Biological hexavalent chromium removal from contaminated groundwater under high and low nitrates content

E. Panousi, D. Mamais, C. Noutsopoulos, K. Mpertoli, C. Kantzavelou, E. Nyktari, I. Kavallari, M. Nasioka
National Technical University of Athens, Faculty of Civil Engineering, Department of Water Resources and Environmental Engineering, 5 Iroon Polytechniou, Zografou, Athens 15780, Greece
(email: mamais@central.ntua.gr)

Abstract
Microbial reduction of Cr(VI) to the much less toxic Cr(III) has been practiced for the treatment of liquid wastes but has not been implemented for the treatment of contaminated, by nitrates, groundwater. The objective of this work is to evaluate biological groundwater treatment systems with high (15 mg/L) and low (5 mg/L) nitrates content that will achieve Cr(VI) removal under anaerobic conditions. The effects of the type of organic substrates added to the contaminated groundwater (milk, sugar, cheese whey), concentrations of organic substrates COD in the feed (100, 150, 200 mg/L) and hydraulic residence time (1.7, 0.9, 0.7 d) on process performance were evaluated through the operation of a series of anaerobic sequential batch reactors (SBR). As a conclusion, biomass acclimatized to Cr(VI) under anaerobic conditions with low nitrates content exhibited a lower tolerance to Cr(VI) compared to biomass acclimatized to Cr(VI) under high nitrates content in accordance with the reduction rate of Cr(VI). The concentration and the type of organic substrate were crucial for the microbial reduction of Cr(VI). The different hydraulic residence time of the reactors did not affect the exhibition of complete hexavalent chromium removal but increased the trivalent particulate chromium in the biomass. This study demonstrates that anaerobic biological systems treating groundwater under low and high nitrates content can provide complete hexavalent chromium removal at initial hexavalent chromium concentrations as high as 200 μg/L, under certain conditions.

Keywords
Biological groundwater treatment; Cr(VI) reduction; anaerobic treatment; high nitrates content; low nitrates content

INTRODUCTION

The release of chromium (Cr) wastes has in many cases resulted to serious contamination of water bodies. In the environment, Cr is usually encountered in the oxidation states of trivalent Cr and hexavalent Cr. Trivalent chromium Cr(III) is considered to be the most stable based on oxidizing step. It is one of the ten most prevalent substances in the earth's crust. It is formed naturally and is considered an essential nutrient for human life (Jacobs and Testa, 2004). Hexavalent chromium is mobile in air and in pure water and is considered mutagenic to bacteria and mutagenic and carcinogenic to humans and animals (Gonzalez et al., 2003; Oze et al., 2007; Contreras et al., 2011). As a conclusion, it is important where Cr(VI) is met to be reduced rapidly to Cr(III) in order to protect the environment and the public health.

USEPA has designated Cr as one of seventeen chemicals causing the greatest threat to human health so that Cr groundwater pollution would be combated. The World Health Organization has proposed a maximum allowable limit for drinking water of 50 μg/L total chromium (WHO, 2004). Similarly in Europe according to the Drinking Water Directive the maximum allowable concentration for total chromium is set at 50 μg/L (Directive 98/83/EU). In USA, EPA has set the maximum contaminant level for total chromium in drinking water at 100 μg/L (EPA, 2009). Until recently, high levels of Cr(VI) in the environment were always attributed to anthropogenic pollution. Industrial wastes from industrial activities such as steelworks, petroleum refining, metal finishing, Cr electroplating and leather tanning (Chen and Gu, 2005a) were the cause of the problem. However, high levels of hexavalent Cr may also exist due to natural geogenic processes, especially in areas where there are relatively high levels of naturally occurring Cr(III) or Cr(VI) in the sediments, and natural processes that can convert Cr(III) to Cr(VI) such as in minerals like crocoite ($PbCrO_4$) and lopezite ($K_2Cr_2O_7$). According to these findings, in ultramafic rocks and serpentinites of

ophiolite complexes, Cr content in groundwater can be as high as 300 ppb (Fantoni et al., 2002; Gonzalez et al., 2005).

In order to tackle the problem of high Cr(VI) concentrations in groundwater, several treatment technologies have been developed. The most often used methods are physicochemical techniques and more specifically: a) chemical oxidation (Barrera-Diaz et al., 2012), b) ion exchange (Ren et al., 2012), c) adsorption via activated carbon (Zhang et al., 2015), or low cost agricultural waste by-products (Kadirvelu et al., 2003), d) membrane separation (Yao et al., 2015), e) electrocoagulation (Emamjomech and Sivakumar, 2009), f) electrodissolution (Mukhopadhyay et al., 2007) and g) photocatalytic reduction (Rivero-Huguet and Marshall, 2009). All these methods present several disadvantages such as high capital and operational cost, production of chemical sludge and sludge disposal problems (Chen and Hao, 1997; Li et al., 2007; Demir and Arisoy, 2007). In recent years, interest has been developed in pursuing biologically-mediated Cr(VI) reduction by enhancing microbial activity. The reduction of Cr(VI) to Cr(III) was shown to be possible, using pure and mixed cultures enriched from wastewaters, under anaerobic conditions (Lovley and Phillips, 1997).

Despite the extensive literature regarding the physicochemical methods for Cr(VI) removal from water, there is very limited literature on the biological treatment of groundwater for Cr(VI) removal. On the other hand, the microbial reduction of Cr(VI) to Cr(III) has been extensively reported in the literature for the treatment of liquid wastes under anaerobic conditions (Ishibashi et al., 1990; Shen and Wang, 1993; Stasinakis et al., 2004; Mamais et al., 2016; Panousi et al., 2016) due to its low operational cost, its effectiveness and its low production of chemical sludge. Many bacterial species have been reported to reduce Cr(VI) to Cr(III) under anaerobic conditions including *Pseudomonas, Escherichia, Achromobacter, Ochrobactrum, Shewanella, Rhodobacter, Brevibacterium, Pannonibacter, Desulfovibrio vulgaris* (Thatoi et al., 2014). The activated sludge technology has been widely applied to treat municipal and some industrial wastewaters since its operation is simple and convenient (Chen and Gu, 2005a; Chen and Gu, 2005b). In contrast to the pure cultures, the activated sludge biomass is easy to acclimate to different environments, it does not need to be manipulated under rigorous conditions, and the wastewater does not need to be sterilized before treatment (Chen and Gu, 2005a; Chen and Gu, 2005b). The main reasons that biological methods have not been implemented for the treatment of groundwater are the absence of organic substrates in groundwater, the lack of applications and experience on biological groundwater treatment systems and the questionable effectiveness of anaerobic treatment systems with high and low nitrates content. In order to optimize the design and operation of the biological Cr(VI) reduction process in sequencing batch reactors, a thorough understanding of the characteristics of microbial transformation of Cr(VI) is needed.

The objective of this work is to evaluate biological groundwater treatment systems that will achieve Cr(VI) removal from contaminated groundwater, under anaerobic conditions with high and low nitrates content. In order to evaluate Cr(VI) removal under these conditions, eight lab scale units operating as sequencing batch reactors (SBR) were employed to study biological Cr(VI) removal from groundwater containing Cr(VI) concentration of 200 μg/L, high nitrates content of the order of 15 mg/L and low nitrates content of the order of 5 mg/L. All systems were compared to their Cr(VI) and soluble NO_3-N removal rates and their general performance under different types/load of substrate and HRT, measured in continuous flow and batch kinetic experiments.

EXPERIMENTAL MATERIALS AND METHODS

Continuous flow and batch experiments

A series of eight bench scale experimental systems operating as SBRs were employed in order to evaluate the effect of several parameters on biological Cr(VI) removal. The quality characteristics of groundwater used are summarized in Table 1.

Table 1: Operational characteristics of the experimental systems

Parameters	LOW NITRATES CONTENT			HIGH NITRATES CONTENT				
	L1	**L2**	**L3**	**H1**	**H2**	**H3**	**H4**	**H5**
Type of substrate	S(90%) M(10%)	S(90%) M(10%)	S(90%) M(10%)	S(90%) M(10%)	M	CW	S(90%) M(10%)	S(90%) M(10%)
Duration of operation (d)	105	30	75	102	30	60	37	30
COD_{in} (mg/L)	200	200	200	200	200	200	150	100
$Cr(VI)_{in}$ (μg/L)	200	200	200	200	200	200	200	200
HRT (d)	1.7	0.9	0.6	1.7	1.7	1.7	1.7	1.7
Sludge age (d)	10	10	10	10	10	10	10	10
Temperature (ºC)	25.2±1.6	30±0.5	22.7±0.4	23.3±1.7	30±0.5	26.2±1	22.2±0.7	20.6±0.9
DO (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
pH	7.1±0.09	7.3±0.04	6.9±0.12	6.95±0.5	7.5±0.06	7.1±0.04	7.0±0.09	7.03±0.4
Redox (mV)	-152±21	-172±26	-163±29	-113±12	-183±22	-188±19	-142±39	-150±23

Five SBR experimental systems (H1, H2, H3, H4, H5) operated under high nitrate contents. More specifically systems H1, H2, H3 operated with one feeding cycle daily consisting of 0.5 h feeding time, 22 h reaction time, 1h settling time and 0.5 h decanting time. They were fed with groundwater of 3 L (V total = 5 L), that was supplemented with a substrate concentration equal to 200 mg/L on a COD basis (consisting of 90% of sugar and 10% of milk, 100% milk and 100% cheese whey, respectively) and sufficient quantities of nutrients ammonium and phosphorus. Systems H1, H4, H5 operated with one feeding cycle daily consisting of 0.5 h feeding time, 22 h reaction time, 1h settling time and 0.5 h decanting time. They were fed with groundwater of 3 L (V total = 5 L), that was supplemented with a substrate concentration equal to 200, 150, 100 mg/L respectively, on a COD basis consisting of 90% of sugar and 10% of milk, and sufficient quantities of nutrients ammonium and phosphorus. Potassium nitrate was added to all five systems in order to reach a nitrate concentration (NO_3-N) equal to 15 mg/L. The nominal hydraulic residence time of the five SBRs was equal to 1.7 d.

Three SBR experimental systems (L1, L2, L3) operated under low nitrate contents with one, two and three feeding cycles daily, respectively. The one feeding cycle was consisting of 0.5 h feeding time, 22 h reaction time, 1h settling time and 0.5 h decanting time. The two feeding cycles/day were consisting of 0.25 h feeding time, 10.5 h reaction time, 1h settling time and 0.25 h decanting time. The three feeding cycles/day were consisting of 0.25 h feeding time, 6.5 h reaction time, 1h settling time and 0.25 h decanting time. The nominal hydraulic residence time of the three SBRs was equal to 1.7, 0.9, 0.6 d, respectively. All three systems were fed with groundwater of 3 L (V total = 5 L), that was supplemented with a substrate concentration equal to 200 mg/L (consisting of 90% of sugar and 10% of milk on a COD basis) and sufficient quantities of nutrients ammonium and phosphorus. Potassium nitrate was added to reach a nitrate concentration (NO_3-N) equal to 5 mg/L.

Groundwater contained an average initial sulfate concentration (SO_4-S) of 40 mg/L that was consider sufficient to support sulfate reducing bacteria. The concentration of hexavalent chromium in treated groundwater was 200 μg/L and a sludge retention time (SRT) of 10 days. Both experimental systems L1, H1 that were the control systems, inoculated with anaerobic activated sludge from Psyttalia Wastewater Treatment Plant (PWTP). All systems received groundwater from the National Technical University of Athens campus water supply network. Furthermore the average temperature of mixed liquor for all systems ranged from 21°C to 30°C.

In order to measure Cr(VI), soluble COD and soluble NO_3-N removal rates, batch experiments were conducted in triplicates at constant temperature, by submerging batch reactors in temperature controlled water baths. These batch assays were conducted with biomass from the SBR systems that was acclimatized to the substrate used, the Cr(VI) initial concentration and the temperature employed in the batch experiments. Following batch feeding, hourly samples from the batch systems were collected and analyzed for soluble COD, soluble NO_3-N, dissolved oxygen (DO), redox (ORP), total and hexavalent chromium for a period of 24 hours.

The performance of the SBR units was assessed by routine measurements of temperature, total and soluble COD, TSS, VSS, NH_4-N, NO_3-N, redox, pH, DO, total and hexavalent chromium throughout the experimental period. All analyses of SBR units and batch assays were performed in accordance with Standard Methods (APHA, 2012). Dissolved oxygen concentration (DO), redox and pH were measured daily using portable equipment (HACH, HQ40d).

RESULTS AND DISCUSSION

The performance characteristics of the eight SBR systems operating under anaerobic conditions with low and high nitrates content are shown in Table 2. The percentage of total effluent chromium achieved under anaerobic conditions with low and high nitrates content is shown in Figure 1.

According to the results of the SBR systems, anaerobic conditions supported almost complete Cr(VI) reduction to Cr(III) (>99%) up to initial groundwater Cr(VI) concentration of 200 μg/L under low and high nitrates content.

Both L1 and H1 systems that were the controls, exhibited a high hexavalent Cr(VI) removal efficiency that averaged over 99%. The results of the batch experiments that were conducted in triplicates at constant temperature to measure Cr(VI) removal rates are presented in Figure 2. Maximum Cr(VI) removal rate averaged 27±0.5 μgCr(VI)/gVSS/h in the high nitrates content system (System H1) and 23±0.4 μgCr(VI)/gVSS/h in the low nitrates content system (System L1). These results demonstrate that nitrates concentration in the 5 – 15 mg/L of NO_3-N do not affect Cr(VI) removal efficiency.

The different hydraulic residence time of the anaerobic-low nitrates content reactors L1, L2, L3 did not affect the exhibition of complete hexavalent chromium removal but increased the trivalent particulate chromium in the biomass. The different hydraulic residence time moreover increased the total particulate trivalent chromium in the effluent to the order of 35%, 57%, 71% respectively, as Figure 1 shows. As shown in Table 2 liquor suspended solids contained a significant amount of chromium solely in the form of Cr(III) that ranged from 1.1±0.3 mg Cr gSS^{-1} to 3.5±0.2 mg Cr gSS^{-1}. Based on this Cr(III) content, biomass should be probably managed as toxic waste due to its high trivalent chromium content. However the quantities of the produced biological sludge are rather limited due to low yield coefficient of microorganisms under anaerobic conditions and the long

sludge being adopted. Therefore, it is anticipated that the cost for management of this sludge is rather insignificant.

Table 2: Experimental results of the lab-scale units-All systems received groundwater with a Cr(VI) content of 200 μg/L.

Parameters	LOW NITRATES CONTENT			HIGH NITRATES CONTENT				
	L1	L2	L3	H1	H2	H3	H4	H5
HRT (d)	1.7	0.9	0.6	1.7	1.7	1.7	1.7	1.7
MLSS (mg/L)	655±71	390±50	957±21	593±27	640±21	444±45	517±65	276±45
MLVSS/MLSS (%)	0.86	0.89	0.98	0.78	0.88	0.82	0.83	0.96
COD_{sol}^{eff} (mg/L)	18±4	25±8	67±6	8±7	25±3	12±3	10±2	16±4
COD_{sol}^{rem}(%)	90.8	87.0	66.5	96.0	97.7	94.0	95.0	92.0
TSS^{eff} (mg/L)	18±13	37±20	40±10	11±8	27±4	32±5	20±8	8.2±5
$Cr(VI)^{eff}$ (μg L^{-1})	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	22±7	65.8±7
$Cr(VI)^{rem}$(%)	>99	>99	>99	>99	>99	>99	88.9	68.4
$Cr(tot)^{eff}$ (μg L^{-1})	13.1±5	84.6	104±5	25±3	45.5±4	50±8	38±3	108±20
$Cr(tot)^{rem}$(%)	93.4	58.0	48.2	87.5	77.3	74.0	80.0	46.0
$Cr(sol)^{eff}$ (μg L^{-1})	6.7±1	34.5±16	60.1±6	16±2	17.1±6	29±11	21±5	105.7±9
Cr(III)part/MLSS (mg Cr g SS^{-1})	1.1±0.3	1.7±0.8	3.5±0.2	2.5±0.3	1.66±0.6	2.7±0.3	3.2±0.3	3.13.2±0.3

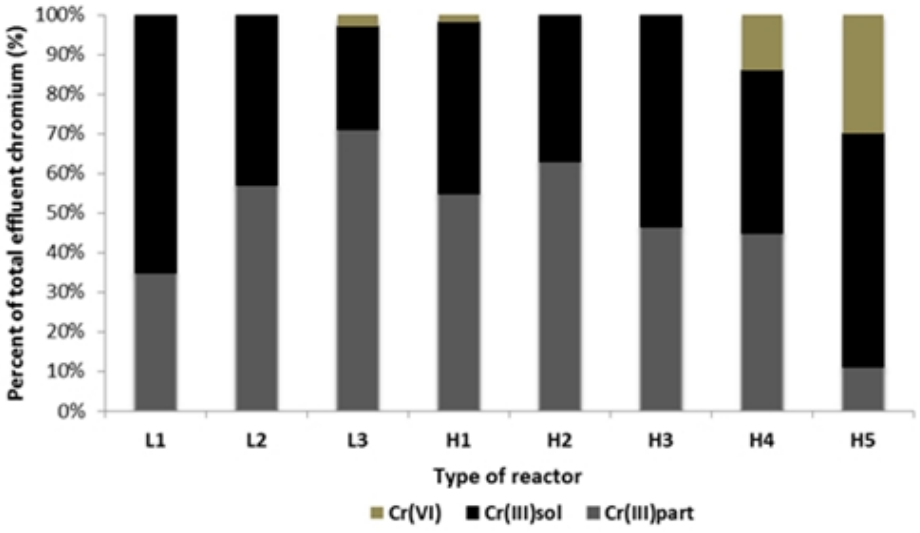

Figure 1. Percentage of total effluent chromium achieved by all systems

Continuous flow assays were performed to study the effect of the type of the carbon source as electron donor on Cr(VI) removal by activated sludge, under anaerobic conditions with high nitrate

contents. The SBRs were operated on three different types of carbon sources: a) glucose, a simple low molecular weight substance that was added as the primary electron donor supplemented with milk (90%-10% ratio on a COD basis) (System H1), b) milk, a complex organic carbon source (System H2), c) liquid cheese whey, a low cost complex organic carbon source (System H3). The experimental data clearly illustrate that biomass under high nitrates content can support practically complete microbial Cr(VI) removal (>99%) with low molecular substrates such as sugar and more complex fermentable substrates such as milk and cheese whey when added to groundwater at a concentration of 200 mg/L.

In order to further assess the effect of the substrate's dosage, experiments were conducted between anaerobic-high nitrates content systems H1, H4, H5 which operated with the same operational characteristics except for the amount of the organic substrate dose that corresponded to 200 mg/L, 150 mg/L, 100 mg/L respectively, on a COD basis. The addition of a relatively low organic substrate dose for the initial Cr(VI) concentration of 200 μg/L, recorded lower Cr(VI) removals to the order of 89% for system H4 and 68% for system H5. Effluent Cr(VI) concentration was less than 2 μg/L (below LOQ) only in system H1. Furthermore Table 2 shows that the lack of sufficient substrate led to the decline of microbial population in system H5 (MLSS=276±45 mg/L), although all three systems started with the same biomass concentration. These results revealed the importance of the substrate's quantity for the purpose of determining the appropriate dosage of substrate for Cr(VI)-reduction.

CONCLUSIONS

This study investigated the efficiency of biological groundwater treatment for total and hexavalent chromium removal under anaerobic conditions with high and low nitrates content. Based on the results from the operation of bench scale units the following conclusions can be drawn:

- Biological treatment systems in the form of SBRs operating under anaerobic conditions with high and low nitrates content, are suitable for practically complete microbial Cr(VI) reduction to Cr(III). Despite the Cr(VI) removal efficiency (over 99%), total chromium removal is not as high because a significant portion of Cr(III) remains in solution.
- Considering the addition of different carbon sources as electron donors for Cr(VI) reduction by activated sludge, results show that the rate and extent of chromate reduction varied insignificantly with the three tested carbon sources. High values of the specific Cr(VI) removal rate were obtained when cheese whey, milk, and glucose, were tested. As a conclusion, simple low molecular substrates such as sugar and more complex fermentable substrates such as milk and cheese whey when added to groundwater at a concentration of 200 mg/L can support practically complete microbial Cr(VI) removal (over 99%), in both forms of SBRs operating (with high and low nitrates content). Therefore, cheese whey may be utilized as a technological alternative due to its low cost because it constitutes a residue from the dairy industries.
- Lower substrate's concentration (100 mg/L, 150 mg/L) of the anaerobic-high nitrates content reactors resulted in microbial Cr(VI) removal inefficiency (68%, 89% respectively), pointing the importance of the adequate organic substrate dosage.
- Different hydraulic residence time of the anaerobic-low nitrates content reactors did not affect the exhibition of complete hexavalent chromium removal (over 99%), but increased the trivalent particulate chromium in the biomass.

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