



# Use of starch and potato peel waste for perchlorate bioreduction in water

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## Abstract

The cost of carbon substrates for microbial reduction of perchlorate ( $\text{ClO}_4^-$ ) is central to the success and competitiveness of a sustainable bioremediation strategy for  $\text{ClO}_4^-$ . This study explored the potential application of starch in combination with an amylolytic bacterial consortia and potato peel waste for  $\text{ClO}_4^-$  bioreduction. We obtained a potent amylolytic bacterial consortium that consisted of a *Citrobacter* sp. S4, *Streptomyces* sp. S2, *Flavobacterium* sp. S6, *Pseudoxanthomonas* sp. S5, *Streptomyces* sp. S7, and an *Aeromonas* sp. S8 identified by 16S rDNA sequencing.  $\text{ClO}_4^-$  concentration substantially decreased in purified starch medium inoculated with the amylolytic bacterial consortium and *Dechlorosoma* sp. perclace. Potato peel waste supported  $\text{ClO}_4^-$  reduction by perclace with the rate of  $\text{ClO}_4^-$  reduction being dependent on the amount of potato peels. Over 90%  $\text{ClO}_4^-$  removal was achieved in 4 days in a single time point experiment with 2% (w/v) potato peels waste.  $\text{ClO}_4^-$  reduction in a non-sterile 0.5% potato peel media inoculated with perclace occurred with an initial concentration of  $10.14 \pm 0.04 \text{ mg L}^{-1}$  to  $2.87 \pm 0.4 \text{ mg L}^{-1}$  (71.7% reduction) within 5 days.  $\text{ClO}_4^-$  was not detected in the cultures in 6 days. In a non-sterile 0.5% potato media without perclace,  $\text{ClO}_4^-$  depletion occurred slowly from an initial value of  $9.99 \pm 0.15 \text{ mg L}^{-1}$  to  $6.33 \pm 0.43 \text{ mg L}^{-1}$  (36.63% reduction) in 5 days. Thereafter,  $\text{ClO}_4^-$  was rapidly degraded achieving 77.1% reduction in 7 days and not detected in 9 days. No substantial reduction of  $\text{ClO}_4^-$  was observed in the sterile potato peel media without perclace in 7 days. Redox potential of the potato peel cultures was favorable for  $\text{ClO}_4^-$  reduction, decreasing to as low as  $-294 \text{ mV}$  in 24 h. Sugar levels remained very low in cultures effectively reducing  $\text{ClO}_4^-$  and was substantially higher in sterilized controls. Our results indicate that potato peel waste in combination with amylolytic microorganisms and *Dechlorosoma* sp. perclace can be economically used to achieve complete  $\text{ClO}_4^-$  removal from water.

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## 1. Introduction

Perchlorate ( $\text{ClO}_4^-$ ) is detected in ground water throughout the United States, because ammonium  $\text{ClO}_4^-$  was extensively used in solid propellants for

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rockets, missiles, explosives, and pyrotechnics (Urbansky, 1998; Gullick et al., 2001; Logan, 2001; Xu et al., 2003).  $\text{ClO}_4^-$  salts are highly soluble in water ( $2000 \text{ g L}^{-1}$  for sodium perchlorate) and adsorbs poorly in soil (Xu et al., 2003). Consequently, natural water sources have been contaminated with  $\text{ClO}_4^-$ .  $\text{ClO}_4^-$  is recalcitrant in the environment and is potentially toxic to various forms of life (Lamm et al., 1999) primarily due to adverse effects on the thyroid gland (Capen, 1994; Von Burg, 1995). Recent studies revealed that low concentrations of  $\text{ClO}_4^-$  inhibit iodide uptake in humans as well as animals (Lawrence et al., 2000; U.S. EPA, 2002).  $\text{ClO}_4^-$  contamination of wildlife and vegetation can have adverse effects on the growth of amphibians (Goleman et al., 2002). The potential impact of  $\text{ClO}_4^-$  on human health has spurred regulatory agencies to regulate  $\text{ClO}_4^-$  concentrations in drinking water. In California, the Department of Health Services (CDHS) established an action level of  $6 \text{ } \mu\text{g L}^{-1}$  for potable water. The CDHS found  $\text{ClO}_4^-$  in drinking water wells in Riverside County up to  $29 \text{ } \mu\text{g L}^{-1}$  and up to  $325 \text{ } \mu\text{g L}^{-1}$  in San Bernardino County. High concentrations of up to  $180,000 \text{ } \mu\text{g L}^{-1}$  in ground water not associated with contamination of public drinking water supplies were, however, found in Santa Clara County (CDHS, 2004a,b).

Remedial strategies for removal of  $\text{ClO}_4^-$  in ground water, include membrane, ion-exchange (IX), and biological technologies. Physicochemical water treatment technologies are expensive and less attractive for  $\text{ClO}_4^-$  removal from ground water. Microbial reduction of  $\text{ClO}_4^-$  to environmentally-acceptable innocuous end products is currently an area of intense interest because this technology is relatively cost-effective and environmentally compatible. Although several studies have addressed bioremediation of  $\text{ClO}_4^-$  (Romanenko et al., 1976; Wallace et al., 1996; Logan, 1998; Herman and Frankenberger, 1998, 1999; Urbansky and Schock, 1999; Logan et al., 2000; Miller and Logan, 2002; Giblin et al., 2000a,b; Frankenberger and Herman, 2002; Giblin et al., 2002; Giblin and Frankenberger, 2001; Losi et al., 2002; Logan et al., 2001; Okeke et al., 2002; Bender et al., 2002; Okeke and Frankenberger, 2003), the cost of substrates will be a major contributor to the economics of bioremediation

of  $\text{ClO}_4^-$ . Organic and inorganic electron donors have been considered for  $\text{ClO}_4^-$  reduction in autotrophic and heterotrophic systems, respectively (Xu et al., 2003). Organic carbon substances used as electron donors include: acetate (Herman and Frankenberger, 1999; Kim and Logan, 2001), acetic acid (Togna et al., 2001), ethanol (Hattinger et al., 2000; Greene and Pitre, 2000; Togna et al., 2001), methanol (Hattinger et al., 2000; Greene and Pitre, 2000), lactate (Brown et al., 2000), pyruvate (Brown et al., 2000), protein nutrients such as brewers yeast, cottonseed protein or whey (Attaway and Smith, 1993), and molasses (Okeke et al., 2002). Hydrogen has been used in autotrophic systems for  $\text{ClO}_4^-$  reduction (Giblin et al., 2000a,b; Miller and Logan, 2000; Nerenberg et al., 2002).

There are many agro-industrial processes that generate wastes that can be used as substrates for microbial growth (Mahmood et al., 1998). Microorganisms are capable of utilizing various components of the organic matter in such wastes as a carbon source for growth and for synthesis of cellular biomass as well. The most abundant solid component of plant materials is carbohydrates. The carbohydrate fraction of plants includes mono- and disaccharide sugars, starch, and structural components such as cellulose, lignin, proteins, pectic substances, and hemicelluloses which are closely related. Starch is the principal reserve polysaccharide found in many economically important crops such as potato, wheat, rice, maize, and tapioca (Nirmala and Muralikrishna, 2003; Van der Maarel et al., 2002). Large-scale industrial starch processing has emerged in the last century (Van der Maarel et al., 2002). A significant portion (30–70%) of the dry weight of these wastes is carbohydrates other than cellulose and is therefore readily assimilable by microorganisms (Forage, 1979). Potato peel waste was chosen for this study. Mahmood et al. (1998) demonstrated that the solid matter of the potato peels is predominantly starch. In processing, 20–59% of the raw product can be generated as waste (Sistrunk et al., 1979). It is thus a suitable source of simple and complex carbohydrates for  $\text{ClO}_4^-$  reduction. This study explores the potential application of starch in combination with an amylolytic bacterial consortium and potato peel waste for  $\text{ClO}_4^-$  reduction in water.

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