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Bioremediation potential of a perchlorate-enriched sewage sludge consortium

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Abstract

The purpose of this work was to explore the reductive bioremediation potential of a perchlorate-enriched facultative anaerobic consortium. Rapid perchlorate reduction and bacterial growth were observed up to $1.84\,g\,l^{-1}$ of perchlorate, but not at $3.82\,g\,l^{-1}$ due to the toxicity. The specific growth rate of the mixed consortium was $0.1\,h^{-1}$. The consortium co-reduced perchlorate and nitrate with acetate as e^- donor and carbon source. The presence of nitrate slowed down the perchlorate reduction rate. The other e^- acceptors utilized include oxygen, chlorate, Cr(VI), and selenate. Over 95% of the $16\,mg\,l^{-1}$ of added Cr(VI) was reduced within 24 h of incubation with a high-density perchlorate-grown consortium. However, the consortium failed to couple growth with reduction of nitrite, sulfate, thiosulfate, and sulfite. During the search for autotrophic perchlorate reduction, many consortia from very diverse natural sources could not use sulfur compounds such as thiosulfate as e^- donor.

Keywords: Electron acceptors; Nitrate; Perchlorate; Autotrophic; Bioremediation

1. Introduction

The use of perchlorate-contaminated water interferes with iodine uptake by human thyroid affecting the vital body functions. Higher doses even result in fatal bone marrow disorders (Achenbach et al., 2001). Additionally perchlorate, contaminated sites often contain variety of pollutants such as chlorate, nitrate, and heavy metals. Perchlorate removal from the contaminated water can be achieved both by physico-chemical processes such as ion exchange and biological methods. Ion exchange is an incomplete process because it is non-selective and only separates these anions from the contaminated sources. Due to very high affinity of the commercially available resins for these anions, the high strength waste brine stream produced during the regeneration of the resins pose additional disposal problems (Batista et al., 2000; Tripp and Clifford, 2000; Venkatesh et al., 2000; Logan et al., 2001a). Under such a scenario, bioremediation offers benign technological solutions, as these pollutants can be eliminated in an environment-friendly manner.

Both the pure and mixed cultures of some denitrifying bacteria are shown to respire perchlorate as terminal e^- acceptor with oxidation of several organic substrates (Attaway and Smith, 1993; Malmqvist et al., 1994; Rikken et al., 1996; Herman and Frankenberger, 1998;

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Logan, 1998). In recent years, information on the microbial diversity, biochemical mechanism, and kinetics of biological perchlorate reduction to non-toxic chloride and oxygen have been reported (Attaway and Smith, 1993; Logan, 1998; Coates et al., 1999; Miller and Logan, 2000; Kim and Logan, 2001; Logan et al., 2001a,b).

In this study, successful enrichment of a consortium possessing bioremediation potential of several commonly encountered pollutants was carried out. Since nitrate from oxidation of ammonium perchlorate leads to co-contamination, the feasibility of nitrate-independent perchlorate removal or co-removal was tested. Similarly, Cr(VI) reduction was tested with the developed consortium, as both Cr(VI) and perchlorate are sometimes used simultaneously by electroplating facilities. Additionally, possibility of the use of reduced sulfur compounds such as sulfur and thiosulfate as e^- donors for autotrophic perchlorate reduction was explored.

2. Materials and methods

2.1. Medium preparation

Standard anaerobic techniques as originally described by Hungate (1950) and their subsequent serum bottle modifications by Miller and Wolin (1974) were followed throughout this study. The medium with low initial concentration of chloride given by Kim and Logan (2001) was used except that $MgSO_4 \cdot 7H_2O$ at $0.01\,g\,l^{-1}$ was added instead of $0.10\,g\,l^{-1}$. The medium was purged with oxygen-free nitrogen with the help of an indigenously fabricated multiple port gassing device to which stainless steel (SS) needles ($18\,G \times 6$ in.) were connected at the end. The anoxic medium was boiled under oxygen-free nitrogen before an appropriate fixed volume was manually dispensed into individual Wheaton crimp top 50–100 ml clear glass serum vials (20 mm rim) under the same gas phase. Each bottle was sealed with a gray butyl rubber stopper (Wheaton, 20 mm) and crimped (Wheaton, 20 mm crimper) using standard aluminum seals (20 mm). The sterilization was carried out by autoclaving the medium contents at 121 °C for 15 min. The anoxic filter-sterilized stock solutions of e^- acceptors, e^- donor, and carbon sources were prepared by passing through 0.2 μm pore size syringe driven filter unit (Millipore Corporation, USA), and used at the time of inoculation. All inoculations were performed either in a clean bench or a Thermo FORMA Anaerobic System (Model 1025, FORMA Scientific, Inc., USA) maintained under N_2:CO_2:H_2 (85:10:5). Resazurin was omitted from the medium to avoid interference during measurement of cell density (OD_{600} nm) and Cr(VI) by Agilent 8453 UV–Vis spectrophotometer.

2.2. Inocula

The liquid and sludge samples from a primary settling tank of a sewage treatment facility in Inchon, South Korea were collected in pre-sterilized (121 °C; 15 min) serum bottles flushed with nitrogen. During transportation to the laboratory, the samples were constantly maintained at low temperature by keeping in ice-box. A 2.5% (by volume) of the seed culture was used to inoculate a 20 ml medium to start the enrichment. For autotrophic perchlorate reduction, several inocula as mentioned in Table 1 were also included along with two heterotrophic perchlorate reducing strains namely KJ and PDX (generous gift by Prof. B.E. Logan, Department of Civil and Environmental Engineering,

Table 1
Summary of the experimental conditions

No.	Experiment	e^- donor	e^- acceptor	Inoculum	(OD_{600} nm) (0 h)	Time (h)
1	Autotrophic perchlorate reduction	$Na_2S_2O_3$, Sulfur, $H_2 + CO_2$	ClO_4^-	STP, SFBR AD, GS, SM, strains KJ & PDX	ND	>600
2	Heterotrophic perchlorate reduction	Acetate	ClO_4^-	STP	0.037	48
3	Susceptibility to ClO_4^-	Acetate	ClO_4^-	STP	0.010	68
4	Possibility of perchlorate reduction in the presence of nitrate	Acetate	$NO_3^- + ClO_4^-$	STP	0.008	72
5	Alternate e^- acceptors	Acetate	$NO_3^-, ClO_3^-, O_2, Se, SO_4^{2-}, S_2O_3^{2-} SO_3^{2-}, NO_2^-$	STP	0.010	240
6	Chromate (VI) reduction	Acetate	Cr(VI)	STP	0.33	167
7	Chlorite dismutation	–	ClO_2^-	STP	–	–

SFBR: sulfur fluidized bed reactor for denitrification; STP: sewage treatment plant; AD: anaerobic digester; GS: garden soil; SM: sea mud; ND: not determined.