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Research article

Carbohydrate based polymeric materials as slow release electron donors for sulphate removal from wastewater

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ABSTRACT

Many industrial sulphate rich wastewaters are deficient in electron donors to achieve complete sulphate removal. Therefore, pure and expensive chemicals are supplied externally. In this study, carbohydrate based polymers (CBP) as potato (2 and 5 mm^3), filter paper (2 and 5 mm^2) and crab shell (2 and 4 mm Ø) were tested as slow release electron donors (SRED) for biological sulphate reduction at 30 °C and initial pH of 7.0. Using the CBP as SRED, sulphate reduction was carried out at different rates: filter paper 0.065 −0.050 > potato 0.022−0.034 > crab shell 0.006−0.009 mg SO_4^{2-}.mg $VSS^{-1}d^{-1}$. These were also affected by the hydrolysis-fermentation rates: potato 0.087−0.070 > filter paper 0.039−0.047 > crab shell 0.011 −0.028 mg COD_S.mg $VSS^{-1}d^{-1}$, respectively. Additionally, the sulphate removal efficiencies using filter paper (cellulose, > 98%), potato (starch, > 82%) and crab shell (chitin, > 32%) were achieved only when using CBP as SRED and in the absence of other easily available electron donors. This study showed that the natural characteristics of the CBP limited the hydrolysis-fermentation step and, therefore, the sulphate reduction rates.

1. Introduction

The water cycle is highly affected by the growing population that demands for goods and facilities. Sulphur species are present in the water cycle by anthropogenic activities; for example, food and electronics production, mineral extraction, pulp and paper and petrochemical industry. Sulphur species are also discharged as sulphate in vinasses (Robles-González et al., 2012) from alcoholic beverage production or in acid mine drainage (AMD) (Nieto et al., 2007), dimethyl sulphoxide in semi-conductor production (Park et al., 2001), sulphite from pulping (Pokhrel and Viraraghavan, 2004) and sulphide from petrochemical industries. Some of these water streams are characterized by the presence of a complex mixture of heavy and toxic metals, *e.g.* from AMD (Borrego et al., 2012; Monterroso and Macıas, 1998) and the electronic industry (Rengaraj et al., 2003).

Another important characteristic of many industrial wastewaters is the very low content of chemical oxygen demand (COD), which is in some cases lower than 100 $mg.L^{-1}$ (Bai et al., 2013; Deng and Lin, 2013) and insufficient to remove sulphate by sulphate reducing bacteria (SRB). Hence, the addition of an external carbon source is required, which in most cases increases the treatment cost due to the use of expensive sources, *e.g.* lactate and formate, or they might require special safety installations, *e.g.* when hydrogen is used (Liamleam and Annachhatre, 2007). The operating costs can be reduced when organic wastes are used as electron donors, which also make the treatment process more sustainable. Cheese whey (Martins et al., 2009), molasses (Wang et al., 2008), plant hydrolyzates (Lakaniemi et al., 2010), horse manure and vegetable compost (Castillo et al., 2012) are some examples of wastes that have been used as alternate electron donors for sulphate removal.

Hydrolysis-fermentation is the rate limiting step in anaerobic digestion of organic solid wastes (Houbron et al., 2008). This low rate of organic matter decomposition provides slow release electron donor (SRED): low molecular weight compounds or soluble COD (COD_S) are provided for to the next trophic levels, including sulphate reduction. Hence, carbohydrate based polymers (CBP),

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such as starch from potato, cellulose from filter paper and chitin from crab shell cannot be directly used as electron donors by SRB, but the hydrolytic-fermentative bacteria can use them to produce a mixture of volatile fatty acids (VFA), alcohols, ketones, and other low molecular weight compounds that can then be used by the SRB to perform sulphate reduction.

Different carbohydrates (starch, cellulose and chitin) have different degrees of complexity in their structure, *e.g.* crystallinity, and are thus hydrolysed and fermented at different rates (Jeihanipour et al., 2011; Labatut et al., 2011). Consequently, the sulphate reduction rate will depend on the CBP supplied as SRED to a bioreactor or permeable barrier. The objective of this research was, therefore, to study the sulphate removal from synthetic wastewater using the COD_S released during the hydrolysis-fermentation of different types of CBP by hydrolytic-fermentative bacteria. Also, the COD_S released from the CBP to the synthetic wastewater without inoculum as well as with inoculum were investigated. In this way, the COD_S released naturally from the CBP, the COD_S released by the hydrolysis-fermentation of the CBP and the COD_S released and used for sulphate reduction using CBP as SRED could be differentiated. Furthermore, the biochemical activities were determined in batch bioreactors.

2. Material and methods

2.1. Inoculum

The inoculum was obtained from an anaerobic reactor treating activated sludge at the municipal wastewater treatment plant, located at Harnaschpolder (The Netherlands). The seed liquid contained a total suspended solid (TSS) concentration of 30.8 (± 2.1) g TSS.L^{-1} and volatile suspended solids (VSS) concentration of 20.4 (± 1.5) g VSS.L^{-1}. TSS and VSS were concentrated (5000×g, 10 min and 4 °C) and the solid phase was used as inoculum in the batch bioreactors to obtain a constant initial concentration of 2 g VSS.L^{-1}.

2.2. CBP as electron donors

The following CBP were used as SRED at two different particle sizes: starch supplied as potato (2 and 5 mm^3), cellulose as filter paper (2 and 5 mm^2) and chitin as crab shell (2 and 4 mm diameter, Ø). CBP samples of potato and filter paper were cut with a kitchen knife and sizes were measured with a ruler. The crab shell was broken with a hammer, properly cleaned with acetone prior to its use, and further sized down with a kitchen blender, the size selection was made with a sieve (mesh 5-6).

The COD was analysed for each CBP, this COD was named recalcitrant COD (COD_R) because it needs to be chemically or biologically hydrolysed prior to the COD analysis (Vaccari et al., 2005), *e.g.* lactate is soluble and gives COD_S. The organic solid samples (1 g of CBP) were hydrolysed with a mixture of sulphuric acid (5 mL) and MiliQ water (5 mL) at 70 °C for 3 h (Lenihan et al., 2011) and the COD_S was determined as described in section 2.7.

2.3. Synthetic wastewater composition

The composition of the synthetic wastewater used in this study was as follows (mg.L^{-1}): NH_4Cl (300), $MgCl_2 \cdot 6H_2O$ (120), KH_2PO_4 (200), KCl (250), $CaCl_2 \cdot 2H_2O$ (15), yeast extract (20) and 0.5 mL of a mixture of micronutrients. The trace elements had the following composition (mg.L^{-1}): $FeCl_2 \cdot 4H_2O$ (1500), $MnCl_2 \cdot 4H_2O$ (100), EDTA (500), H_3BO_3 (62), $ZnCl_2$ (70), $NaMoO_4 \cdot 2H_2O$ (36), $AlCl_3 \cdot 6H_2O$ (40), $NiCl_3 \cdot 6H_2O$ (24), $CoCl_2 \cdot 6H_2O$ (70), $CuCl_2 \cdot 2H_2O$ (20) and HCl 36% (1 mL) (Villa-Gomez et al., 2011). Sodium lactate (was solely used for sulphate reducing activity tests of the biomass) and sodium sulphate were also used as, respectively, the electron donor and acceptor when needed. All reagents used in this study were of analytical grade.

2.4. Sulphate reducing and methanogenic activity test of anaerobic sludge

Methanogenic activity of the biomass was determined as described by Angelidaki et al. (2009) and glucose was used as the electron donor. The sulphate reducing activity of the biomass was determined as described by Villa-Gomez et al. (2011) using sulphate and lactate (as COD_S) as electron acceptor and donor, respectively, at a constant ratio of 1:1. The experiments were performed in batch (serum bottles of 500 mL), filled up to 0.3 L of mineral media and 0.2 L of headspace, covered with an airtight rubber stopper and done in triplicates. The initial pH was adjusted to 7.0. Each batch bioreactor was flushed with nitrogen and the initial pressure in the serum bottle was kept constant at 1 bar. The batch bioreactors were maintained at 30 °C and agitated at 160 rpm on an orbital shaker (New Brunswick Scientific Innova 2100 platform shaker, Eppendorf, USA). Methanogenic activity was evaluated as a response of the pressure increment in the head space. The pressure increment of each batch incubation was recorded with a manometer (LEO 1 digital manometer, Winterthur, Switzerland) and used to calculate the volume of biogas produced considering a theoretical biogas composition (CH_4/CO_2 = 70/30% *v/v*, Yentekakis, 2006) and following the calculation explained by de Lemos Chernicharo (2007). Biogas was represented as mg COD-CH_4.L^{-1} in the plots, however, the composition was not analysed, but only simulated.

2.5. SRED experiments

The SRED experiments were done in triplicate and carried out in batch, 500 mL serum bottles fitted with airtight rubber stoppers, as described above. CBP (1.02 ± 0.01 g potato, filter paper or crab shell) were added individually as the sole source of electron donor and under no circumstance lactate was added. The CBP were investigated as follows: Test 1 for COD_S release in synthetic wastewater under anaerobic conditions without inoculum and under non-sterile conditions. Test 2 for COD_S release with inoculum (hydrolysis-fermentation), and test 3, for COD_S release and sulphate reduction (by addition of sulphate, 760 mg.L^{-1} to the synthetic wastewater) with inoculum.

2.6. Estimation of volumetric and specific rates

The volumetric rates (V_r), specific rates (S_r) and sulphate removal efficiencies (SRE) were evaluated using the following equations:

$$V_r = \frac{(y_0 - y_1)}{(t_1 - t_0)} \quad (1)$$

$$S_r = \frac{V_r}{[VSS]} \quad (2)$$

$$SRE = \left(\frac{\left(SO_{4\ initial}^{2-} - SO_{4\ final}^{2-}\right)}{SO_{4\ initial}^{2-}}\right) \times 100 \quad (3)$$

where, y_0 is the concentration of any parameter at the beginning of the experiment (t_0) and y_1 is the concentration of the same