^{o}C in an orbital shaking incubator (200 rpm). Appropriate sterilisation techniques were followed at all times to avoid contamination of the pure culture.

APPENDIX B **Comparative performance of an anion exchange membrane (AEM) and a cation exchange membrane (CEM)**

Introduction

To date, previous studies with Cr(VI) reducing MFCs have mainly used CEMs as separators, with the exception of the work conducted by Pandit et al. (2011), who studied the performance of various oxidisers, including Cr(VI), in an AEM containing MFC. However, data related to Cr(VI) removal and Coulombic efficiencies are lacking from this latter work, thus knowledge on using AEMs in Cr(VI) reducing MFCs remains incomplete.

The aim of this study was to highlight issues that might be related to the use of AEMs in Cr(VI) reducing MFCs, such as low cathodic efficiency, Cr(VI) migration to the anode and membrane sensitivity. These issues have not been sufficiently raised before and are essential for complete evaluation of such systems. For this reason, a longer study than the previous one was conducted (20 d instead of 3 d) and data related to Cr(VI) removal and Coulombic efficiency are also reported.

Experimental plan

A dual-cathode MFC configuration was used, as shown in Figure A-1. Such an arrangement with a common anode in the middle is electrically equivalent to two single-cathode MFCs connected in parallel, with different currents adding up to give the total current (Aelterman et al., 2006, Kim et al., 2011). By using two external resistors as in Figure A-1, two distinct currents are created, where electrons leaving the anode will follow the path of least resistance towards the terminal electron acceptor.

Both CMI-7000 and AMI-7001 used had a 4 cm^2 active diameter, same thickness and polymer structure and were pre-treated as described in Section 3.1. *Shewanella oneidensis* MR-1 was used as the anodic biocatalyst and Na-DL-lactate was the electron donor. A solution containing 100 mM NaCl, 21 mM KH_2PO_4 and 29 mM K_2HPO_4 was amended to pH 2 using a 5 N HCl solution and was used in both cathodes. This phosphate containing catholyte was used in this study to have a comparable catholyte to the one used by Pandit et al. (2011). $K_2Cr_2O_7$ from a stock solution was spiked into both cathode chambers to give an initial Cr(VI) concentration of 270 mg L^{-1}. Slow mixing was applied in all chambers at all times and the cathodes were sparged

with N_2 before start and at intervals. Potential drops over 100 Ω external loads were measured as shown in Figure A-1 and current *I* was calculated as previously described in Section 3.7.

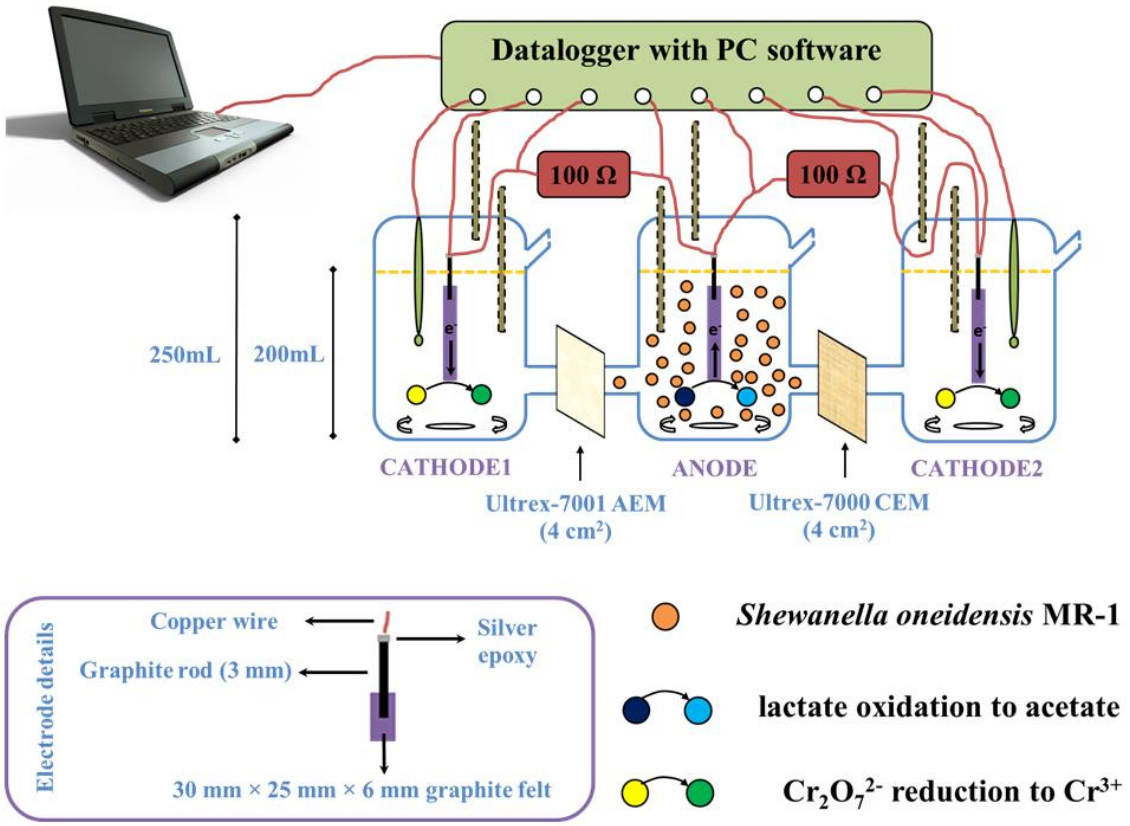

Figure A-1: Dual-cathode MFC assembly and data recording.

Results and Discussion

To ensure electron donor availability, lactate in the anode was kept at concentrations over 18 mM at all times (Figure A-2). After inoculation, open circuit voltage equilibrated at approximately 1,150 mV within 20 min. Both cathode open circuit potentials were 635 mV vs. Ag/AgCl and the anode equilibrated at an open circuit anode potential of -515 mV vs. Ag/AgCl. After the start, an initial total current of 0.23 A m^{-2} almost equally distributed to both C1 and C2 evolved, followed by a gradual total current decrease (Figure A-3). This decrease can be partially attributed to the pH increase observed in both cathodic chambers (Figure A-4) but also to the Cr(VI) concentration decrease over time (Figure A-5). In addition, Cr(VI) escaped from C1 to the anode, inhibiting anodic current as already discussed in Section 4.3.2.4. This Cr(VI) migration might have also caused blocking and deterioration of the AEM and could be considered responsible for the total current inhibition observed after 15 days of operation (Figure A-3).

As seen in Figure A-4, the AEM managed to keep a better pH balance than the CEM did and this is important for highly proton consuming processes like Cr(VI) reduction. On the contrary, pH in C2 kept increasing regardless of the low Cr(VI) reduction observed after the 3rd day, and this was mainly due to protons migration to the anode through the CEM. Better pH balance maintained by AEMs was also observed by Pandit et al. (2011), when they used permanganate as the electron acceptor at pH 2.5.

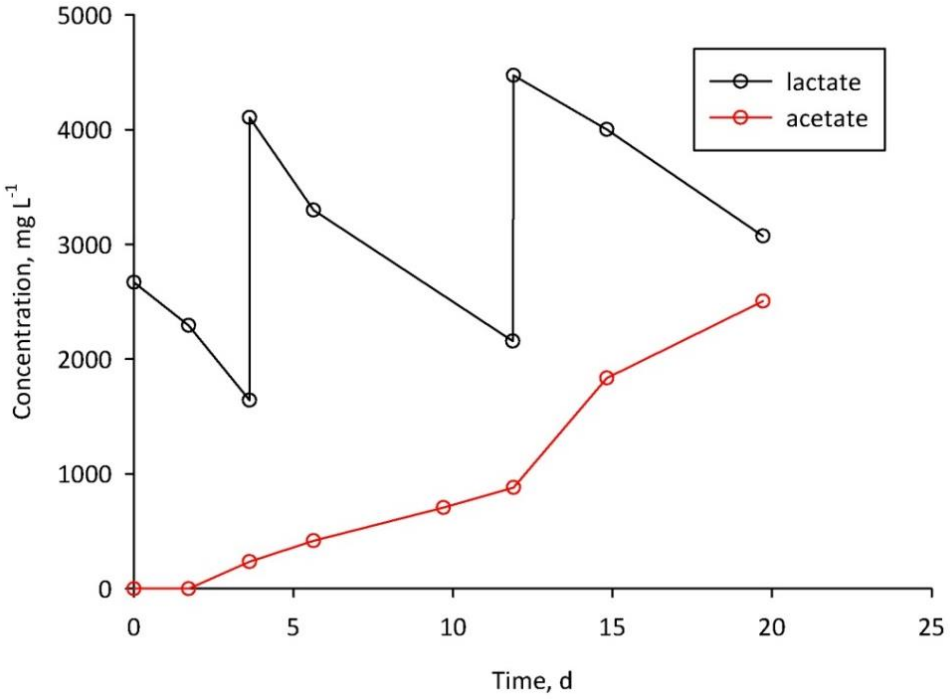

Figure A-2: Lactate and acetate concentrations in the dual-cathode MFC anode.

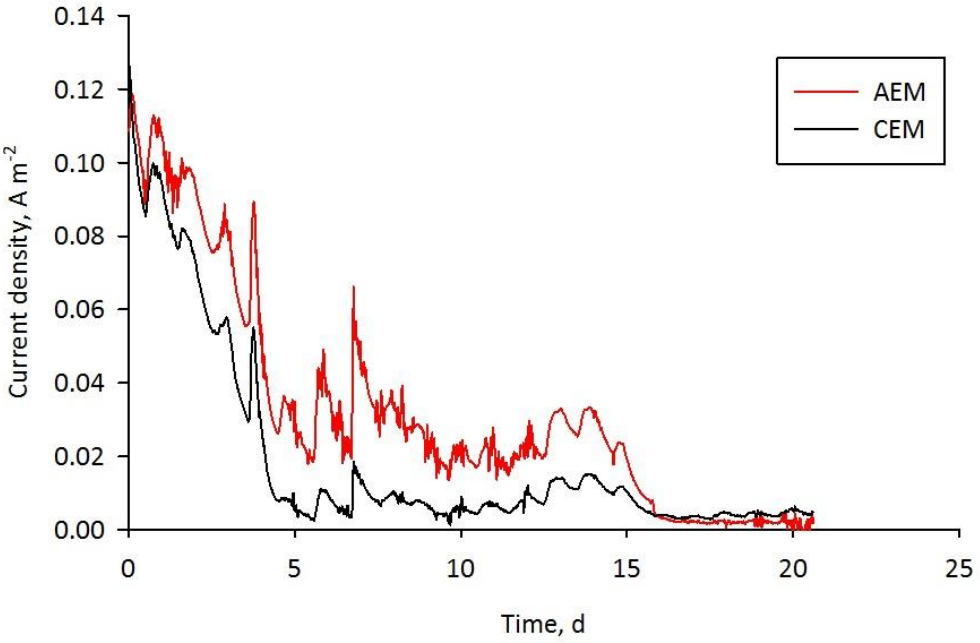

Figure A-3: Current evolution towards both parts of the dual-cathode MFC.

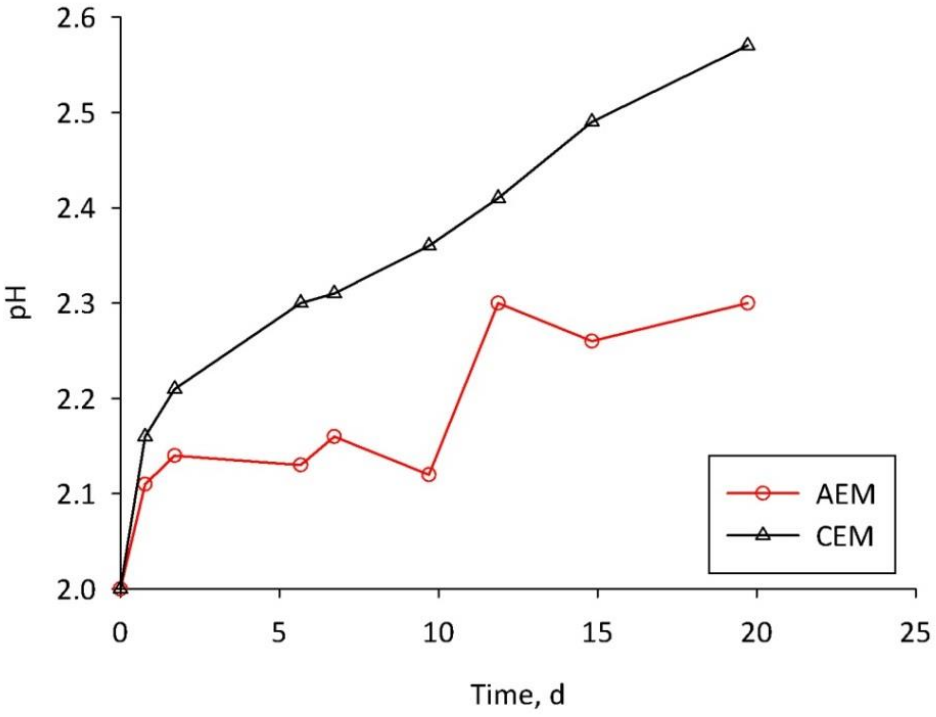

Figure A-4: pH evolution in both cathodes of the dual-cathode MFC.

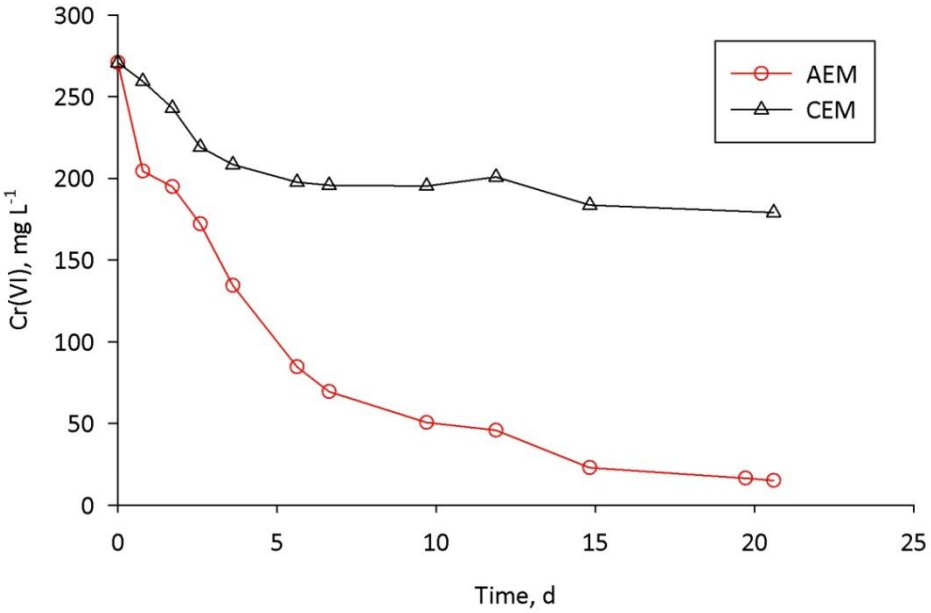

Figure A-5: Cr(VI) removal from both cathodes of the dual-cathode MFC.

Regarding the proportion of the current produced by C1, this increased despite the lower Cr(VI) concentration in C1 and was 77±5% of the total current produced after the 4th day of operation. This indicates a preferred electron pathway towards C1 and higher internal resistance for C2, however this cannot necessarily be generalised for dual-chamber MFCs, as in that case the cathode's effect on the anode must be studied individually for the two membranes. Furthermore, even if C1 was the major electron sink and there seems to be a higher Cr(VI) removal from C1 (Figure A-5), calculations on cathodic efficiency showed that 161 mg/l of Cr(VI) removed in C1 (63% of the total Cr(VI) removal in C1) was not due to electricity production. This compared to 33 mg/l (36% of the total Cr(VI) removal) for C2 (Figure A-6).

A maximum Cr(VI) concentration of 3.3 mg L^{-1} was also detected in the anode (Figure A-7, Figure A-8), indicating that Cr(VI) anions ($HCrO_4^-$ and $Cr_2O_7^{2-}$) escaped from C1. Cr(VI) reduction in the anode was also indicated by the light green colour evolved by the end of the run, which was due to the presence of Cr(III), the product of Cr(VI) reduction. No Cr(VI) or colour change was noticed for the same time period in anodes of dual-chamber MFCs operating with only a cation or a proton exchange membrane, as described in the previous paragraphs.

Apart from the low CE, a serious deterioration of the AEM was also observed after the MFC disassembly, which was not observed on the CEM (Figure A-9). This was not unexpected, as quaternary ammonium, a common functional group of AEMs like AMI-7001 used in this study

and RALEX™ used by Pandit et al. (2011), can bind to Cr(VI) anions (Jinhua et al., 2010) and probably to other similar strong oxidising oxyanions like permanganate (MnO_4^-) and persulfate ($S_2O_8^{2-}$). This will finally cause a membrane deterioration, it will increase the operation costs, it will limit the electron acceptor availability for power production, and it will most probably affect the anodic biocatalyst and inhibit current production.

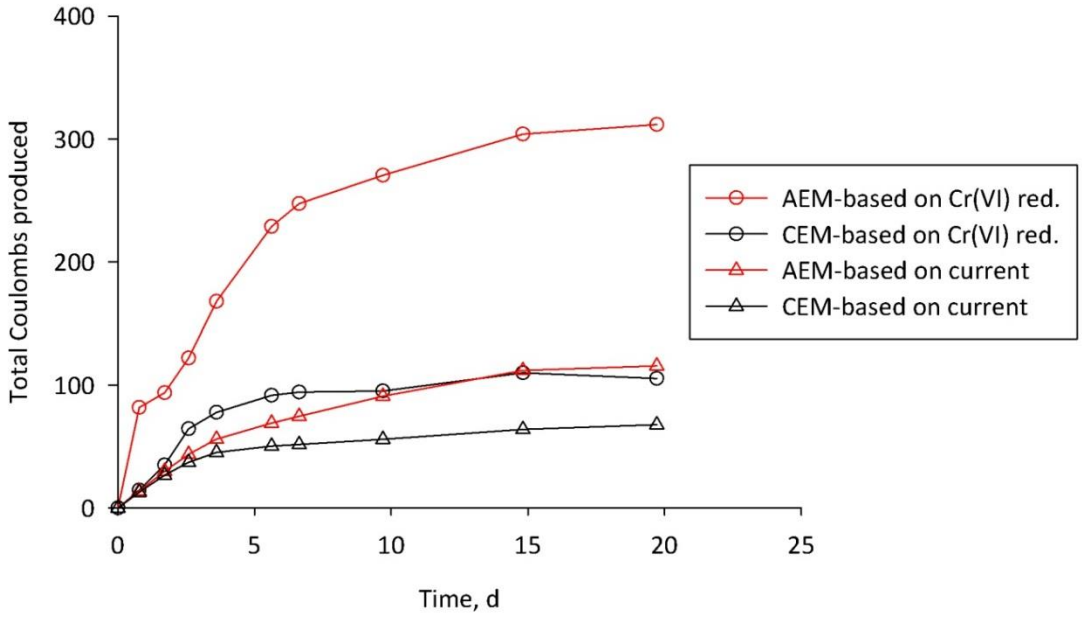

Figure A-6: Coulombs produced by both parts of the dual-cathode MFC, calculated based on both Cr(VI) reduction and current production.

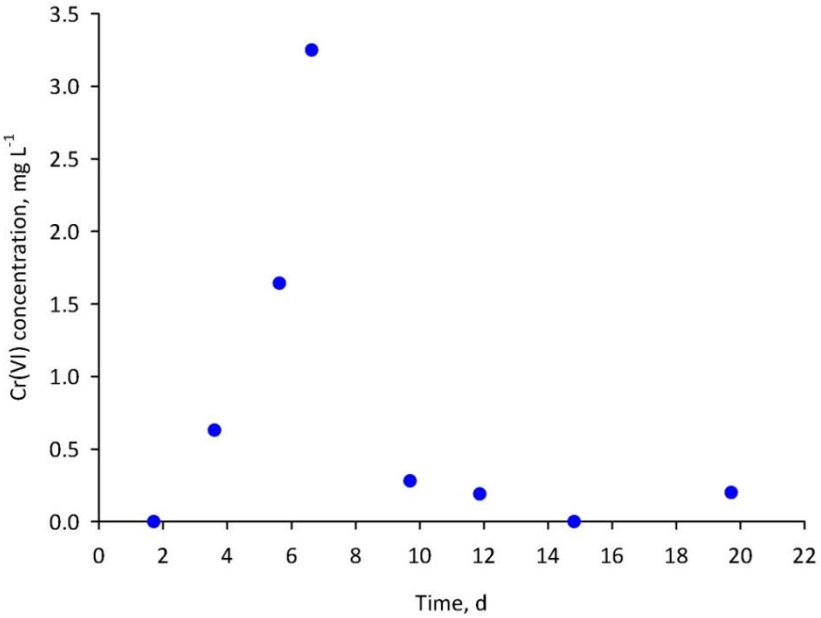

Figure A-7: Cr(VI) detected in the anode chamber of the dual-cathode MFC.

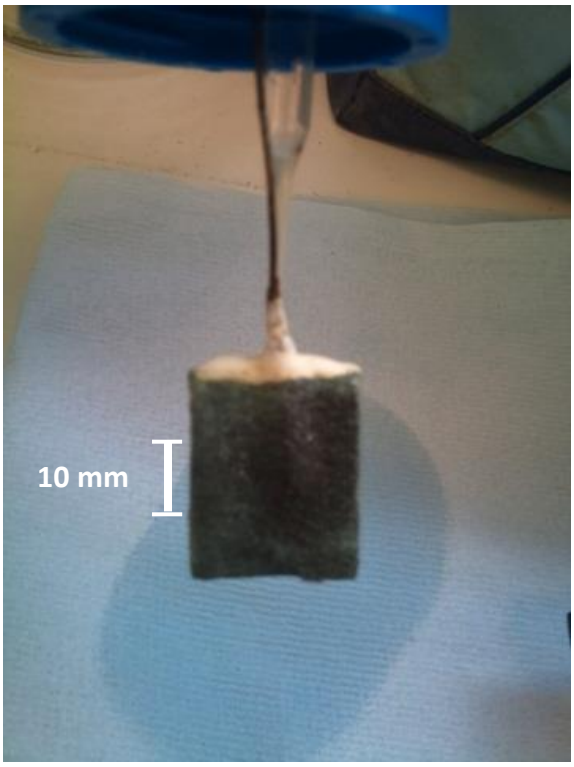

Figure A-8: Anode electrode after disassembly of the dual-cathode MFC showing green Cr(III) precipitates on the electrode's surface.

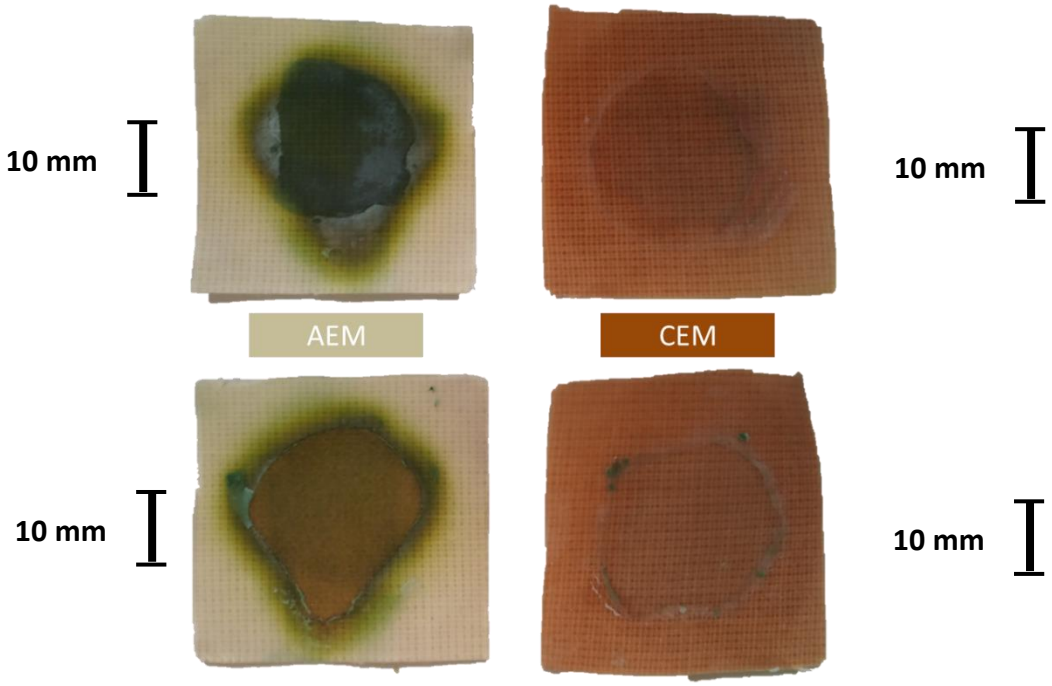

Figure A-9: Membranes of the dual-cathode reactor after reactor disassembly; top: cathode facing, bottom: anode facing.

Conclusions

The presence of Cr(VI) in the anode of bioelectrochemical systems must be prevented as it has been demonstrated to have an irreversible inhibitory effect on anodic current production. This can be done by selecting appropriate membrane separators which prevent the transport of Cr(VI) from the cathode to the anode. AEMs have been shown incapable of this task and therefore, despite being more capable of keeping the pH stable in the cathode, their use should be avoided. The use of PEMs or CEMs with appropriate chemical control of the pH might be a more viable solution in this instance.