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Hydrophobic mixed culture for 1,2-dichloroethane biodegradation: Batch-mode biodegradability and application performance in two-phase partitioning airlift bioreactors

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ABSTRACT

Recent studies highlighted that hydrophobic microorganisms played key roles in VOC removal performance of two-phase partitioning bioreactors (TPPBs). The objective of this study was to evaluate the performance of a hydrophobic mixed culture for 1,2-dichloroethane (1,2-DCA) biodegradation in both batch and TPPB tests. The hydrophobic mixed culture with cell surface hydrophobicity of 79% was acclimated from an activated sludge able to efficiently degrade 1,2-DCA as sole carbon and energy source. It was primarily composed of *Xanthobacter* genus (62%). Nearly complete degradation with coefficients of 0.80 mg_{CO2} $mg_{1,2\text{-}DCA}^{-1}$ and 0.66 mg_{Cl^-} $mg_{1,2\text{-}DCA}^{-1}$ was achieved for a wide range of 1,2-DCA concentrations (114.1–1141.5 mg L^{-1}) in the presence of silicone oil. The maximum specific growth rate was 0.247 h^{-1} which was approximately two times the values of previous pure strains. The two-phase partitioning airlift bioreactors inoculated with the hydrophobic mixed culture was robust against step changes of gas flow rates and spike changes of inlet 1,2-DCA concentrations. All data proved the potential of the hydrophobic mixed culture for toxic-VOC removal in the TPPBs.

1. Introduction

1,2-Dichloroethane (1,2-DCA) is industry-wide raw materials, organic solvents and intermediates. It is easily released to atmospheric environment due to its high volatility and low sorption coefficient, which is potentially toxic, mutagenic and carcinogenic for organisms (Chen et al., 2015; Field and Sierra-Alvarez, 2004). Compared with physical and chemical approaches, biotechnologies are more attractive due to their low cost, easy operation and non-secondary pollution. However, the toxicity and volatility of 1,2-DCA have been the major limitations for current biotechnologies (Estrada et al., 2012; Gordon et al., 2002). Two-phase partitioning bioreactors (TPPBs) with non-aqueous phases (NAPs) can protect microorganisms from inhibition effect of pollutant toxicity and minimize volatility losses simultaneously. They have been successfully applied for the removal of toxic volatile organic compounds (VOCs) (Bailon et al., 2009; Rehmann and Daugulis, 2008).

Removal performance of the TPPBs is determined by the selection of NAPs, reactor configurations and microorganisms. The combination of these three factors is critical for physical mass transfer and biodegradation kinetics for the treatment of VOCs. So far, intensive researches have been devoted to exploring effective bioreactor configurations and NAPs, especially selecting polymers for the TPPBs (Bacon et al., 2015; Hernandez et al., 2011; Littlejohns and Daugulis, 2009). Unfortunately, some work confirmed that removal performance of VOCs was not enhanced in the presence of solid polymers under steady conditions, although the polymers had affinities with target VOCs (Littlejohns and Daugulis, 2009; Montes et al., 2015). These studies suggested that specialized microorganisms with efficient VOC-biodegradability and their affinities with the NAPs were also crucial for VOC removal. In particular, microorganisms with cell surface hydrophobicity (CSH) could ingest target VOCs from the NAPs directly, which could introduce further enhancement for mass transfer in the TPPBs (Hernández et al., 2012).

Up to now, several studies have been conducted to isolate specialized microorganisms with 1,2-DCA biodegradability. However, only a few pure strains have been isolated under aerobic condition (Janssen et al., 1985; Saiyood et al., 2016; Stucki et al., 1983; van den

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Wijngaard et al., 1993), such as *Pseudomonas* sp. strain DE2, *Xanthobacter autotrophicus* GJ10 and *Ancylobacter aquaticus* AD25. Only *Pseudomonas* sp. strain DCA1 and a mixed culture were found previously able to degrade solo 1,2-DCA without additional carbon and energy sources (Hage and Hartmans, 1999; Herbst and Wiesmann, 1996). Actually, an application of specific pure strains was a risk strategy for stable removal performance in industrial situations, because practical implementation was accompanied by nonsterile long-term operation, concentration fluctuations, and waste accumulation. Compared with the pure strains, the mixed culture had better adaptive capacity to environmental deterioration due to the diversity of microbial community (Baptista et al., 2006; Wang et al., 2015a). Therefore, an inoculation of the specialized mixed culture with VOC biodegradability has been identified as a promising and effective strategy for large-scale application of the TPPBs, especially the hydrophobic microorganisms for the TPPBs (Hernández et al., 2012; Muñoz et al., 2012). However, little information is available on the hydrophobic mixed culture for 1,2-DCA biodegradation with the NAPs as buffer, or their application for 1,2-DCA removal in the TPPBs.

In this study, a hydrophobic mixed culture able to efficiently degrade solo 1,2-DCA without additional carbon and energy sources was acclimated from an activated sludge of a resin wastewater treatment plant. Batch-mode performance of 1,2-DCA biodegradation and microbial growth were evaluated in the absence/presence of silicone oil. Besides, zero-order model, first-order model and pseudo first-order model were used for kinetic analysis of 1,2-DCA biodegradation, while Haldane-Andrews model for microbial-growth kinetics. An application evaluation of the hydrophobic mixed culture was finally conducted in an airlift bioreactor (ALR) with silicone oil as the NAP. All the results would enhance the development of the TPPB design and operation for the toxic-VOC removal.

2. Materials and methods

2.1. Chemicals and medium components

1,2-DCA ($C_2H_4Cl_2$) was bought from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Silicone oil DC 200 (dimethyl silicone oil PMX-200, $[\text{-Si}(CH_3)_6\text{O-}]_n$) was purchased from Aladdin Reagent Co., Ltd (Shanghai, China). The density and viscosity of silicone oil DC 200 at 25 °C were 0.963 g m^{-3} and 10 mPa s, respectively. The partition coefficients of 1,2-DCA at 30 °C in mineral salt medium (MSM) and MSM mixture with 7% (v/v) of silicone oil were 210 ± 14 and 96 ± 8 Pa m^3 mol^{-1}, respectively.

The components of the MSM for microbial growth were prepared according to a previous report of Hartmans and Tramper (1991). All of chemical reagents with an analytical purity were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

2.2. Enrichment of a hydrophobic mixed culture

An activated sludge from a resin wastewater treatment plant (Ningbo, China) was used for an enrichment of a hydrophobic mixed culture. For an acclimation of 1,2-DCA, 1 L of the activated sludge was mixed with 2 L of MSM in a 5-L sealed bottle. 1,2-DCA was introduced continuously from the bottom of the bottle for three months. Ten milliliters of the acclimated sludge was transferred into a 640-mL serum bottle with 90 mL MSM for the culture enrichment. The airtight bottle was gradually supplied with 47.9–239.7 mg L^{-1} of 1,2-DCA and refreshed the MSM simultaneously. The bottle was kept in a rotary shaker with 150 r min^{-1} at 30 °C until the enriched culture had 1,2-DCA biodegradability.

Biodegradability and biocompatibility tests of silicone oil were conducted using the modified procedures from the report of Arriaga et al. (2006). Then ten milliliters of the enriched culture was cultivated in a 640-mL serum bottle containing 83 mL MSM and 7 mL silicone oil in order to obtain a CSH characteristic. The initial concentration of 1,2-DCA was 524.0 mg L^{-1}. The MSM and silicone oil were refreshed for every acclimation cycles. After each acclimation cycle, the CSH of the culture was measured in triplicate according to the previous procedures (Lebrero et al., 2014). The mixed culture with obvious hydrophobicity was obtained after a 20-day enrichment.

2.3. Identification of the hydrophobic mixed culture

Microbial compositions of the hydrophobic mixed culture were identified by high-throughput sequencing. The biomass sample was firstly centrifuged at 10,000 rpm for 10 min to separate the biomass from silicone oil. Genomic DNA was collected using Power Soil DNA Isolation Kit (Sangon, China) with proper concentrations. Each DNA extraction was examined by the agarose electrophoresis analysis to check the integrity and concentration of genome DNA. DNA quantitative analysis was conducted by Qubit 2.0 DNA Detection Kit (Sangon, China).

Polymerase chain reaction (PCR) was conducted by using the universal primers for V3-V4 region of 16S rRNA bacterial gene. After the PCR and purification process, the sequencing was performed using an Illumina Miseq platform (Sangon, China). The taxonomic position of the sequences from microbial samples was obtained using the Naive Bayesian classifier tool in the RDP database (http://rdp.cme.msu.edu/) with a confidence level of 95%.

2.4. Batch 1,2-DCA biodegradation tests

Batch experiments were conducted to evaluate 1,2-DCA biodegradation and microbial growth of the hydrophobic mixed culture. One milliliter of microbial suspension, 3.5 mL of silicone oil and 45.5 mL of MSM were put into a 320-mL sterile serum bottle. The bottle was sealed with Teflon-faced silicone rubber septa. Different amounts of liquid 1,2-DCA were added to the bottle through the septa by an airtight micro-syringe. The initial concentrations of 1,2-DCA in the liquid phase were controlled at 114.1, 228.3, 342.4, 456.6, 570.7, 684.9 and 1141.5 mg L^{-1}, respectively, with 17.4–19.8 mg dry cell weight (DCW)·L^{-1} of initial cell concentrations. A contrast experiment without silicone oil was carried out to evaluate the effect of silicone oil on the 1,2-DCA biodegradation and microbial growth. The amount of 1,2-DCA in the contrast experiment was five microliters, corresponding to 93.3 and 114.1 mg L^{-1} of initial 1,2-DCA concentration in the MSM and MSM/silicone oil mixture, respectively. Gaseous 1,2-DCA and CO_2 concentrations in the headspace of bottles, and cell concentrations in the liquid phase were measured periodically. Chloridion (Cl^-) concentrations in the liquid phase were measured at the end of the experiments. All the experiments were performed in duplicate.

2.5. Kinetic analysis for batch 1,2-DCA biodegradation tests

Kinetic constants of biodegradation were evaluated using zero-order model, first-order model and pseudo first-order model which have been extensively applied for modeling the degradation kinetics of organic compounds (Raghuvanshi and Babu, 2010; Wang et al., 2015b,c). The equation for each model was presented in Table 1. In all equations, S_t and S_0 are the liquid-phase concentrations of 1,2-DCA at the time zero and t (mg L^{-1}), respectively. K_0, K_1 and K_{1p} are the zero-order, first-order and pseudo first-order degradation rate constants (h^{-1}), respectively.