Bio-induced reduction of Cr(VI) in aquifers by organic substrates injection

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Abstract

Hexavalent chromium is a primary toxic element used in galvanic processes, in metallurgical industry and for the production of dyes and pigments. Conventional methods for Cr(VI) remediation, pump&treat and excavation, are expensive and require a large amount of energy and time. Innovative technologies include bio-induced reduction, that is Cr(VI) reduction to Cr(III) by injection of organic substrates that are readily biodegraded by autochthonous microorganisms in the aquifer, resulting in reducing conditions.

Lab scale batch tests were carried out, with two different soil (A and B) and solid/liquid ratios (25% and 50% on weight basis). Initial Cr(VI) concentrations were 5000 or 10000 µg/L. Ultrafiltration permeates of cheese whey and beer distillation residues were used as the organic substrates.

In all microcosms, dissolved oxygen decreased from about 6 mg/L to values <1 mg/L after 1-2 d incubation, and the redox potential from approximately +250 mV to -400 mV by 11 d. After about 40 days, the highest Cr(VI) abatements were obtained in soil A microcosms fed with beer distillation residues, as soil A had an initial total heterotrophic bacteria concentration three orders of magnitude higher than soil B. Fe(II) availability was also a key factor in Cr(III) co-precipitation.

Keywords: cheese whey permeate; beer distillation residue; chromium; bioremediation

1. Introduction

Chromium can have several oxidation states, but the most common forms in subsoil are Cr(III) and Cr(VI). The hexavalent form is very soluble and mobile, and carcinogenic to humans. Cr(VI) can be reduced to the trivalent form by redox reactions involving organic substances in the soil (carbohydrates, proteins, and humic acids) or as part of metabolism of certain microbial species (Dhal *et al*., 2013).

Traditionally, groundwater pump & treat has been used to remediate chromium-contaminated plumes; this method requires long-term application to meet Cr(VI) remediation goals. In the last decade, innovative in situ clean up technologies for Cr(VI) have been tested. Taking into consideration sustainability, the most interesting technologies are based on biological or chemical mechanisms (Fruchter *et al*., 2002).

This experimental work has focused on bio-induced reduction in the saturated soil zone. During bio-induced reduction, an organic substrate is injected into the aquifer to create a negative redox potential zone. In fact, the heterotrophic microorganisms in the aquifer rapidly degrade the injected carbon substrate, consuming the dissolved oxygen. After oxygen, nitrates, manganese and iron oxides, sulphates and carbon dioxide are consumed. The resulting environmental conditions make Cr(VI) reduction possible (USEPA, 2013); reduced chromium, less mobile than Cr(VI) and non-carcinogenic, tends to precipitate in the form of hydroxides.

Two different soils, two different organic substrates from food industry, and two different Cr(VI) initial concentrations were investigated to assess the dissolved Cr(VI) abatement and the kinetics of the process.

2. Materials and methods

Microcosms were prepared in batch mode, using tap water similar in composition to groundwater (Table 1) and two soils (named “A” and “B”), collected from two different sites in Italy (Table 2).

Permeate from cheese whey ultrafiltration or a waste from the brewing process were used as organic substrates in the tests (Table 3). The dose of ultrafiltration permeate of cheese whey to consume the available electron acceptors in the microcosms was calculated according to theoretical oxidation of the reference molecule (lactose) and a safety factor of 1.25 (third method in Parsons (2010)), resulting in 5 ml of permeate per liter of aqueous phase. As for the waste from the brewing process, the dosage was set in order to have the same initial chemical oxygen demand (COD) as for the microcosms with cheese whey permeate (300 mg COD/l of aqueous phase), resulting in 2.5 ml substrate per liter of aqueous phase.

Cr(VI) contamination in the microcosms was carried out with a 0.2 N potassium dichromate solution, dosed to

obtain different initial Cr(VI) concentrations (5000 and 10000 μg/l of water).

The details about the tests (soil, organic substrate, initial chromium concentration, solid to liquid ratio, temperature, Fe (II) addition, number of replicates, and sampling times) are summarized in Table 4.

During the experiments, redox potential (ORP), dissolved oxygen (DO) and pH, as well as Cr(VI) dissolved concentration, were monitored.

3. Results and Discussion

3.1. DO, ORP and pH behavior

DO rapidly (1-2 d) decreased in all tests beyond 1 mg/l (Table 5), whereas the ORP values were still above 200 mV. With soil A, at 5 mg/l initial Cr(VI) concentration, ORP values of about -400 mV were obtained after 3-4 d incubation, with a very steep decrease after 3 d of treatment. In microcosms with the initial concentration of 10 mg Cr(VI)/l, the ORP underwent slower decrease, reaching slightly negative values in 6-8 d treatment. In tests with the brewery substrate, the ORP decreased to -400 mV after 9 d, whereas –300 mV were reached in 11 d with the permeate of cheese whey.

Table 1. Features of water used in the laboratory tests

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

Table 2. Soils used in the experiments

Parameter	**Soil "A"**	**Soil "B"**	**Method**
Particle size distribution	Slightly silty sand with gravel	Sand	ISO 11277 (2009)
Dry bulk density (kg/m^3)	1606±63	1478±22	ISO 11272 (1998)
Organic carbon (%)	0.59±0.03	0.27±0.02	UNI EN 15169 (2007)
pH (-)	8.52±0.01	8.5±0.1	Rayment and Higginson (1992)
Total heterotrophic bacteria (CFU/g d.w.)	10^4	10	Plate counting
Parameter	**Ultrafiltration permeate of cheese whey**	**Waste from brewing process**	**Method**
COD (g/l)	60±12	122±24	MU 201 (2006)
Total heterotrophic bacteria (CFU/100 ml)	10^6	10^3	Plate counting

Table 3. Organic substrates used in the experiments

Parameter	**Ultrafiltration permeate of cheese whey**	**Waste from brewing process**	**Method**
COD (g/l)	60±12	122±24	MU 201 (2006)
Total heterotrophic bacteria (CFU/100 ml)	10^6	10^3	Plate counting

Table 4. Details about the tests (C_0: initial Cr(VI) concentration; S: Organic substrate - ultrafiltration permeate of cheese whey (P), and waste from brewing process (B); S/L: solid to liquid ratio; T: temperature; Nr R: number of replicates; ST: sampling times). After 7 d incubation, microcosms nr. 17 to nr. 19 were added of 10 mg/L of Fe(II) and incubated for further 31 d (*).

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

In tests with soil B and 50% S/L, redox potential decreased to values of about -400 mV in 5.2 d of incubation with the substrate from brewery and initial Cr(VI) concentration of 5 mg/l; this was the test with the fastest ORP decreasing rate in the first week of treatment. In the other microcosms, after 10 d, -200 mV (permeate of cheese whey, 10 mg Cr(VI)/l), -400 mV (brewery substrate, 10 mg Cr(VI)/l), and -600 mV (permeate of cheese whey, 5 mg Cr(VI)/l) were obtained. Reducing conditions were registered within 5 d of incubation in all the investigated microcosms with 25% S/L, with ORP values <-400 mV in 5-7 d. The depletion of electron acceptors occurred at a faster rate with soil A (DO <1 mg/l in 1.1 d, ORP <0 mV in 5 d, and ORP <-400 mV in 5.6 d) than with soil B (DO <1 mg/l in 1.6 d, ORP <0 mV in 6.4 d, ORP <-400 mV in 8.1 d), as soil B had total heterotrophic biomass three orders of magnitude lower than soil A. With smaller S/L and a higher temperature (tests 13-16), reducing conditions were reached in a shorter incubation time: DO <1 mg/l after 0.9 d, ORP <0 mV after 4.2 d, ORP <-400 mV after 5.6 d.

Table 5. Time (d) to achieve specific conditions in the microcosms. -: not measured; no: not reached by 11 d.

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

Comparing the results of the tests with 50% S/L, the kinetics was faster in the case of brewery substrate (4.3 d on average for achieving negative redox) than with permeate of cheese whey (6.1 d). DO and ORP trends over time were not significantly affected by Cr(VI) initial concentration.

As an example, Figure 1 shows the ORP versus time from continuous monitoring in tests 7 and 8.

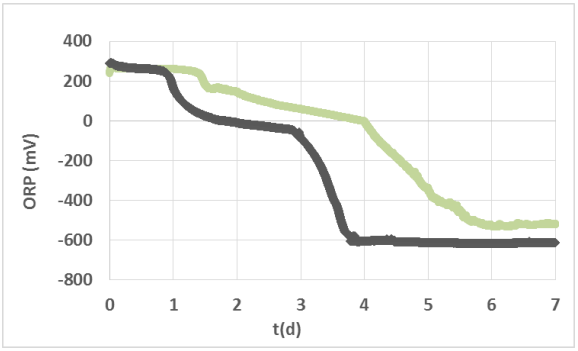

Figure 1. ORP trends in test 4 and 8 (waste from the brewing process, Cr(VI) initial concentration of 5 mg/l).

In all microcosms, the initial pH value was in the range 7.0 ± 0.5 and no significant variations were registered during the tests.

3.2. Cr(VI) reduction

The effect of the different soil on chromium reduction was observed in test 1-8, performed at 17±1 °C for 36 d and a 50% solid to liquid ratio.

In microcosms with soil A (Figure 2), dissolved Cr(VI) removal started after 8 d incubation. Following 36 d of incubation, residual values of about 1.3 µg/l were registered in all the microcosms, except in tests with 10 mg/l initial Cr(VI) concentration and permeate of whey as substrate; in this latter, the residual Cr(VI) concentration was about 2 mg/l.

With soil B, as shown in Figure 3, 11 d incubation were necessary to observe a decrease in the Cr(VI) concentrations. The residual values after 36 d of treatment, ranged between 5 µg/l and 5500 µg/l. In particular, in the test with 10 mg/l initial Cr(VI) concentration, the final values were approximately 5.4 mg/l both with the permeate of whey cheese and the waste from brewing process as substrates. Final values of 394 µg/l (permeate of whey cheese) and 5 µg/l (waste from brewing process) were obtained starting from 5 mg Cr(VI)/l.

Comparing the microcosms at the same Cr(VI) initial concentration and organic substrate, the best performance was obtained with soil A.

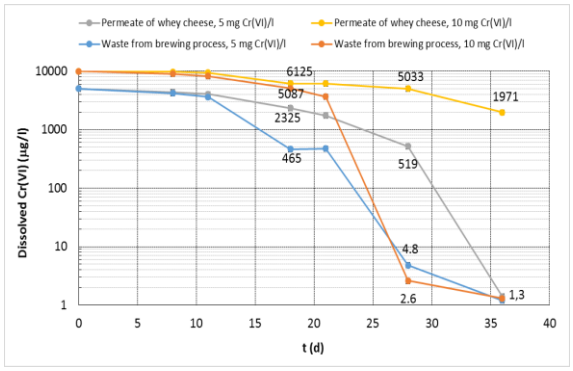

Figure 2. Dissolved Cr(VI) over time t, in microcosms with soil A and 50% S/L.

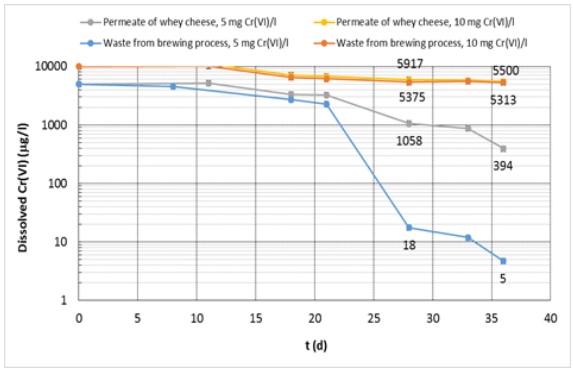

Figure 3. Dissolved Cr(VI) over time t, in microcosms with soil B and 50% S/L.

3.3. Cr(VI) reduction - iron effect

Iron has a key role in the Cr(III) co-precipitation. Therefore, to investigate potential causes for the different results obtained with the two soils, the release of Fe(II) by the two soils as a function of time was assessed with tests 9-12, performed at 25±2 °C and a 25% solid to liquid ratio on weight basis.

With both substrates, kinetics of Fe(II) release by soil B were slower than by soil A; with soil B, more than 20 d were required to trigger a significant iron release. In fact Fe(II) dissolved concentration in microcosms with soil A was more than 1 mg Fe/l, whereas it was <100 µg/l till 10 d with soil B.

To further highlight the Fe(II) role in Cr(VI) reduction, test 13-20 were performed for 38 d at 25 °C, 25% S/L, using only soil B. In tests 17-20, after 7 d incubation, Fe(II) was added; the dose was set according to stoichiometric calculation to provide Fe(II) sufficient for almost complete Cr precipitation in the microcosms with 5 mg Cr(VI)/l ad the initial concentration (He, 2003):

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

In Figure 4 the dissolved Cr(VI) removal in the different microcosms, calculated with respect to the initial concentration, are shown. At the end of the tests (38 d), major abatements were observed in microcosms with lower initial Cr(VI) values, and where Fe(II) had been added

during incubation (71% with cheese whey, 79% with waste from brewing process). Lower abatements (38% with whey permeate, 48% with brewing residue) were registered in microcosms with 10 mg Cr(VI)/l initial concentration, even in case of Fe(II) addition. In all the other tests, Cr(VI) removal fell in the range 6%-24%. About 45% removal was observed in tests at 10 mg Cr(VI)/l initial concentration and 50% S/L. Very high efficiency (>90%) resulted with 5 mg Cr(VI)/l and 50% S/L.

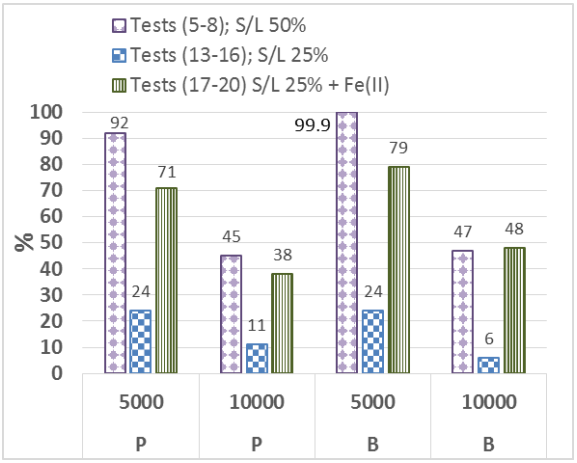

Figure 4. Cr(VI) removal at the end of the tests. 5000 µg/l and 10000 µg/l initial Cr(VI) concentration; P: Permeate of cheese whey; B: waste from brewing process.

Conclusions

Hexavalent chromium is a highly carcinogenic metal and a wide spread contaminant in groundwater. Bio-induced reduction, an innovative clean-up technologies for Cr(VI) reduction, involves the injection of readily biodegradable organic substrates. Biodegradation reactions by heterotrophic microorganisms in the aquifer causes sequential depletion of the different electron acceptors and promote reducing conditions.

Lab scale tests for Cr(VI) reduction under bio-induced reducing conditions were performed to select a proper organic substrate, to study the kinetics of the process and to evaluate iron involvement in Cr precipitation.

A comparison between two different soils showed that the electron acceptors consumption was quicker for the soil with the highest total heterotrophic bacteria initial concentration.

Cr(VI) abatement in all the microcosms started after about 10 d incubation. Reduction/precipitation efficiency was affected by the substrate used and the initial Cr(VI) concentration. In the microcosms with soil A (50% S/L), Cr(VI) residual values lower than 5 µg/l were obtained, except in tests with 10 mg/l initial Cr(VI) concentration and cheese whey. With soil B at 50% w/w, Cr(VI) values <5 µg/l were obtained only in the test with 5 mg Cr(VI)/l initial concentration and the waste from the brewing process. Tests at 25% S/L resulted in lower Cr(VI) abatements after 38 days than tests at 50% S/L; the Fe(II) dosing significantly increased the removal efficiency.

The presence of Fe (II) in microcosms was a key factor, as well as a limiting parameter, in the process of Cr removal from water.

In conclusion, chromium reduction was observed after strongly reducing conditions have been reached and maintained for a few days. Redox potentials <-200 mV were necessary in order to get Cr(VI) reduction. In several tests, Cr(VI) removals were high, resulting in values up to 100% after about 40 d incubation and final concentration lower than the Italian regulatory limit (5 µg Cr(VI)/l).

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