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Study of microbial perchlorate reduction: Considering of multiple pH, electron acceptors and donors

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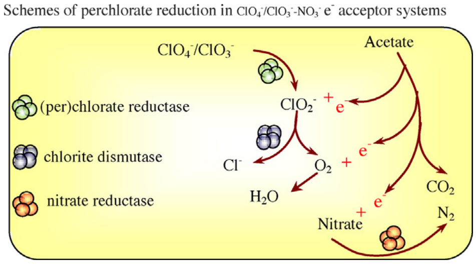
HIGHLIGHTS

- We created a multiple electron acceptor/donor system for ClO_4^- reduction.
- Nitrate reduction was inhibited when using perchlorate-grown *Azospira* sp. KJ.
- Reduction proceeded as an order of ClO_3^-, ClO_4^- and NO_3^-.
- Oxidation of acetate was inhibited by succinate in acetate–succinate series.

GRAPHICAL ABSTRACT

Schemes of perchlorate reduction in $ClO_4^-/ClO_3^-–NO_3^-$ e^-acceptor systems

Schemes of perchlorate reduction in ClO_4^-/ClO_3^--NO_3^- e^- acceptor systems



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ABSTRACT

Bioremediation of perchlorate-cotaminated water by a heterotrophic perchlorate reducing bacterium creates a multiple electron acceptor-donor system. We experimentally determined the perchlorate reduction by *Azospira* sp. KJ at multiple pH, electron acceptors and donors systems; this was the aim of this study. Perchlorate reduction was drastically inhibited at the pH 6.0, and the maximum reduction of perchlorate by *Azospira* sp. KJ was observed at pH value of 8.0. Perchlorate reduction was retarded in $ClO_4^-–ClO_3^-$, $ClO_4^-–ClO_3^-–NO_3^-$,and $ClO_4^-–NO_3^-$ acceptor systems, while being completely inhibited by the additional O_2 in the $ClO_4^-–O_2$ acceptor system. The reduction proceeded as an order of ClO_3^-, ClO_4^-, and NO_3^- in the $ClO_4^-–ClO_3^-–NO_3^-$ system. K_S, v_{max}, and q_{max} obtained at different e^- acceptor and donor conditions are calculated as 140.5–190.6 mg/L, 8.7–13.2 mg-perchlorate/L-h, and 0.094–0.16 mg-perchlorate/mg-DW-h, respectively.

1. Introduction

Perchlorate (ClO_4^-) is one of the major inorganic contaminants in surface water, and groundwater due to the use of perchlorate salts in solid rocket fuel propellants, explosives, and manufacture of commercial products (ranging from electronics to pharmaceuticals) [1–3]. Perchlorate has adverse health effect on humans by interfering with iodine intake of body, thus inhibiting the production of thyroid hormones [4]. In 2009, US Environmental Protection Agency released an interim health advisory level of perchlorate at 15 μg/L.

Strategies for treating perchlorate-contaminated waters include ion exchange, activated carbon absorption, chemical or electrochemical reduction, biological reduction, membrane filtration, and reverse-osmosis [2,5,6]. Of all these technologies, ion-exchange, and biological treatment are most widely used.

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Although ion exchange and some new adsorption technologies have shown high capacities for perchlorate removal from water, the costs were high [6]. Another shortcoming of these processes is the innocuous disposal of the saturated adsorbents, and eluents after regeneration. Owing to its solubility and non-reactivity, bioremediation has been targeted as the most promising method for reduction of perchlorate contamination. Evidences/researches have shown that perchlorate can be easily metabolised by perchlorate-reducing bacteria (PCRB) and biological approaches using perchlorate as an electron acceptor can completely reduce perchlorate without any waste disposal production; this has been proven to be the most effective and economically attractive approach for treating perchlorate-contaminated waters [7].

The ClO_4^-/Cl^- pair has a high electrode reduction potential ($E^{0\prime}$) of 1.29 V, which makes ClO_4^- ideal electron acceptor for microbial metabolism [8–10]. Recent studies have reported that a wide variety of organic (e.g. acetate, lactate, pyruvate, fumarate, succinate, methanol, ethanol) and inorganic (H_2, reduced iron, elemental sulphur, H_2S, and $S_2O_3^{2-}$) substances can serve as electron donors for biological perchlorate reduction [11–14]. Autotrophic PCRB use inorganics as electron donors for perchlorate reduction [11–14], and conversely, heterotrophic PCRB use organic electron donors for perchlorate reduction [9,10].

In a biological perchlorate reduction system, there may be a number of organic and inorganic compounds co-existing in ClO_4^- contaminated wastewater [15]. All these chemical species could create a complicate multiple electron donor and acceptor system for PCRB, which might regulate or inhibit the growing pathways and stoichiometric reactions. Multiple researches concerning the effects of different competitors including nitrate, nitrite, oxygen, and chlorate on the microbial reduction of perchlorate have been reported [16–21]. Key points of these related reports indicated that significant perchlorate reduction through PCRB occurred only after complete removal of O_2. In addition, many PCRB differed significantly in the response towards the e^- acceptors of nitrate and perchlorate. Both simultaneous reduction of perchlorate and nitrate [16–19] and sequential reduction of the two e^- acceptors [19–21] have been extensively reported in the literature. However, these researches seemed to be scattered, and rare literature was reported regarding to the reduction kinetic of multiple e^- acceptors systems, and donors by a sole pure PCRB.

In this study, *Azospira* sp. KJ (ATCC® BAA-592), a heterotrophic PCRB was employed to study the perchlorate reduction with respect to the complicated e^- acceptors (ClO_4^-, ClO_4^-–O_2, ClO_4^-–NO_3^-, ClO_4^-–ClO_3^- ,and ClO_4^-–ClO_3^-–NO_3^-) and donors (acetate, succinate, propionate, glucose, glycerol and benzoate). The five multiple e^- acceptor systems would give a better understanding on oxygen, chlorate, and nitrate reduction in perchlorate-contaminated wastewaters, since many perchlorate-contaminated wastewaters that are being considered for bioremediation contain high concentrations of the above mentioned e^- acceptors. Our objective is to understand how these multiple e^- acceptors, and donors could affect the growth of *Azospira* sp. KJ, and capability of *Azospira* sp. KJ for perchlorate reduction. Reduction kinetic of perchlorate in multiple e^- acceptors systems, and donors were also evaluated. We also analyzed the growth of *Azospira* sp. KJ as well as its reduction capability for perchlorate under different pH conditions.

2. Materials and methods

2.1. Bacterium and medium

Azospira sp. KJ, originally isolated by Logan and the co-workers in 2000 [22,23], was used in this study. It was purchased from American type culture collection (ATCC®, BAA-592).

The basal medium contained (per liter) [22,23]: 1.60 g $K_2HPO_4 \cdot 3H_2O$, 0.85 g $NaH_2PO_4 \cdot H_2O$, 0.5 g $NH_4H_2PO_4$ and 10 ml trace mineral solution (TMS). The composition of TMS was EDTA 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 3.0 g/L, $MnSO_4 \cdot H_2O$ 0.5 g/L, NaCl 0.5 g/L, $FeSO_4 \cdot 7H_2O$ 0.1 g/L, $Co(NO_3)_2 \cdot 6H_2O$ 0.1 g/L, $CaCl_2$ (anhydrous) 0.1 g/L, $ZnSO_4 \cdot 7H_2O$ 0.1 g/L, $CuSO_4 \cdot 5H_2O$ 0.01 g/L, $AlK(SO_4)_2$ 0.01 g/L, H_3BO_3 0.01 g/L, $Na_2MoO_4 \cdot 2H_2O$ 0.01 g/L, $Na_2WO_4 \cdot 2H_2O$ 0.01 g/L and $NiCl_2 \cdot 6H_2O$ 0.02 g/L. Unless stated otherwise, perchlorate (ClO_4^-, 0.5 g/L) salt and sodium acetate (CH_3COO^-, 2 g/L) were used as the electron acceptor and donor in study tests. All these chemical reagents were analytical pure.

2.2. Bacterial cell preparation

A 125 mL glass bottle containing 100 mL of the medium was inoculated with *Azospira* sp. KJ from the agar plate (the same composition as basal medium with addition of 1.8% agar). The inoculated medium was then cultivated in a 2.5 L anaerobic jar (HP0031, Thermo Fisher Scientific Inc., USA) at 26 °C. The anaerobic gas atmosphere was created by ANAEROGEN (AN2005, Thermo Fisher Scientific Inc., USA) in the anaerobic jar. Cells were harvested at late-log-phase (optical density at 600 nm (OD_{600}) of 0.2), first centrifuged at 4000 × *g* for 20 min, and then resuspended in medium to the same OD_{600}.

2.3. Experimental set-up

2.3.1. pH experiments

Media with original pH at 6.2, 6.7, 7.3, 7.7, 8.0 and 8.3 were employed in pH experiments. Actual pH values (6.2, 6.6, 7.2, 7.6, 7.8 and 8.0) varied slightly from the targets after sterilization. Sixty test tubes (total volume of 28 ml) containing 1 ml of cell suspension was evenly divided into 6 groups. They were filled with 19 ml of media containing 500 mg/L of perchlorate and 2000 mg/L of acetate. All test tubes were sealed in the anaerobic jar and kept anaerobic at 26 °C. Each test tube from these testing groups was analyzed to measure the remaining perchlorate concentrations and OD_{600} values at different times. Each test was performed in triplicate.

2.3.2. Electron acceptor experiments

Five pairs electron-acceptor systems (ClO_4^-, ClO_4^-–NO_3^-, ClO_4^-–ClO_3^-, ClO_4^-–ClO_3^-–NO_3^- and ClO_4^-–O_2) were tested for their potential to receive the electrons from acetate. The concentrations of ClO_4^-, ClO_3^- and NO_3^- in all these systems were 500 mg/L. Except the ClO_4^-–O_2 group, other groups of test tubes were incubated anaerobically in the anaerobic jar. For the ClO_4^-–O_2 group, all the test tubes were incubated in an aerobic condition at 26 °C. Each test tube was then analysed to measure the remaining perchlorate, and OD_{600} values.

2.3.3. Electron donor experiments

Acetate, succinate, propionate, glucose, glycerol, and benzoate were served as organic electron donors for perchlorate reduction by *Azospira* sp. KJ. One milliliter of cell suspension was transferred to the test tubes containing 19 ml of media with different electron donors (2000 mg/L) and 500 mg/L of perchlorate. The pH of media was adjusted to 8.0 with 1.0 M NaOH. In addition, two sets of experiments were performed to study the perchlorate reduction at multiple electron donor systems (acetate–succinate; acetate–propionate, 1000–1000 mg/L). They were incubated according to the same method in Section 2.3.2.

2.3.4. Kinetic analysis

Perchlorate reduction kinetics was analyzed and determined at different accepter systems (sole ClO_4^-, ClO_4^-–NO_3^-, ClO_4^-–ClO_3^-)