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Microbial ecology of a perchlorate-reducing, hydrogen-based membrane biofilm reactor

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ABSTRACT

The hydrogen-based membrane biofilm reactor (MBfR) has been shown to reduce perchlorate to below 4 μg/L, but little is known about the microbial ecology of this or other hydrogen-based reactors, especially when influent perchlorate concentrations are much lower than the influent oxygen and nitrate concentrations. Dissimilatory (per)chlorate-reducing bacteria (PCRB) can use oxygen as an electron acceptor, and most can also use nitrate. Since oxygen and nitrate can be reduced concurrently with perchlorate, they may serve as primary electron acceptors, sustaining PCRB when the perchlorate concentrations are very low. We studied five identical MBfRs, all seeded with the same inoculum and initially supplied with oxygen, or oxygen plus nitrate, in the influent. After 20 days, perchlorate was added to four MBfRs at influent concentrations of 100–10,000 μg/L, while the fifth was maintained as a control. One day after perchlorate addition, the MBfRs displayed limited perchlorate reduction, suggesting a low initial abundance of PCRB. However, perchlorate reduction improved significantly over time, and denaturing gradient gel electrophoresis (DGGE) analyses suggested an increasing abundance of a single *Dechloromonas* species. Fluorescence *in-situ* hybridization (FISH) tests showed that the *Dechloromonas* species accounted for 14% of the bacterial count in the control MBfR, and 22%, 31%, and 49% in the MBfRs receiving nitrate plus 100, 1000, and 10,000 μg/L perchlorate, respectively. The abundance was 34% in the MBfR receiving oxygen plus 1000 μg/L perchlorate. These results suggest that oxygen is more favorable than nitrate as a primary electron acceptor for PCRB, that PCRB are present at low levels even without perchlorate, and that the presence of perchlorate, even at low levels relative to nitrate or oxygen, significantly enhances selection for PCRB.

1. Introduction

Perchlorate (ClO_4^-) contamination of surface and ground waters has become a significant environmental concern, with detections throughout the United States (Gullick et al., 2001). Although perchlorate is used in a wide range of industrial applications (Kirk et al., 1991), the contamination has mainly been attributed to two sources: defense-related facilities,

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where synthetically manufactured ammonium perchlorate was used in rocket fuels (Motzer, 2001; Wallace et al., 1996), and fertilizers made with perchlorate-containing nitrates imported from Chile (Ericksen, 1983; Schilt, 1979). Recent studies show that perchlorate is present in rain and snow, suggesting that it is naturally formed in the atmosphere (Dasgupta et al., 2005; Rao et al., 2007). Perchlorate is a concern due to its inhibition of thyroid function (Clark, 2000; Stanbury and Wyngaarden, 1952). It is on the US Contaminant Candidate List (Scharfenaker, 2005), and a recently published reference dose of 0.7 mg/kg day translates to a drinking-water equivalent level of 24.5 μg/L if water were the only source of perchlorate exposure (Dahl, 2005; Ginsberg and Rice, 2005). Several states have perchlorate advisory levels for drinking water at 6 μg/L or less (EPA, 2005).

Perchlorate is very soluble and stable in water, making it difficult to remove by conventional water-treatment processes (Schilt, 1979; Urbansky, 1998; Xu et al., 2003). However, biological reduction is a promising treatment approach (Hatzinger, 2005; Urbansky and Schock, 1999; Xu et al., 2003), as perchlorate can be reduced to chloride and water by dissimilatory (per)chlorate-reducing bacteria (PCRB), i.e., bacteria that reduce perchlorate and chlorate (ClO_3^-) as electron acceptors that provide energy for growth (Coates and Achenbach, 2004; Xu et al., 2003). PCRB are ubiquitous in the environment, and perchlorate is highly energetic, with a redox potential similar to that of nitrate (Schilt, 1979). Perchlorate's reduction pathway is believed to include two specialized enzymes, (per)chlorate reductase and chlorite (ClO_2^-) dismutase (Bender et al., 2002, 2005; Bruce et al., 1999; Coates and Achenbach, 2004; Kengen et al., 1999; Nerenberg et al., 2006; van Ginkel et al., 1996). The first reduces perchlorate to chlorate, and then chlorate to chorite, with a total transfer of four electrons. The second transforms chlorite into chloride and oxygen via disproportionation. The produced oxygen is subsequently reduced via the conventional pathway with a transfer of four more electrons. All PCRB are facultative aerobes or microaerophiles (Coates and Achenbach, 2004). Although oxygen at high concentrations inhibits perchlorate reduction (Chaudhuri et al., 2002), all PCRB reduce oxygen produced from perchlorate reduction concurrently with perchlorate, and perchlorate reduction has been found to occur with measurable oxygen concentrations (Shrout and Parkin, 2006). Most PCRB can also use nitrate as an electron acceptor, although in most cases nitrate inhibits perchlorate reduction when its concentration is high enough (Chaudhuri et al., 2002; Coates et al., 1999a; Giblin and Frankenberger, 2001; Wallace et al., 1996). Chlorate also inhibits perchlorate reduction (Nerenberg et al., 2006).

In most water-treatment applications, perchlorate concentrations in the influent water are in the μg/L range, while oxygen and nitrate are in the mg/L range. On the one hand, respiration of nitrate and oxygen may select for non-PCRB, since it is not clear whether respiration of trace levels of perchlorate can exert a sufficient selective pressure for PCRB. On the other hand, nitrate and oxygen respirations may be beneficial to the perchlorate-reducing processes when they act as primary substrates that support PCRB when the perchlorate concentration is low or below the growth threshold (Nerenberg et al., 2006). In these cases, perchlorate acts as a secondary substrate and is utilized concurrently with oxygen and nitrate (Namkung and Rittmann, 1987; Rittmann et al., 1994). Thus, the ways in which nitrate and oxygen respirations affect the microbial ecology of PCRB are of the utmost importance when perchlorate reduction is the goal.

Some ecology tests have been carried out on heterotrophic perchlorate-reducing bioreactors. Zhang et al. (2005) explored the abundance and spatial distribution of *Dechloromonas* and *Dechlorosoma* species in a pilot-scale packed-bed reactor supplied with 50–120 mg/L perchlorate, 9 mg/L dissolved oxygen, and 4 mgN/L nitrate. Acetate was added as an electron donor. The reactor was operated under plug-flow conditions, with electron donor and acceptor concentrations varying with bed depth. Using ribosomal intergenic spacer analysis (RISA) and fluorescence *in-situ* hybridization (FISH), they found that *Dechloromonas* and *Dechlorosoma* species accounted for less than 5% of total bacteria.

Nerenberg et al. (2002) studied a novel, hydrogen-based membrane biofilm reactor (MBfR), which reduced influent perchlorate concentrations of up to 1000 μg/L to below 4 μg/L. The reactor influent contained 5 mgN/L nitrate. In these studies, the addition of 1 mg/L perchlorate to a denitrifying MBfR resulted in an initial perchlorate reduction of around 40%, and then a gradual increase to 99% over 2 weeks. These results suggest that some PCRB were present under denitrifying conditions, but that adding perchlorate to the influent increased their abundance.

Our objective is to understand how and why PCRB become members of a microbial community of an MBfR biofilm when perchlorate concentrations are low. This understanding is at the core of understanding the microbial ecology in hydrogen-based biofilms. The understanding also is key for reliable MBfR operation. Closely related is the question of whether nitrate is required as a primary acceptor for trace perchlorate reduction, or if oxygen can also serve as a primary acceptor. In this research, we study the microbial ecology of a hydrogen-based, autotrophic bioreactor, with special emphasis on the role of oxygen and nitrate in selecting for perchlorate-reducing species.

2. Methods

Five bench-scale, hydrogen-based MBfRs, designated R1–R5, were used to study the effect of perchlorate on the microbial ecology of a mixed-culture biofilm. Reactors R1–R4 had oxygen plus nitrate as primary electron acceptors, while R5 had only oxygen. The MBfRs were identical in physical configuration, and the experiments were conducted concurrently. Hydrogen was supplied in excess to prevent it from becoming rate limiting. The main focus of this research was to understand the effect of perchlorate at low influent concentrations. Higher influent perchlorate concentrations were included to allow its potentially subtle effects on the microbial ecology to be more clearly visualized.

2.1. *Reactor configuration*

Details of the MBfR configuration are described by Chung et al. (2006). Fig. 1 shows the configuration schematically, and