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Review

Progress Towards Bioelectrochemical Remediation of Hexavalent Chromium

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Received: 1 October 2019; Accepted: 3 November 2019; Published: 7 November 2019

Abstract: Chromium is one of the most frequently used metal contaminants. Its hexavalent form Cr(VI), which is exploited in many industrial activities, is highly toxic, is water-soluble in the full pH range, and is a major threat to groundwater resources. Alongside traditional approaches to Cr(VI) treatment based on physical-chemical methods, technologies exploiting the ability of several microorganisms to reduce toxic and mobile Cr(VI) to the less toxic and stable Cr(III) form have been developed to improve the cost-effectiveness and sustainability of remediating hexavalent chromium-contaminated groundwater. Bioelectrochemical systems (BESs), principally investigated for wastewater treatment, may represent an innovative option for groundwater remediation. By using electrodes as virtually inexhaustible electron donors and acceptors to promote microbial oxidation-reduction reactions, in in situ remediation, BESs may offer the advantage of limited energy and chemicals requirements in comparison to other bioremediation technologies, which rely on external supplies of limiting inorganic nutrients and electron acceptors or donors to ensure proper conditions for microbial activity. Electron transfer is continuously promoted/controlled in terms of current or voltage application between the electrodes, close to which electrochemically active microorganisms are located. Therefore, this enhances the options of process real-time monitoring and control, which are often limited in in situ treatment schemes. This paper reviews research with BESs for treating chromium-contaminated wastewater, by focusing on the perspectives for Cr(VI) bioelectrochemical remediation and open research issues.

Keywords: bioelectrochemical systems (BESs); hexavalent chromium; electrobioremediation; groundwater treatment

1. Introduction

Hexavalent Cr(VI) and trivalent Cr(III) chromium are the most common forms of this element in the environment. Cr(VI) is water-soluble in the full pH range and extremely toxic to human health and all living organisms because of its mutagenic and carcinogenic properties [1]. The U.S. EPA has classified Cr(VI) as one of the 17 most dangerous elements for human health [2,3]. In aqueous systems, Cr(VI) can be present in different species: primarily as chromic acid [H_2CrO_4] and its salts, the hydrogen chromate ion [$(HCrO_4)^-$], and the chromate ion [$(CrO_4)^{2-}$]. The chemical equilibrium of the different chromium species depends on Cr(VI) concentration, oxidation-reduction potential (ORP),

and pH of the system [3]. Cr(III) is less toxic and more stable than Cr(VI) in aquatic environments, since under natural conditions, at moderately acidic or alkaline pH, it tends to precipitate as chromium hydroxide or oxide ($Cr(OH)_3$ or Cr_2O_3) [4,5].

Hexavalent chromium has been, and it is still used, in many industrial activities such as chromite ore processing, electroplating, in the production of dyes and pigments, pharmaceuticals, in leather tanning, in wood preservation/processing, and in the metallurgical industry for alloy preparation [6–8]. Improper management of Cr(VI)-containing effluents or wastes led to widespread chromium environmental contamination around the world. As a consequence of its subsequent mobility in water, groundwater resources are especially vulnerable to Cr(VI) contamination, with levels often shown to exceed the internationally acceptable exposure limit in water of 0.05 mg Cr(VI)/L [9,10].

Hexavalent chromium in industrial wastewater is typically treated by reduction, and subsequent precipitation, to the non-toxic Cr(III) by means of reductants (for instance, $FeSO_4$, $Na_2S_2O_5$, and SO_2) [11] or electrochemical processes (including electrocoagulation, electro-reduction, electrodialysis, and electro-deionization) [12]. Generally, these processes are easy to implement and efficient at high or moderate Cr(VI) concentrations. However, the use of reductants produces a large amount of metallic sludge, and both the above-mentioned approaches are either ineffective or not cost-effective when applied for trace Cr(VI) treatment. Adsorption on adsorbents, such as activated carbons or zeolites, is also adopted, since this process exhibits several advantages of simple operation, low cost, and high efficiency [13,14]. Nevertheless, this strategy presents some disadvantages such as needing regeneration of adsorption media and no degradation/detoxification of Cr(VI) to Cr(III) is achieved [15].

Conventional methods for Cr(VI) contaminated site remediation, soil excavation, and groundwater pump and treat (P&T), despite still being widely applied, are energy and chemical-intensive and entail high costs [16]. Furthermore, the P&T method, which involves the extraction of contaminated water from the aquifer and above-ground treatment, tends to be poorly efficient when dealing with contaminated plumes at relatively low Cr(VI) concentrations [17].

In situ chemical strategies are applied using Fe(0) permeable reactive barriers or by injecting nano-Fe(0), $Na_2S_2O_4$ or hydrogen sulphide to develop reactive zones in the aquifer [7,18,19]. The applicability and effectiveness of these approaches are limited by high costs, poor chemical distributions, and undesired side reactions in the subsurface [20].

Recent studies have shown that microbial activity can indirectly promote Cr(VI) reduction and immobilization in contaminated groundwater and/or soil. Bio-reduction relies on injections of biodegradable organic substrates (e.g., molasses), whose rapid microbial degradation by indigenous heterotrophic microorganisms prompts anaerobic conditions in the subsurface and produces reductants, such as S^{2-}, Fe(II), and fermentation metabolites, which are able to mediate Cr(VI) chemical reduction and precipitation [21–24].

Selected microorganisms can directly bio-reduce [25,26] and bio-sorb [27] Cr(VI) as well. Nevertheless, the presence of suitable microorganisms in conjunction with favorable environmental conditions is essential. The performance of in situ Cr(VI) bio-remediation, by relying on either induced—or direct—bioreduction/biosorption mechanisms, is, therefore, influenced by the abundance of suitable electron acceptors and donors, carbon sources, and nutrients for the microorganisms, which often require external supplements for a balance [20]. The control of supply rates can be a crucial step to avoid unwanted side reactions and the accumulation of undesired substances (e.g., excess substrates or nutrients, fermentation products) that contribute to the deterioration of soil and water quality.

Cr(VI), like any metal, is not actually removed by in situ remediation, but only changes its form and valence. This requires careful evaluation of the long-term stability of reduced products, post-treatment, and of possible re-oxidation mechanisms [28,29]. Cr(III) in the precipitates formed during remediation, whether exposed to environmentally common Mn oxides, such as the birnessite, have the potential of rapid re-oxidation to Cr(VI). Even highly oxidized Mn(III, IV) oxides are likely unstable under reducing conditions, in case these are caused by the addition of electron donors as part

of the Cr(VI) remediation strategy. Cr could be re-mobilized when the donor addition stops and the site returns to natural oxidizing conditions [28].

A promising strategy to be explored for in situ Cr(VI) bioremediation is the application of bioelectrochemical systems (BESs), which is an emerging platform technology combining microbial processes with electrochemical systems. In BESs, the ability of electrochemically active microorganisms (EAM) to use electrodes as inexhaustible electron acceptors/donors are exploited, via a process typically referred to as extracellular electron transfer [30–32]. BES reactors essentially consist of electrodes, an anode and a cathode, immersed in an electrolytic medium/solution and an optional ion-exchange membrane to separate the compartments. At the anode, oxidation of reduced species generates a flow of electrons to the cathode, where reduction reactions take place. At least one or both reactions are microbially mediated. Membranes provide a separation structure to isolate different bulk liquids in the anode and cathode chambers, to optimize the operating condition without affecting the microbial community, to prevent undesired substrate transport, and to facilitate transfer of ionic species from one chamber to another for charge balance, increasing, however, the internal resistance of the system [33]. In case of thermodynamically favorable redox reactions, BESs can result in direct electricity production (microbial fuel cells, MFCs) [30,34,35] or, by external energy supply, in enhancement of thermodynamically unfavorable processes [36], with production of less toxic or value-added chemicals (such as hydrogen, H_2O_2, methane, or even organic molecules) (in microbial electrolysis cells, MECs) [37].

BESs have been extensively studied and intensively developed, especially during the last 10 years, for wastewater treatment, valorization, and reuse [38,39]. In environmental remediation, BESs, through biologically-mediated oxidation (at the anode) and reduction (at the cathode), potentially provide a flexible platform for treating many pollutants frequently found at contaminated sites, in co-contamination cases [40,41]. Solid electrodes can serve, in fact, either as an electron sink, for the oxidation of petroleum hydrocarbons [42–44] or As(III) [45], or as electron donor, for reduction of chlorinated hydrocarbons [46,47], nitrate [48], or oxidized metals, including Cr(VI) [49].

In in situ treatments, it would be possible to directly introduce the electrodes in the aquifer and stimulate biologic activity with no external chemicals or a minimal external chemical supply [50], which creates an advantage in terms of cost-effectiveness and sustainability in comparison to current approaches [51]. The electrical signal generated in BESs provides opportunities for real-time monitoring of Cr(VI) concentration [52–54] and in situ microbial activity [55]. Cr(III) deposition next to the electrode theoretically offers the chance of recovering the metal itself through the electrode [39].

Several experimental works investigated Cr(VI) reduction in MFCs with bioanodes and either abiotic cathodes, relying on Cr(VI) electrochemical reduction, or biocathodes, while taking advantage of biological activity. However, no research, to our knowledge, has yet specifically addressed Cr(VI) contaminated groundwater remediation. This review provides a comprehensive analysis of the current knowledge and experiences in bio-electrochemical treatment of Cr(VI) contaminated water streams, in order to explore BESs opportunities for in situ groundwater bioremediation.

2. Principles of Cr(VI) Reduction in Bio-Electrochemical Systems

Bio-electrochemical Cr(VI) reduction essentially relies on cathodic reduction (Figure 1), with most research focused on wastewater treatment coupled with energy recovery in MFCs, with biotic anodes and either abiotic or biotic cathodes [56]. Only a single study evaluated Cr(VI) detoxification at the bioanode, via bacterial protection mechanisms [57].

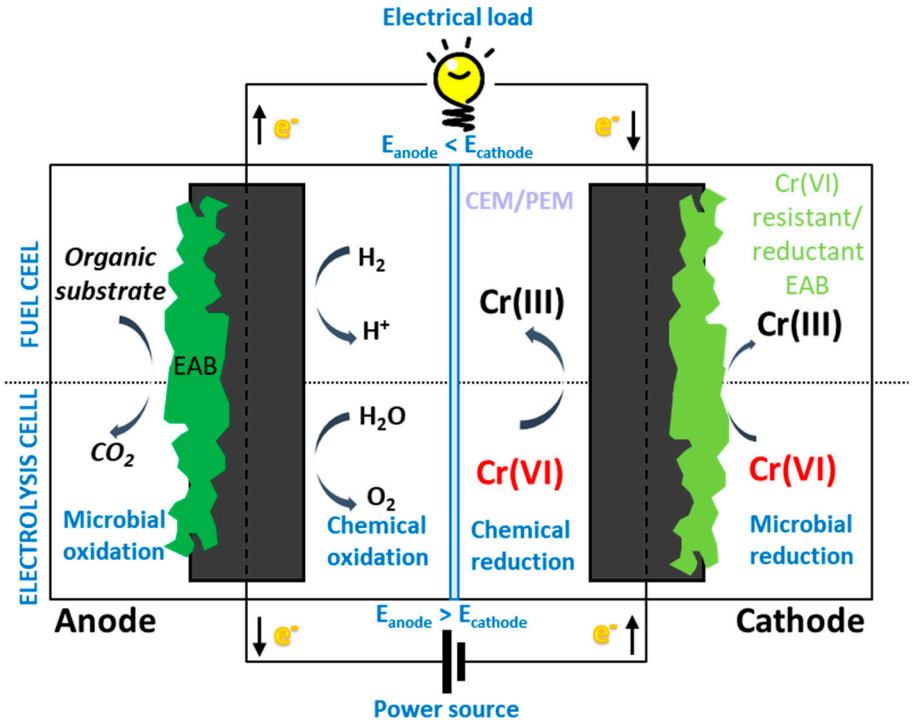

Figure 1. Schematic overview of a BES for Cr(VI) reduction as MFC with energy harvesting or MEC with external supply ([58], modified). In MFCs, oxidation of the electron donor at the anode is coupled with a reduction of species with comparable or higher redox potential at the cathode. The net potential of the MFC, as the sum of anodic and cathodic potentials, is positive. Therefore, spontaneous electron flow from the anode to the cathode occurs. Conversely, in MEC, thanks to external power input to force electron flow, the oxidation of an electron donor at the anode can be coupled with the reduction of lower redox potential species at the cathode. CEM/PEM: Cation/Proton Exchange Membrane. EAB: Electrochemically Active Bacteria

2.1. Electrochemical Reduction of Cr(VI)

Due to its high standard reduction potential, which is comparable, or, in certain conditions, even higher than those of other commonly used electron acceptors in BESs, Cr(VI) has been initially investigated as a theoretically favorable electron acceptor, to get reduced at abiotic cathodes in a typical MFC configuration for power production. This concept has been demonstrated for the first time in a dual-chamber MFC (2CMFC) by Wang et al. [59] who, using acetate as electron donor and Cr(VI) solution at a pH of 2 as acceptor, observed higher power densities than for O_2 and hexacyanoferrate.

The half-cell Cr(VI) reduction potential and the stoichiometry of the reaction are, however, strongly dependent on chromium species, concentration, and pH conditions (Table 1).

In water solutions, the dichromate $Cr_2O_7^{2-}$ form prevails for total chromium concentrations above approximately 1 g/L [3,17]. At lower concentrations, which typically occurs in groundwater plumes or natural surface water, the dominant species is $HCrO_4^-$ at a pH between 1 and about 6 to 6.5, and CrO_4^{2-} at neutral or alkaline conditions [60]. High positive standard reduction potentials (Eh^0 vs. SHE) for both $Cr_2O_7^{2-}$ and $HCrO_4^-$ indicate a thermodynamically favorable reaction, conducive to high power density generation in BESs, only in acidic environments [39,59,61]. On the contrary, CrO_4^{2-} lower potential limits chromium electrochemical reduction in the neutral pH range, which often makes the external energy supply necessary. Furthermore, at pH < 4, the predominant form of chromium reduction is dissolved Cr^{3+}, whereas in the 5–8 pH range, soluble $Cr(OH)_2^+$ and $Cr(OH)^{2+}$, coexist with $Cr(OH)_3$ or Cr_2O_3 precipitates, which are responsible for the progressive deterioration of the reduction rates as Cr(III) deposits onto the cathode surface, especially at a pH above 6.5 [62,63].

Table 1. Standard potentials (E_h^0) at 25 °C (V vs. Standard Hydrogen Electrode, SHE) and theoretical potential (E_h') at pH = 7 and 25 °C (V vs. SHE) of Cr(VI) and other selected species of interest for BESs application in Cr(VI) groundwater remediation, sourced from [32,39,59].

Reaction	E_h^0 (V vs. SHE)	E_h' at pH 7 (V vs. SHE)
$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$	1.33	0.33
$HCrO_4- + 7H^+ + 3e^- \rightarrow Cr^{3+} + 4H_2O$	1.35	0.35
$HCrO_4- + 4H^+ + 3e^- \rightarrow Cr(OH)_2^+ + 3H_2O$	1.31	0.76
$CrO_4^{2-} + 4H_2O + 3e^- \rightarrow Cr(OH)_{3(s,\ hydrated)} + 5OH^-$	−0.13	0.21
$O_2 + 4H^+ + 4e^- \rightarrow H_2O$	1.23	0.805 (pO_2 = 0.2 bar)
$NO_3^- + 6H^+ + 5e^- \rightarrow 1/2\ N_2 + 3H_2O$	1.24	0.71 (pN_2 = 0.8 bar)
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.77	0.77
$SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O$	0.30	−0.21
$HCO_3^- + 9H_+ + 8e^- \rightarrow CH_{4(g)} + 3H_2O$	0.20	−0.26
$2HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O$	0.19	−0.29
$2H^+ + 2e^- \rightarrow H_{2(g)}$	0	−0.41 (pH_2 = 1 bar)
$MnO_{2(s)} + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$	1.23	0.402

Effective abiotic electrochemical reduction requires strongly acidic conditions (optimally at a pH of 2) that greatly limit its applicability to environmental remediation.

Biologically mediated Cr(VI) reduction, relying on several microbiological mechanisms, may overcome the current issues of abiotic electrochemical reduction, by offering opportunities to hexavalent chromium treatment in the environmentally compatible neutral pH range. Moreover, the biofilm on the cathode may somewhat protect and improve the long-term efficiency of the electrode by preventing or delaying Cr(III) deposition [64,65].

2.2. Microbiological Mechanisms of Cr(VI) Reduction

Several mechanisms of bacterial Cr(VI) reduction have been described both under aerobic and anaerobic conditions [66,67].

In the presence of oxygen, the reduction of Cr(VI) is commonly associated with soluble chromate reductases and requires reduced nicotinamide-adenine dinucleotide phosphate (NAD(P)H) as electron donor [66,68]. The mechanisms associated with Cr(VI) reduction can involve a direct, one-step or two-step electron transfer. *Escherichia coli* YieF Cr(VI) reductase transfers three electrons to Cr(VI) in one step to produce Cr(III), and one to molecular oxygen generating reactive oxygen species (ROS) [69]. The Cr(VI) reductase ChrR from *Pseudomonas putida* involves a one/two steps mechanism in which one/two electrons are donated from NAD(P)H to generate the intermediate Cr(V)/Cr(IV) that is further reduced to Cr(III) by one/two additional electrons [66,70]. Under aerobic conditions, most Cr(VI)-resistant microorganisms tolerate up to 1500 mg Cr(VI)/L [71]. However, the rate of chromium reduction is directly related to the concentration of the contaminant and physical parameters, such as pH and temperature [71,72]. One of the first studies with Cr(VI)-reducing bacteria, achieved almost 100% of chromate reduction in 2.0 mg/L Cr(VI) solution within 90 h by *P. putida* PRS2000 and *P. fluorescens* LB303 [73]. Similar results were obtained by the soil-isolated strains *Bacillus* sp. E29 and *Arthrobacter crystallopoietes* strain ES32 that achieved reductions of 82% and 90% of Cr(VI) in less than 6 h and 12 h, respectively [71]. Much higher Cr(VI) concentrations were removed by *Serratia proteamaculans*. Within 48 h, 100 mg Cr(VI)/L were reduced (corresponding to 100% of dichromate added) under aerobic conditions [72]. In the same study, the authors demonstrated that *S. proteamaculans* was also able to reduce chromate anaerobically, but the process was more efficient in the presence of oxygen.

Chromium-resistant microorganisms have also been found in marine environments. A Cr(VI)-resistant bacterium isolated from seawater and identified as *Exiguobacterium indicum* achieved nearly 92%, 50%, and 46% reduction for 100, 500, and 1000 mg Cr(VI)/L, respectively, after 192 h of incubation [74].

Under anaerobic conditions, Cr(VI) can serve as the final electron acceptor in a process that usually involves membrane-bound reductases [66], but also soluble enzymes (e.g., soluble cytochrome c_3

from *Desulfovibrio vulgaris*) were observed to reduce Cr(VI) [75]. The overall reaction is provided in Equation (1), with glucose as electron donor [76].

$$C_6H_{12}O_6 + 8CrO_4{}^{2-}{}_{(aq)} + 14H_2O \rightarrow 8Cr(OH)_{3(s)} + 10OH^-{}_{(aq)} + 6HCO^-{}_{(aq)} \quad (1)$$

Cr(VI) reduction in anaerobic conditions was reported in several microorganisms. Both *P. dechromaticans* and in *Enterobacter cloacae* are capable to use Cr(VI) as terminal electron acceptor [66,77]. Gene expression of *Shewanella oneidensis* MR-1 during Cr(VI) reduction was studied [78]. Under Cr(VI) reducing conditions, 83 genes were upregulated. Among the others, genes involved in the reduction of Fe(III) and Mn(IV) were also upregulated. Further studies with mutant strains confirmed the involvement of *mtrA*, *mtrB*, *mtrC*, and *omcA* in the reduction of Cr(VI) [78,79].

In anaerobic environments, iron(II) and sulphide can also play a role in Cr(VI) reduction [66,80]. Iron-reducing bacteria (IRB) reduce Fe(III) to Fe(II), and biologically produced Fe(II) can be re-oxidized by reducing Cr(VI) to Cr(III) [76]. Sulphate is an electron acceptor widely used by several bacterial groups for the degradation of the organic matter in anaerobic environments [81]. In sulphate-rich environments, Cr(VI) can react with sulphide produced by sulphate-reducing bacteria (SRB) to produce Cr(III) that precipitates [76].

In addition to laboratory studies focused on the elucidation of possible mechanisms used by bacteria for Cr(VI) reduction, the ability to reduce Cr(VI) in soil-aquifer systems has been reported but needs to be further investigated. *Clostridium chromiireducens* sp. a Cr(VI)-resistant, Gram-positive, spore-forming, obligate anaerobe, was identified for its ability to reduce Cr(VI) at a contaminated site [82]. Many previous reports also confirmed autotrophic reduction of chromium, by mostly using hydrogen as electron donor [83,84]. A Gram-negative bacterium, capable of reducing hexavalent chromium, was also isolated from a contaminated site. 16S rRNA analysis revealed that it belonged to the *Pseudomonas* genus, with high similarity to *P. synxantha* [85,86]. Marsh and McInerney [2] demonstrated reduction of Cr(VI) with hydrogen and carbon dioxide/$NaHCO_3$ as an electron donor and carbon source, respectively, carried out by an anaerobic mixed culture developed from aquifer sediment.

As previously reported, the reduction of the Cr(VI) can occur through the action of soluble cytochromes [66], membrane reductase mtrCAB, and omcA [78,79]. Kracke and colleagues [87] report that the mtrCAB terminal reductase complex and the omcA cytochrome are able to interact with the electrode directly or via mediators (flavins). This scientific evidence combined with the well documented ability of IRB (i.e., *Shewanella oneidensis* MR-1) [78] and SRB (i.e., *Desulfovibrio desulfuricans* 27774) [88] to reduce Cr(VI) and to exchange electrons with solid materials are good reasons to consider the feasibility of a bio-electrochemical system for reducing Cr(VI).

All the previously described biological mechanisms can take place in BESs. The inoculated cathode may act as an electron donor for electrochemical or bio-electrochemical Cr(VI) reduction. Electroactive Cr-reducing microorganisms in the cathodic biofilm or the production of hydrogen at the cathode of a BES could also favor the autotrophic reduction of Cr(VI) by hydrogenotrophic bacteria [2,83,84], or IRB or SRB involved in bio-electrochemical processes may facilitate indirect reduction of Cr(VI).

3. Cr(VI) Biocathodic Reduction

Table 2 summarizes the available research experiences of Cr(VI) reduction with biocathodes, by focusing on the electrode materials and inoculum, cathode potential, pH, Cr(VI) concentrations, the observed Cr(VI) removal rates, and efficiencies.

Table 2. Summary of key studies regarding Cr(VI) reduction in BESs.

BES Type [1]	Anode Material	Anodic Inoculum/Mediator [2]	Cathode Material	Cathodic Inoculum/Mediator	Cathode Potential (V vs. SHE)	Initial Cr(VI) (mg/L)	Initial pH	Test Period (h) [3]	Cr^{VI} Removal (%)	Rate ($mgCr^{VI}/L/h$) (Specific Rate ($mgCr^{VI}/g_{VSS}/h$)) [4]	References
2CMFC-PEM, H(b)	graphite plate	anaerobic sludge. ED: acetate	graphite plate	Cr(VI) enriched denitrifying and anaerobic mixed culture	NA	22	7.2–7.6	552	100	0.14 (0.18)	[89]
						31				(0.22)	
						40				(0.36)	
						63				0.45 (0.46)	
2CMFC, PEM, H(b)	graphite plate	MFC effluent. ED: acetate	graphite plate and granules	Anaerobic mixed culture from contaminated soil	NA	13	≈7	7	100	3.8 (2)	[90]
					NA	39				5.3 (2.4)	
2CMFC, CEM, TR(b)	graphite fiber	effluent and acclimated anode from MFC	graphite fiber (C/A = 3)	MFC anaerobic effluent	0.013	20	5	3	98.5	6.57	[65]
					0.03		8		61	4.07	
					NA	40	7	3.5	75	8.57	
					−0.05	50		3	NA	(5.2)	
					NA	20		5	100	4.08 (12.4)	
			graphite fiber (C/A = 10)					4		5.12 (15.5)	
			graphite fiber (C/A = 20)					3		6.8 (20.6)	
			graphite fiber (C/A = 3)					5	90.25	3.61 ± 0.1 (11.3 ± 2.2)	
			graphite felt (C/A = 3)						76.75	3.07 ± 0.12 (9.5 ± 1)	
			graphite granules (C/A = 3)						86.5	3.46 ± 0.09 (5.6 ± 0.6)	
2CMFC, CEM, TR(b)	graphite brush	anaerobic wastewater ED: acetate	graphite granules	WWTP primary clarifier effluent	NA	20	7	24	70	0.6	[64]
2CBES (poised cathode), CEM, TR(b)	graphite brush		graphite granules		−0.45				55	0.43	
					−0.3				100	0.83	
					−0.15				100	0.83	
					0.2				43	0.36	

Table 2. *Cont.*

BES Type [1]	Anode Material	Anodic Inoculum/Mediator [2]	Cathode Material	Cathodic Inoculum/Mediator	Cathode Potential [V vs. SHE]	Initial Cr(VI) (mg/L)	Initial pH	Test Period (h) [3]	Cr^{VI} Removal (%)	Rate ($mgCr^{VI}$/L/h) (Specific Rate ($mgCr^{VI}$/ g_{VSS} /h)) [4]	References
2CMFC, CEM, H(sb)	reticulated vitreous carbon	*Shewanella oneidensis* MR-1. ED: lactate	reticulated vitreous carbon	*Shewanella oneidensis* MR1	NA	2.5 (3 cycles)	7	72 (each cycle)	60–100	0.02–0.04	[91]
				Shewanella putrefaciens					92–100	0.03–0.04	
				Shewanella amazonensis					72–100	0.025–0.04	
				Shewanella sp ANA3					32–100	0.01–0.04	
				Shewanella loihica					44–100	0.015–0.04	
				Shewanella sp MR-4					20–100	0.007–0.04	
2CMFC, PEM, H(b)	graphite felt	*S.oneidensis* MR-1. ED: lactate	graphite felt	*Shewanella oneidensis* MR-1	NA	10 (6 cycles)	7	300	90	0.1–1.1	[92]
2CBES (poised cathode), PEM, H(b)	graphite felt	*Shewanella oneidensis* MR-1. ED: lactate	graphite felt	*Shewanella oneidensis* MR1					10	0.5	
				Shewanella oneidensis MR1; MED: riboflavin					13	0.65	
				Shewanella oneidensis MR1; ED: lactate	−0.3	20	7	4	45	2.25	
SCMFC (b)	carbon brush	municipal wastewater. ED: acetate	carbon cloth with Pt	municipal wastewater; ED: acetate	2.93	1	6.5	120	89	0.01	[93]
					3.03	3			95.7	0.02	
					3.13	10			98.8	0.08	
2CMFC, CEM, TR(b)	graphite brush	MFC anodic effluent. ED: acetate	graphite felt	WWTP primary clarifier effluent acclimated to Cr(VI)	−0.074	5	5.8	4	100	1.21	[94]
					NA	5 (with Cu(II), Cd(II))			39	0.49	
				WWTP primary clarifier effluent, acclimated to Cr(VI), Cu(II) and Cd(II)					100	1.24	
2CMFC, PEM, H(b)	graphite felt	*Shewanella oneidensis*	graphite felt	*Shewanella oneidensis*	NA	10 (8 cycles)	8	840	90–100	0.9–1.95	[95]

Table 2. *Cont.*

BES Type [1]	Anode Material	Anodic Inoculum/Mediator [2]	Cathode Material	Cathodic Inoculum/Mediator	Cathode Potential [V vs. SHE]	Initial Cr(VI) (mg/L)	Initial pH	Test Period (h) [3]	Cr^{VI} Removal (%)	Rate ($mgCr^{VI}$/L/h) (Specific Rate ($mgCr^{VI}$/ g_{VSS} /h)) [4]	References
2CMFC, PEM, C(b)	graphite felt	anaerobic sludge. ED: glucose	graphite felt	mixed culture from MFC anode (ex situ)	NA	20	7	24	79.3	0.66	[96]
				anaerobic digester sludge enriched in presence of Cr(VI) (*in situ*)					20.2	0.17	
SCMEC, Cyl(c)	carbon rod	anaerobic sludge	graphite felt	anaerobic sludge	NA	100		16 HRT	43.12–96.68	2.69–6.04	[97]
						100 (200 NO_3^-, 100 p-FNB)			41.38	2.59	
						100 (200 NO_3^-, 150 p-FNB)			49.14	3.07	
						100 (200 NO_3^-, 200 p-FNB)			55.21	3.45	
						100 (200 NO_3^-, 300 p-FNB)			58.93	3.68	
2CMFC-PEM, C(b)	graphite felt	anaerobic sludge. ED: glucose	graphite felt	acclimated MFC anode	NA	20	7	5	28.3	1.13 ± 0.01	[98]
			graphite felt /NaX		NA				69	2.76 ± 0.09	
			graphite felt /NaX-HNO_3		NA			3	100	10.39 ± 0.28	
2CMFC-PEM, H(b)	graphite felt	sewage sludge. ED: glucose	graphite felt	acclimated MFC	NA	40	7	48	58.3	0.49	[99]
			graphene-modified felt						100	0.83	
2CMFC, CEM, Cyl(b)	graphite felt	MFC anodic effluent. ED: acetate	graphite felt	*Stenotrophomonas* sp., *S. maltophilia*, *Serratia marcescens*, *Achromobacter*	−0.04	20	5.8	5	74–83	2.96–3.32	[100]
					−0.05	20 (20 mg/L Cd(II))			63–71	2.52–2.84	
SCMEC, Cyl(c)	graphite rod	activated sludge	carbon felt	*xylosoxidans* activated sludge	NA	30 (20 mg/L NO_3^-)	6	20 HRT	58.96	0.88	[101]
							7		72.65	1.09	
							8		65.08	0.98	
2CMFC-CEM, H(b)	graphite felt	anaerobic sludge	graphite felt	*Bacillus cereus*	NA	27	7	25	100	2.56	[102]

Notes: [1] 2CMFC: Double chamber Microbial Fuel Cell. 2CBES-poised cathode: potentiostatically controlled bio-electrochemical systems. SCMFC: Single-Chamber Microbial Fuel Cell. SCMEC: Single-Chamber Microbial Electrolysis Cell. 3CMDC: three-chamber Microbial Desalinization Cell. 3CMFC: three-chamber Microbial Fuel Cell (anode and double cathode). PEM: Proton Exchange Membrane. CEM: Cation Exchange Membrane. BPM: Bipolar membrane. H = H-type reactor. Cyl = Cylindrical reactor. C = cubic chambers reactor. TR = tubular reactor. (b) = batch mode operation. (sb) = semi-batch mode operation, i.e., continuous flow anode chamber and batch cathodic chamber. (c) continuous flow operation [2] ED: Electron Donor. MED: mediator for chemical or biological activity. WWTP (Wastewater Treatment Plant). PPy (polypyrrole). AQS (9,10-anthraquinone-2-sulfonic acid sodium salt). AQDS (anthraquinone-2-sulfonate) [3] HRT: Hydraulic Retention Time. [4] Specific rate normalized by the mass of volatile suspended solids (VSS) in the cathode compartment. NA: no available information.

These experiments evaluated Cr(VI) reduction under anaerobic conditions. So far, no literature report a reduction of Cr(VI) in open-air biocathodes. Oxygen is unquestionably the preferred final electron acceptor for microorganisms. However, possible advantages to Cr(VI)-reducing bacteria with an aerobic cathode may exist. Cr(VI)-reducing bacteria may be favored over other species in an environment with specific toxicity, even though tolerance to high Cr(VI) concentrations, up to 1 g Cr(VI)/L or even above, have often been documented [103].

The first test with Cr(VI) reducing biocathode was performed by Tandukar et al. [89], who inoculated the cathodic compartment of a 2CMFC (PEM membrane) with a mix of a denitrifying and methanogenic mixed culture, dosing bicarbonate as sole carbon source. Anaerobic mixed culture fed with acetate served as anode inoculum. With graphite plate electrodes and an external 1000 Ω resistor, the authors reported power densities of 7.0 mW/m^2 and 55.5 mW/m^2 depending on initial Cr(VI) concentration (22 and 63 mg/L, respectively). The maximum specific Cr(VI) reduction rate, about 0.46 mgCrVI/g$_{VSS}$/h, was registered at a 63 mgCr(VI)/L initial concentration. Analysis of the Cr(VI) reduction community by 16S rRNA gene sequences showed a predominance of phylotypes related to *Trichococcus pasteurii* and *P. aeruginosa*. Even considering the small amount of substrate that can leak from the anode, even when an ion-exchange membrane is used [104], and organic carbon released in cell lysis, most Cr(VI) reduction was obtained with autotrophic conditions.

In a batch-fed 2CMFC, the cathode is inoculated with a mixed microbial consortium from a Cr(VI) contaminated site and 39.2 mg Cr(VI)/L. Huang et al. [90] observed a specific reduction rate of about 2.4 mgCrVI/g$_{VSS}$/h, and 3.9 W/m^2 maximum power production at a current density of 11.1 mA/m^2.

Anaerobic pure cultures were also tested [91,92,102]. Hsu et al. [91] compared Cr(VI) reduction by six *Shewanella* strains at the cathode of MFCs in repeated cycles, observing initially the use of the electrode as the sole electron source in all tested strains. The variability in Cr(VI) reduction was associated with different mechanisms of chromium reduction, not identified, for each *Shewanella* strain evaluated, and other factors such as biofilm attachment to the electrode. Repeated Cr(VI) injections resulted in a general decrease in the MFCs performances and high residual Cr(VI) concentrations, which were explained with microorganisms' finite tolerance limit to Cr(VI) exposure and gradual fouling of the system by biological or reduced chromium species, which limit the active surface area of the cathode. Xafenias et al. [92] inoculated the cathode of an MFC and a MEC with *S. oneidensis* MR-1 fed with lactate. The combined use of the electrode and lactate as electron donors allowed bio-electrochemical and non-bio-electrochemical Cr(VI) reduction at the same time, even the contribution of the two different mechanisms to the overall process was not recognized. In Wu et al. [102], *Bacillus* sp. showed efficient Cr(VI)-reducing ability in both heterotrophic and autotrophic environments. The Cr(VI) removal rate reached 2.56 mg/L/h, which was 1.75 times higher than that of the MFC with the sterile control cathode.

3.1. Effects of pH and Cr(VI) Concentration

Extreme pH values (indicatively pH <5 or >8) and/or high chromium concentrations, typically 10–100 mg/L, can inhibit microbial activity. Tandukar et al. [89] reported that initial Cr(VI) concentrations above 80 mg/L inhibited the reduction rates in a denitrifying community. Li et al. [93] observed 10 mg Cr(VI)/L to irreversibly inhibit microbial activity in a single chamber MFC inoculated with municipal wastewater. Below toxic levels, increased initial Cr(VI) concentration, and following thermodynamics, was associated with an improved specific chromium reduction rate and MFC's power production [90,93].

The pH, with its effects on the surface properties of the cells, including cell surface hydrophobicity, net surface electrostatic charge, and biofilm structure, may also heavily affect complex biological and electrochemical reactions at the biocathode. Variation in pH may also affect enzymatic activity, and produced Cr(III) precipitation or bio-adsorption [105]. In Huang et al. [64,65], 50 mg/L initial Cr(VI) concentration inhibited the catalytic activity of electrochemical bacteria in the biocathode, whereas, at a 20 mg/L Cr(VI) concentration, chromium reduction efficiencies increased (+27.3%) and decreased (−21%) in acidic (pH = 5) and alkaline catholyte (pH = 8), with respect to neutral pH. A 0.22 cell net

potential increase, from 0.54 V at a pH of 8.0 to 0.76 V at a pH of 5.0, beyond the theoretical value of 0.177 V derived by Nernst's law, was associated with a pH decrease in the cathodic compartment, which actually indicates a positive response of microorganisms' activity associated with a pH decline [64]. Similar effects have been reported for denitrifying biocathodes [106]. Clearly, pH also affected the Cr(III) precipitation, with 9.3 mg/L dissolved Cr(III) at the end of the test at pH 5.0, in comparison to 0.3 mg/L at a pH of 8.0.

3.2. Effects of Cathode Potential

Tests with potentiostatically controlled cathodes pointed out an optimal potential range that typically exists for enhancing Cr(VI) reduction performances in biocathodes [95,107]. Theoretically, from Nernst's law, in MFC with a chromium-reducing cathode and acetate-oxidizing bioanode, the open-circuit voltage at pH 7.0 and 25 °C is about 0.68 V, which results in about 0.4 V theoretical cathode potential [90].

Lower set cathode potentials would promote the Cr(VI) reduction process. Huang et al. [64] compared the behavior of a potentiostatically controlled BES (with cathode operated at 200, −150, −300, and −450 mV vs. SHE) to an MFC operating with 200 Ω external load. Cathode at −150/−300 mV set potential promoted fast start-up time (19 days compared to 26 days in the uncontrolled MFC or 28 days in +200 mV set cathode system) and Cr(VI) reduction, with almost complete removal of 20 mg/L in 24 h, with respect to 43–70% with the other systems. Furthermore, +200 mV and −450 mV poised cathode limited bacterial growth, whereas −150 and −300 mV had beneficial effects. In all the tests, the reduction of Cr(VI) was attributed to microorganisms by directly accepting electrons from the electrode surface and transferring them to Cr(VI), as, even in the test, at the most negative potential, no production of hydrogen gas was observed. Optimal set potential can provide an appropriate selective pressure for adaptation of the microbial community in the system, which leads to enhancements of microbial electrochemical interaction with the cathode. The difference between Cr(VI) reduction potential and the cathode set potential represents the maximum energy to be gained by the cathodic microorganisms. Thus, the lower the set cathode potential is, the more energy microorganisms will potentially obtain. However, in case the cathode potential is set too low and goes beyond the self-regulation capability of microbial consortia, the energy gain by the cathodic microorganisms gets lost. Likely −150 and −300 mV set potentials allowed the biomass to gain more energy than when 200 mV set the potential. Although, theoretically most favorable, −450 mV may have exceeded the self-regulation capability of the microbial consortia, with no positive effect on power generation and Cr(VI) reduction [64].

Xafenias et al. [92] demonstrated the positive impact on Cr(VI) reduction of riboflavin, which is a naturally produced mediator, in potentiostatically-controlled Shewanella oneidensis MR-1 biocathodes. Different configurations, with lactate supplied as electron donor in inoculated and abiotic systems, with or without riboflavin addiction, were tested. At 20 mg/L initial Cr(VI) concentration, in a −300 mV poised biocathode fed with lactate (30 mM, or equivalently 2700 mg/L), up to 45% Cr(VI) reduction was observed in 4 h in comparison with 5% Cr(VI) reduction in a biotic system with no lactate and 15% reduction in abiotic systems with lactate. In 2CMFC with *S. oneidensis* MR-1 fed with lactate in both anodic and cathodic compartments, Cr(VI) reduction at the cathode (10 mgCr(VI)/L initial concentration) was coupled with 32.5 mA/m^2 maximum current density production [92].

3.3. Effects of Materials, Reactor Design, and Other Operational Parameters

Huang et al. [90] identified, together with Cr(VI) concentration, high conductivity of the electrolyte, (i.e., improved ion transport between the biofilm and bulk phase), as a key factor for efficient Cr(VI) reduction and power production. Increased conductivity of the solution, from 1.5 mS/cm to 10.6 mS/cm, increased the specific Cr(VI) reduction rate by about 25%, from 2.4 mg/(L g_{VSS} h) to about 3.0 mg/(L g_{VSS} h).

As to electrode materials, most experiences tested graphite or carbon-based electrodes. Huang et al. [90] tested graphite electrodes' specific surface, by covering the cathode with graphite granules, to

promote bacterial attachment and electrical connection between bacteria and the electrode surface. In a tubular 2CMFC, with a cathode to anode surface ratio (C/A) of 3, at a pH of 7, and 22 °C, the graphite fiber biocathode showed a higher specific Cr(VI) reduction rate and power generation than either graphite felt and granular graphite ones [65]. Specific Cr(VI) reduction rates on the graphite fiber cathodes, 12.4–20.6 mg/ g_{VSS} /h, were about 10–100 folds higher than the values reported for biocatalyzed carbon plate or graphite granule cathode in H-type MFCs with about the same Cr(VI) concentrations [89,90].

These results underline the coordinated role of the cathode surface area and reactor architecture on the biocathode performance. In Wu et al. [108], NaX zeolite-modified graphite felts were used as electrodes (anode and cathode) in 2CMFCs. NaX zeolite proved to enhance the hydrophilicity of the graphite felt by facilitating bacterial adhesion and electrochemical reaction, and by decreasing mass transport resistances. Two different fabrication methods for the NaX zeolite-modified graphite felts were testedin which the first one was tested without any pre-treatment of the felt and the second one was tested with HNO_3 pre-treatment. Both methods, especially the latter, resulted in excellent performance, with significant improvement in both electricity generation and Cr(VI) reduction rates, in comparison with graphite felts MFC. The HNO_3 pre-process remarkably enhanced NaX loading mass on the graphite felt, by decreasing the organic residues on the graphite surface. NaX zeolite-modified graphite felts MFC at an initial Cr(VI) concentration of 20 mg/L resulted in more than 410 mV maximum voltage, 29 mW/m^2 power density, and complete removal of Cr(VI) in 3 h, with an 8.2 times faster rate than simple graphite felts MFC. Nanostructured graphene also reduced Cr(VI) [99]. The maximum power density in an MFC with graphene biocathode was 5.7 times higher than the one produced with graphite felt biocathode. Electricity production, in fact, increased from 28.6 to 164 mW/m^2. Furthermore, improved efficiency in Cr(VI) reduction was obtained, with 100% reduction in a 40 mgCr(VI)/L solution within 48 h, in comparison to only 58% reduction with graphite felt.

The most widely tested configuration with biocathodes is the 2CMFC. Even a study with a single-chamber reactor exists [93]. Organic substrate removal at the anode and cathodic chromium reduction were reflected in the open circuit potential of the system and Cr(III) deposition on the cathode, as revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy [93]. Cr(VI) conversion efficiencies ranged from 89% to 99% depending on initial Cr(VI) concentrations (89% at 1.1 mg/L, 95% at 3 mg/L, and 99% at 10 mg/L). In the open-circuit control, Cr(VI) conversion efficiency was lower and decreased with Cr(VI) concentrations (88% at 1.1mg/L, 63% at 3 mg/L, and 28% at 10 mg/L). This means the SCMFC took advantage of electroactive bacteria using Cr(VI) as electron acceptor, as the other Cr(VI) reduction mechanisms, including bio-adsorption or bio-reduction by inelectroactive bacteria, as the open-circuit control pointed out, were inhibited at high Cr(VI) concentrations [93].

To increase microbial concentration and prevent premature cathode passivation due to Cr(III) precipitates during the system set-up, Wu et al. [96] proposed an ex situ acclimatization method for Cr(VI)-reducing biocathodes. The electrode was initially enriched with exoelectrogenic biofilm as an MFC anode, and the system was subsequently established using the anode as biocathode. This method allowed for the development of a mature biofilm in a shorter period of acclimatization (<19 days in the authors' experience) compared to traditional in situ methods, with Cr(VI) removal reaching 79% in 24 h, which is about four times higher than the one observed in the MFC with an in situ acclimated cathode. The improved performance was attributed not only to avoidance of premature formation of Cr(III) precipitates on the electrode, during biofilm acclimatization, but also to the enhanced bacterial growth rates in the heterotrophic anodic environment, which leads to high microbial density and bacterial coverage of the electrode. This may limit the effects of Cr(VI) toxicity on the microorganisms, at the anode/cathode inversion.

3.4. Simultaneous Reduction of Cr(VI) and Other Metals

As abiotic cathodes, biocathodes are also being tested for the simultaneous reduction of multiple metals, usually present in a variety of metal-processing wastewaters. Huang et al. [94] demonstrated that bacterial communities in biocathodes could adaptively evolve to utilize solutions containing mixtures of metals. Reduction rates of Cr(VI), Cu(II), and Cd(III) in BESs with biocathodes individually acclimated to the three different metals or acclimated to increased concentrations of a mixture of metalswere compared. In a Cr(VI) acclimated biocathode, the Cr(VI) reduction rate decreased from about 1.21 mg/L/h to 0.49 mg/L/h in the presence of 5 mg/L Cu(II) and 5 mg/L Cd(II). Acclimatization, by gradually increasing concentrations of mixed metals, allowed complete removal of Cr(VI) at a rate similar to that originally obtained with the reactor acclimated to Cr(VI) only. Analysis of bacterial communities showed different communities on the biocathodes of the reactors acclimated to the mixed metal solutions, compared to reactors acclimated only to a single metal. The decrease in diversity of the microbial communities was likely due to the greater toxicity of the mixed metals compared to only single metals. At the phylum level, compared to Cr(VI) acclimated biofilm, the relative abundance of Proteobacteria, Actinobacteria, Firmicutes, and Tenericutes increased in biofilms acclimated to mixed metals. Synergistetes, to the contrary, were absent following acclimation to the mixed metals.

Huang et al. [100] examined Cr(VI) and Cd(II) reductions on biocathode in pure culture MFC experiments with known electrochemically active bacteria, *Stenotrophomonas* sp. YS1, *Stenotrophomonas maltophilia* YS2, *Serratia marcescens* YS3, and *Achromobacter xylosoxidans* YS8. Cr(VI) reduction in the MFCs decreased in the presence of Cd(II) for all the pure cultures, with removals in a 5-h period ranging from 63% to 71%, depending on the species, compared to a range of 73–82% when Cd(II) was absent. Cr(VI) removal in biocathodes was higher than in the abiotic cathode, limited to about 39%. Cd(II) removal, on the contrary, was not impacted by the presence of Cr(VI).

4. Cr(VI) Reduction at Bioanode

Yeon et al. [57] enriched electroactive Cr(VI)-reducing bacteria in the anode compartment of MFC with the air cathode, using Cr(VI)-containing sludge from a leather tanning wastewater treatment plant fed with synthetic wastewater. At the end of the enrichment procedure, Cr(VI) removal capability of such biofilm was observed with a 93% reduction of 5 mg/L Cr(VI) and 61% of 25 mg/L Cr(VI). MFC-mediated Cr(VI) removal was attributed to either physical adsorption on the carbon felt anode (about 20%) and biological reactions including biosorption or reduction to Cr(III).

The bacterial community analysis by polymerase chain reaction—denaturing gradient gel electrophoresis (PCR-DGGE) of 16S rDNA, after enrichment, pointed out the microbial consortium is composed of both Cr(VI) reducers with either electrochemical activity (such as *Clostridium* sp.) or not (like *Acinetobacter* sp.), and non-Cr(VI) reducers with/without electrochemical activity (as *Actinobacteria* sp). Electroactive bacteria were responsible for electricity production in the MFC. However, reducers with electrochemical activity used Cr(VI) as an electron acceptor instead of interacting with the electrode, which causes a decrease in the current. Cr(VI) reduction was likely performed also by Cr(VI) reducers without electrochemical activity, with the required protons supplied by the metabolism of fermentative bacteria.

5. Cr(VI) Bio-Electrochemical Remediation

This review of published research, targeting treatment of Cr(VI) contaminated wastewater/industrial effluents, offers the first proof of concept for the chance of bio-electrochemical Cr(VI) remediation. Experiences with conventional bioreduction processes, under both aerobic and anaerobic conditions, by either pure cultures or mixed consortia, refer of Cr(VI) bioreduction rates in a 0.1–13.5 mg/L/h range [22,62,109–111], which are fully comparable to the values, 0.1–6.6 mg/L/h, observed in Cr(VI) reducing biocathodes.

Therefore, with comparable results, bioelectrochemical Cr(VI) remediation over the conventional bioremediation presents the following advantages: (i) the chance of excluding any external chemical supply to support the microorganisms' actions, (ii) ease of monitoring and control of the process, and (iii) the potential recovery of reduced chromium deposited on the electrode.

It should be noted that all the reviewed studies were batch laboratory tests, under conditions quite different from natural Cr(VI) contaminated water/groundwater. It would, therefore, be useful to perform evaluations under dynamic water flow conditions as in contaminated aquifers, with real groundwater, as reported by Gregory and Lovley [49] for uranium-contaminated aquifers.

For groundwater remediation, it is important to take into consideration specific properties that potentially affect BES operation [20]. Although Cr(VI) contamination likely increases the specific conductivity, typical low specific conductivity value of groundwater (well below 2 mS/cm) can be negatively impacted on BESs by implying higher ohmic and transport losses [30]. Moreover, pH shifts due to electrochemical Cr(VI) reduction in low buffering capacity systems may directly harm the electroactive bacteria and their removal performance [59]. Another challenge for bio-electrochemical treatment of contaminated groundwater is the presence, in addition to the contaminants, of a mixture of various naturally occurring inorganic (calcium, magnesium, carbonate, nitrates and sulphates, metals) and organic chemicals (e.g., humic acids) [112]. Magnesium and calcium can produce precipitates that could passivate the cathode with the consequent reduction of surface exchange active area [113]. Bio-electrochemical reduction has been recently reported for nitrate [97,101,114] and sulphate [115]. So far, the study of co-contaminants with BESs is limited, but theoretically, since reduction potentials of nitrate and sulphate are similar to the reduction potential of several pollutants, they can be electron competitors in the remediation process and affect the microbial community at the biocathode [116]. Wang et al. [101] evaluated the simultaneous autotrophic denitrification and the reduction of Cr(VI) under different pH conditions (6, 7, and 8). The highest removal efficiencies for nitrates (97%) and Cr(VI) (73%) were obtained at a pH of 7. The stable combined reduction was mainly ascribed to *Pseudomonas*, *Halomonas*, and *Thauera* species.

Chen and colleagues [97] used a 3 L cylindrical single-chamber reactor with a graphite felt cathode and a central carbon rod anode. The reactor was filled with sulphur granules and inoculated with anaerobic sludge. The reactor was continuously fed with 100 mg/L Cr(VI) synthetic wastewater with no organic C source (16 h of a hydraulic retention time) and run in the galvanostatic mode (current 10–60 mA). Cr(VI) reduction in the effluent ranged between 43% and 97%, which is proportional to the externally supplied current. This observation, together with SO_4^{2-} in the effluent, highlighted both sulphur and hydrogen autotrophic bacteria were responsible for Cr(VI) reduction by using the S granules in the reactor and H_2 produced by the cathode as electron donors. A similar system, which is a single chamber cylindrical reactor operated in galvanostatic (200 mA) continuous flow mode (20 h HRT), was adopted by Wang et al. [101] for removing Cr(VI) and nitrates from synthetic wastewater.

A continuous-flow BES was proposed for the simultaneous removal of p-fluoronitrobenzene (p-FNB), nitrates, and hexavalent chromium from synthetic wastewater as well [97]. In this co-contaminated system, the competition for electrons, for the carbon source and metabolism of microorganisms negatively influenced the degradation rates in comparison with the single pollutant control tests. The biodegradation of p-FNB in the co-contaminated system produced an additional organic carbon source to the microorganisms that promoted Cr(VI) and nitrates removal (nitrate and Cr(VI) removal through degradation of p-FNB). Instead, the p-FNB removal rate was controlled by electron availability (p-FNB degradation increased at currents above 40 mA) [97].

It is also interesting to consider the full cycle of sulphur in BESs. The role of the sulphur cycle during the electro-bioremediation of oil spills has been recently reviewed [43]. Sulphide produced by sulphate reducers can be oxidized to elemental sulphur on the anode surface [117]. Elemental sulphur can be back oxidized to sulphate [118] or can be reduced again to sulphide [119]. The sulphur cycle in BESs can, thus, be effective in enhancing current production (i.e., via sulphide recycling) or in supplying electron acceptors for biodegradation of reduced pollutants, such as hydrocarbons (i.e.,

via back oxidation of sulphur to sulphate). In this context, it is crucial to understand the possible role of Cr(VI) in environments in which co-contamination occurs. Reduction of Cr(VI) to Cr(III) can represent a sink for sulphide oxidation, which affects the performance of the process. Whether the effect is positive or negative on the anodic oxidation is still an open question. During oxidation of BTEX (benzene, toluene, ethylbenzene, xylenes) mixtures in BESs where sulphate was present in the medium (250 mg/L), the bacterial communities enriched on the anodes were dominated by microorganisms linked to the sulphur cycle. However, bacteria able to oxidize hydrocarbons and to perform direct electron transfer to the electrode (i.e., *Geobacter* spp.) were also detected [120]. In similar conditions, the competition between the anode and chromium for the scavenging of sulphide could facilitate the enrichment on the electrode of microorganisms not directly linked to the sulphur cycle. No study of Cr(VI) bioelectrochemical remediation in the presence of nitrate and sulphate is available. However, interferences with chromium reduction are likely to occur. Therefore, further understanding of chemical species that coexist with the target pollutant in groundwater is required.

In view of in situ applications, the effects of soil particles on pollutant partitioning and bioavailability, as well as system conductance need to be assessed. Soil type and external resistance significantly affected the current and Cr(VI) removal efficiency in soil MFCs tests operated at external resistances of 100 and 1000 Ω for 16 days [121]. The current production and Cr(VI) reduction in red soil and fluvo-aquic soil MFCs were compared. Red soil MFC performed better in the current production, but showed a lower Cr(VI) removal than fluvo-aquic soil MFC, which implies red soil may contain more electron acceptors that competed with the Cr(VI) reduction reaction [121]. About 60% to 90% of Cr(VI) was removed in 16 days of operation of a soil MFCs, while only 32–46% was removed in the open circuit control. Experiences integrating plants, microbes, and electrochemistry revealed promising applications of BESs to shallow contaminations [122,123], since plants can rely on atmospheric CO_2 for photosynthesis and secrete root exudates that can serve as carbon sources and electron donors for microbes in the rhizosphere to promote biodegradation/biostabilization.

6. Conclusions

Ever since the discovery of microbial remediation methods for Cr(VI), many technological approaches have been developed, and some are already used in full-scale treatments. As compared to other energy-intensive technologies, bioremediation is considered a promising cost-efficient and sustainable option. Microbial electrochemical systems have been recently proposed as an alternative platform for bioremediation of Cr(VI) and other toxic chemicals. BESs, in comparison with other bioremediation techniques, are particularly appealing for in situ applications, since they do not require relevant chemical addition in the subsurface and may entail a low energy supply. Reducing energy costs and chemical soil amendments implies lower operating costs for the BESs, which is particularly valuable considering the typical timeframe required for groundwater remediation. Moreover, in the Cr(VI) contamination treatment, the deposition of Cr(III) onto the electrode may enable extraction and recovery of Cr(III).

In lab-scale studies, BESs were competent in reducing at the cathode Cr(VI) in contaminated water streams with initial concentrations as low as 1 mg/L up to about 1 g/L. Even though positive results have been reported with abiotic cathode systems, biocathodes offer several advantages in Cr(VI) reduction from the perspective of groundwater remediation, such as the effectiveness in the natural waters' pH range and the exploitation of microbial catalysis, which limits cathode passivation due to Cr(III) precipitation.

Nonetheless, much work is still needed to improve the Cr(VI) reduction rate at the biocathode to maximize the advantages of biocathode BESs over conventional biological processes. A lack in the long-term pilot and scale-up research suggests that more focus should be given to key factors that need to be considered for fully-practiced feasibility studies and full-scale applications. For example, the stability of processes and equipment and the area of influence that each BES module can effectively cover have to be addressed. In addition, flexible configurations to adapt to different

site-specific characteristics (for example, water table depth, soil types, etc.) are required for in situ remediation. No preliminary models have been developed to start addressing this issue. Further research is needed to understand the mechanisms driving BES function and, thus, to fully exploit BES potential in real contaminated soil and groundwater. Many challenges, including the selection of microorganisms, cheap materials, and an optimal reactor configuration, need to be addressed during the technology scale-up.

Author Contributions: Conceptualization, G.B., A.F.M. and E.S.; literature review of microbial mechanisms of Cr(VI) reduction G.B., A.E.T. and M.D.; literature review of electrochemical and bio-cathodic Cr(VI) reduction, G.B., A.F.M. and E.S.; organization of the information, synthesis and critical analysis, G.B., A.E.T., A.F.M. and E.S.; writing—original draft preparation, A.F.M. and E.S.; writing—review and editing, G.B., A.E.T., M.D. and E.S.; project supervision, S.S. and A.F.

Funding: The Fondazione Cariplo in the framework of the project BEvERAGE—BioElEctrochemical RemediAtion of Groundwater plumes (2015-0195) supported this work.

Conflicts of Interest: The authors declare no conflict of interest.

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