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Enhanced sulfate reduction and trichloroethylene (TCE) biodegradation in a UASB reactor operated with a sludge developed from hydrothermal vents sediments: Process and microbial ecology

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A B S T R A C T

Most Trichloroethylene (TCE) biodegradation reports refer to methanogenic conditions, however, in this work, enhanced sulfidogenesis and TCE biodegradation were achieved in an upflow anaerobic sludge blanket (UASB) reactor in which a completely sulfidogenic sludge, from hydrothermal vents sediments, was developed. The work was divided in three stages, (i) sludge development and sulfate reducing activity (SRA) evaluation, (ii) TCE biodegradation and (iii) SRA evaluation after TCE biodegradation. For (i) SR was 98 ± 0.1%, 84% as sulfide (H_2S, 1200 ± 28 mg/L), sulfate reducing activity (SRA) was 188 ± 50 mg COD H_2S/g VSS*d. For (ii) The reactor reached 74% of TCE removal, concentrations of vinyl chloride of 16 ± 0.3 μM (5% of the TCE added) and ethene 202 ± 81 μM (67% of the TCE added), SRA of 161 ± 7 mg COD H_2S/g VSS*d, 68% of sulfide (H_2S) production and 93% of COD removal. For (iii) SRA was of 248 ± 22 mg COD H_2S/g VSS*d demonstrating no adverse effects due to TCE.

Among the genera of the microorganisms identified in the sludge during TCE biodegradation were: *Dehalobacter*, *Desulfotomaculum*, *Sulfospirillum*, *Desulfitobacterium*, *Desulfovibrio* and *Clostridium*. To the best of our knowledge, this is the first report using a sulfidogenic UASB reactor to biodegrade TCE. The overall conclusions of this work are that the reactor is efficient on both, sulfate and TCE biodegradation and it could be used to decontaminate wastewater containing organic solvents and relatively high concentrations of sulfate.

1. Introduction

High concentrations of sulfate are released to the environment through several anthropogenic activities such as those derived from mining and mineral processing and various other types of industries, related mainly to the petrochemistry, tannery, pharmaceutical and chemical manufacturing (Lens et al., 2002; Silva et al., 2002; Boshoff et al., 2004; Thabet et al., 2009; Tang and Nemati, 2009; Sarti and Zaiat, 2011). Derived from mining industry, acid mine drainage (AMD) leads to high sulfate concentrations and all the related problems that this may present by increasing the total dissolved solids and heavy metals content of water streams (Medírcio et al., 2007; Tang and Nemati, 2009).

It is widely known that in northern Mexico mining activities have led to serious contamination problems in soil and water (Meza-Figueroa et al., 2009). In addition to the environmental threat that the release of sulfate may cause, in many cases reported along the US-Mexico border, surface water and groundwater

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streams are also contaminated with heavy metals and a great variety of organic chemicals (pesticides, aromatics and chlorinated compounds) (Gutiérrez-Galindo et al., 1998; Meza-Figueroa et al., 2009; NOM-014-CONAGUA-2003, 2009). As an example, in some sites from Matamoros, Tamaulipas, Mexico, the sulfate concentrations are between 170 and 412 mg/L and may become higher (Owens and Niemeyer, 2006). Additionally, the requirements of drinking and crop water are fulfilled (~70%) with groundwater along the country and treated wastewater is used for groundwater recharge. In this regard, regulations have stated that certain toxic chlorinated compounds reach low concentrations in the treated water used for recharge, for example 0.002, 0.007, 0.07, 0.1 and 0.005 mg/L for vinyl chloride (VC), 1,1, dichloroethelylene (1,1-DCE), *cis* 1,2-dichloroethylene (*cis*, 1,2-DCE), *trans* 1,2- dichloroethylene (*trans*, 1,2-DCE) and trichloroethylene (TCE), respectively (NOM-014-CONAGUA-2003, 2009). These chlorinated solvents are frequently found in several Super Fund sites in the U.S. and some of them such as TCE are known for its high toxicity and included as priority pollutants in groundwater streams in the US (EPA, 2000).

Particularly in Mexico, the water pollution caused by heavy metals, hydrocarbons and toxic organic chlorinated compounds coming from industrial wastewater, justifies the development of a biological process to remove simultaneously several types of contaminants at low cost. In this work, TCE was chosen as the model pollutant.

Microbial sulfate reduction (MSR) is a process widely used in wastewater treatment techniques such as: removal of organic matter and heavy metals (Lens et al., 2002; Jong and Parry, 2003; Hollingsworth et al., 2005; Thabet et al., 2009; Gallegos-García, 2009; Lu et al., 2011), biodegradation of xenobiotics such as hydrocarbons (Kleikemper et al., 2002; Widdel et al., 2007; Kleinsteuber et al., 2008; Selesi and Meckenstock, 2009; Tang and Nemati, 2009), as well as in biodegradation of nitroaromatic compounds and in bioremediation of explosives (Boopathy, 2007), and decreasing of acidity in wastewater streams (Lens et al., 2002; Jong and Parry, 2003; Kaksonen et al., 2006; Tang and Nemati, 2009). This biological reaction is carried out in bioreactors such as the UASB and EGSB (Dries et al., 1998; Lens et al., 2002; Tang and Nemati, 2009) and bioreactors with other configurations (Esposito et al., 2003; Kaksonen et al., 2004; Celis-García et al., 2007; Sarti and Zaiat, 2011). The typical electron donors used in MSR are short chain fatty acids, alcohols, hydrogen as well as several petroleum hydrocarbons in both, natural and engineered environments (Kleikemper et al., 2002; Liamleam and Annachhatre, 2007; Thauer et al., 2007; Widdel et al., 2007).

Under certain conditions, i.e. using hydrogen and acetate and mixed inocula, competition between sulfate reducing bacteria (SRB) and methanogens may occur. One of the most important factors that influence the competition between SRB and methanogens is the ratio COD/SO_4^{-2}. Under COD/SO_4^{-2} ratios $\leq$1.7, SRB outcompete methanogens. A competition between SRB and methanogens occurs at COD/SO_4^{-2} ratios between 1.7 and 2.7. Meanwhile methanogens outcompete SRB at COD/SO_4^{-2} ratios $\geq$2.7 (Colleran et al., 1995; Lens et al., 2002). Other factors besides the ratio COD/SO_4^{-2} that may determine the outcome of competition between SRB and methanogens in anaerobic reactors are: inoculum composition, operational conditions such as pH, temperature and substrate concentration, influent composition, substrate and product gradient in the biofilms (Yoda et al., 1987; Hulshoff Pol et al., 1998; Lens et al., 2002; Shageyan et al., 2005; Lopes et al., 2007, 2010a, 2010b; Velasco et al., 2008).

On the other hand, reports on biodegradation of chlorinated compounds (mainly chlorophenols) using anaerobic bioreactors (UASB or EGSB) are mostly related to methanogenic conditions (Sponza, 2001a,b, 2003; Sponza and Atalay, 2005; Garibay-Orijel et al., 2006; Majumder and Gupta, 2008; Sponza and Uluköy, 2008; Bhatt et al., 2008; Sponza and Cigal, 2008; Puyol et al., 2009, 2011; Basu and Gupta, 2010). The main use of microbial sulfate reduction in lab and large scale bioreactors has been the treatment of scrubbing waters, the removal of heavy metals from wastewater streams and degradation of aromatic hydrocarbons such as BTEX (Lens et al., 2002; Bhagat et al., 2004; Hollingsworth et al., 2005; Bayrakdar et al., 2009; Tang and Nemati, 2009).

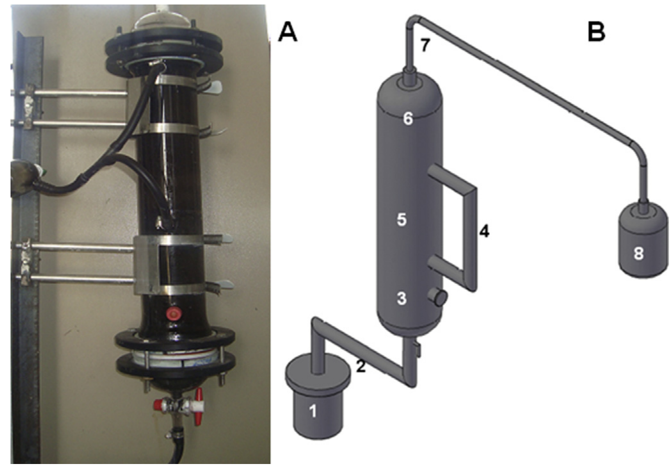

Fig. 1. UASB reactor. **(A)** Photograph of the reactor **(B)** Scheme of the UASB reactor: **(1)** basal medium feeding, **(2)** peristaltic pump, **(3)** sludge, **(4)** recirculation pump, **(5)** basal medium and fluidized sludge, **(6)** gas/liquid separator, **(7)** gas outlet and **(8)** displacement gas column.

One of the advantages of MSR over methanogenesis is the possibility to remove simultaneously COD, heavy metals and organic toxic compounds at a convenient pH range (i.e., 5–7).

To the best of our knowledge, there are no reports on microbial sulfate reduction in reactors (sulfidogenic UASB, EGSB or upflow fluidized bed reactors) used to degrade chlorinated compounds with inocula different from adapted methanogenic sludge to sulfidogenesis.

An important factor for a good performance in a UASB reactor is the quality of the granular sludge. According to Tauseef et al. (2013) the development of methanogenic granules can be very slow or not always occurs, hence, sulfidogenic granules development from methanogenic sludge is a much harder task (Omil et al., 1997). Moreover, the characteristics of sludge change in response to the wastewater source whether a robust granular sludge is used or not. For instance, in the present work it was decided to use hydrothermal marine sediment to develop sulfidogenic sludge (although not granular). Hydrothermal marine sediment is highly available in Mexico and also offers a variety of sulfate reducing bacteria. Previous works carried out by our research group have demonstrated that TCE can be degraded using sludge developed from hydrothermal vents sediments (Guerrero-Barajas and García-Peña, 2010; Guerrero-Barajas et al., 2011; García-Solares et al., 2013).

Therefore, the objectives of this work were: (i) develop a sulfidogenic sludge from hydrothermal marine sediments (ii) evaluate the sulfate reducing activity (SRA) of the sludge in a UASB reactor before and after TCE addition (iii) conduct biodegradation of TCE in the bioreactor and (iv) identify the microorganisms involved after the TCE biodegradation.

The approach to SR and TCE biodegradation of the present work intended to develop a simple but efficient system to obtain a good SR efficiency and TCE biodegradation, taking into account the resources we considered available such as a sludge developed from marine sediments and a UASB reactor configuration although there