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Removal of gaseous dichloromethane using a solid–liquid partitioning bioreactor under gradual and stepped load increase

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ABSTRACT

Silicone rubber was used as the solid non-aqueous phase of two-phase partitioning bioreactor (TPPB) for dichloromethane (DCM) removal. When the load gradually increased from 150 g m^{-3} h^{-1}–485 g m^{-3} h^{-1} by increasing the gaseous DCM concentration, a steady elimination capacity (EC) of approximately 130 g m^{-3} h^{-1} was achieved in one-liquid-phase completely mixed stirred tank bioreactor (CSTB), whereas the EC of TPPB was enhanced from 155 g m^{-3} h^{-1}–366 g m^{-3} h^{-1}. This result indicated that DCM removal was controlled by the biological process in CSTB and by mass transfer in TPPB. Meanwhile, the biomass decreased from 534 mg l^{-1} to 381 mg l^{-1} in CSTB compared with that maintained at around 580 mg l^{-1} in TPPB due to the different resistance capabilities to DCM toxicity. Dehalogenase activity was maintained at a relatively high level under high DCM stress with the addition of silicon rubber. TPPB was shown to be more dependable in the overload from 120 g m^{-3} h^{-1}–450 g m^{-3} h^{-1}. This paper is the first to report that the crucial step in high-load pollutant removal can be converted from the biological process to mass transfer by adding solid non-aqueous phase.

1. Introduction

Dichloromethane (DCM) is an important organic solvent that has been widely used in paint removal, acetate film production, metal degreasing, and pharmaceutical industries (Kaefferlein et al., 2011). However, it has become one of the most widespread volatile contaminants because of its high persistence in water (half-life of over 700 years) and atmosphere (half-life of 79 days) (Pittman et al., 2015). DCM has been listed by the U.S. Environmental Protection Agency as a toxic pollutant that must be primarily controlled. Thus, exploring effective DCM treatment approaches, especially for DCM-containing waste gas, has great environmental and social significance.

Traditional technologies that are used to eliminate DCM from waste gases, including incineration, adsorption, and wet scrubbing, are costly, especially in the case of low pollutant concentrations. Biofiltration of air polluted with volatile organic compounds (VOCs) is an attractive alternative technology due to its advantages, such as low energy loss and low operating cost (Chen et al., 2018; Iranpour et al., 2005; Kennes and Thalasso, 1998; Popat and Deshusses, 2009). We previously isolated an effective DCM utilizer *Methylobacterium rhodesianum* H13, and the obtained high specific growth rate demonstrated its great potential in further full-scale DCM biodegradation (Chen et al., 2014). However, the removal efficiency of DCM in waste gas is usually limited because of its low water solubility, which results in poor mass transfer (Bailon et al., 2009). Moreover, the microbial inhibition by the toxic substrate causes low performance of DCM biodegradation.

In recent studies, two-phase partitioning bioreactors (TPPBs) have been frequently employed for the removal of hydrophobic substances (Darracq et al., 2012). TPPBs are based on the addition of a non-aqueous phase (NAP) with a high affinity for the target VOCs to overcome mass transfer limitations (Daugulis, 2001; Nguyen et al., 2017). The presence of a NAP can provide an overall higher driving force for mass transfer and an increase in the gas interfacial areas (Quijano et al., 2010), which ultimately enhances the transfer of hydrophobic VOCs and therefore, their removal rates (Bechohra et al., 2015; Dorado et al., 2015). Many immiscible liquid organic phases, such as silicone oil, hexadecane, and 1-octadecene, can be utilized to absorb and partition large concentrations of toxic compounds from aqueous phase (Montes et al., 2012; Munoz et al., 2008), and among them, silicone oil was the most popular liquid NAP, which met all the required characteristics of an optimum NAP (Munoz et al., 2012). However, several limitations still exist to worsen the performance of TPPBs, including the formation

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of a large number of bubbles due to the small surface tension and subsequently, the loss of silicone oil (Tuckermann, 2007). Therefore, TPPBs are expensive when applied in industrial processes compared with traditional technologies, and secondary pollution should be considered because of the oil waste during the reactor operation (Muñoz et al., 2007).

The use of solid polymers instead of liquid organic solvents has been proposed for the removal of some hydrophobic VOCs (Hernandez et al., 2010). Solid non-aqueous phase (SNAP) has shown a partition capability similar to that of an immiscible liquid solvent and has the significant advantages of low cost, separation facilitation, and recycling during VOC treatment (Angelucci and Tomei, 2015). In addition, polymers can be tailored to a particular target molecule and formed into many shapes and sizes; most importantly, they are biocompatible and nonbiodegradable (Daugulis and Boudreau, 2008). However, some researchers reported that solid–liquid TPPBs show lower VOC removal performance than liquid–liquid TPPBs, especially under steady-state conditions. According to the result of Hernandez et al. (2010), the addition of Kraton did not enhance hexane biodegradation, whereas silicone oil supported a twofold increase in biodegradation rate.

Fluctuant loading, which easily occurs in realistic scenarios, such as waste gas treatment, may inhibit microbial activity and worse the performance of the bioreactor. Some researchers adopted an oil absorber, which was placed prior to a one-liquid-phase bioreactor, to stand the shock loads. The oil absorber can buffer various loads and avoid biomass inhibition (Koutinas et al., 2006). TPPB, which incorporates absorber and reactor into one system, has also been reported to enhance process robustness by buffering the microorganisms against VOC loading surges. In such a reactor, NAP can act as a VOC reservoir, which temporarily decreases the high VOC concentration in the aqueous phase and releases VOC to the aqueous phase when loading is relatively low (Munoz et al., 2012). Thus, cells are protected from toxic substrate levels, and microbial inhibition can be precluded or alleviated. Bailon et al. (2009) observed a more stable DCM removal process in the presence of NAP, irrespective of the reactor forms. However, the microbial activity was not evaluated, and thus, whether substrate inhibition was alleviated remains unknown.

In the present study, a suitable SNAP, silicone rubber, was selected for enhancing DCM removal. Its performance in the treatment of waste gas in TPPB inoculated by *M. rhodesianum* H13 was assessed with a completely mixed and stirred tank bioreactor (CSTB) as the control. The biomass in both bioreactors was determined by the gradual increase of the DCM load. The activity of DCM dehalogenase under high substrate concentration in the presence and absence of silicone rubber was also analyzed. Different rate-control steps in relation to the biomass were issued in CSTB and TPPB with loading increase.

2. Materials and methods

2.1. Bacterium

The DCM-degrading bacterium *M. rhodesianum* H13 (CCTCC M 2010121), which was previously isolated and identified in our laboratory (Chen et al., 2014), was used for this work. It could utilize DCM as the sole carbon source with high specific growth rate in the carbon-free minimal medium (MM) proposed by Chen et al. (2014).

2.2. Analytical methods

The DCM gas concentration was determined with an Agilent 6890 gas chromatograph equipped with a flame ionization detector (FID). An HP-Innowax column of 30 m length and 0.32 mm I.D. was used. The injector and detector temperatures were 200 °C and 300 °C, respectively, with the oven temperature set at 80 °C. Nitrogen was used as the carrier gas at a flow rate of 1 ml min^{-1} and split ratio of 5:1. DCM concentrations in liquid phase were calculated according to the Henry coefficient, after the measurement of the headspace gas concentrations with a GC-FID.

The bacterial growth was monitored by measuring the optical density (OD) at 600 nm using an ultraviolet spectrometer (HITACHI U-2910 Double Beam UV/Vis spectrophotometer, Tokyo, Japan), and concentrations were determined from previously constructed calibration curves.

2.3. Partition coefficients of SNAPs

The SNAPs tested include thermoplastic polyurethanes (TPU 6124, Hersbit), ethylene–ethyl acetate copolymer (EEAC 2050, Hanwha Chemical), silicone rubber (Hansheng Rubber and Plastic Products Co., Xiamen, China), polymethyl methacrylate (PMMA DR-101, Hersbit, China), ethylene propylene diene terpolymer (Royalene 694, Lion Copolymer), and high-impact polystyrene (HIPS 466F, Hersbit, China).

To determine the partition coefficient between SNAPs and aqueous phase, 5 g of SNAPs (cut into similarly sized pieces) were added to 300 ml serum bottles, which were filled with distilled water with different DCM concentrations (~5 mM–50 mM), and one bottle without SNAP was used as control. These vials were mixed using a magnetic stirring apparatus for 3 h, and then 50 ml of liquid was removed to measure the DCM concentration. The amount of DCM in the SNAP was estimated based on mass balance calculations. A similar procedure was followed for the determination of the partitioning of DCM between the polymer and the gas phase but without distilled water. Partition coefficient can be defined herein as the ratio of concentration in SNAPs to that in water or gas, which is expressed as

$$K = \frac{[DCM]SNAP}{[DCM]water/gas} \quad (1)$$

The diffusivity values of DCM were tested in the batch experiments with different kinds of SNAPs without water. These were determined experimentally by adding 5 g of SNAP into 300 ml sealed serum bottles. DCM was injected into the bottles and then agitated on a shaker (34 °C) at 160 rpm. The gas phase was measured using a gas-tight syringe every 5 min through a sealed septum at the top of the bottle.

2.4. Assays of the DCM dehalogenase activity

Cells grown on 10 mM DCM with the addition of silicon rubber (0%, 5%, and 10% v/v) were harvested and washed with 50 mM Tris-hydrochloride (pH 7.5). Then they were disrupted with an ultrasonic cell disruptor for 100 times (300 w, 5-s interval), and the crude extract supernatant was obtained after centrifugation to remove cell debris at 15000 rpm and 4 °C for 45 min.

A coupled enzymatic reaction was conducted to estimate DCM dehalogenase activity by assaying the formaldehyde production rate (Gong et al., 2015). A reaction mixture containing 50 mM K_2HPO_4, 5 mM reduced glutathione, 1 mM nicotinamide adenine dinucleotide (NAD^+), 0.05 units formaldehyde dehydrogenase (Sigma), 0.5 ml of cell-free extract from *M. rhodesianum* H13, and 12.5 mM DCM, was incubated at 40 °C in gas-tight spectrophotometric cuvettes at a total volume of 3 ml. The produced formaldehyde was determined spectrophotometrically as NADH at 340 nm over a 5-min period. One unit of DCM dehalogenase was defined as the milligram of protein in the cell-free extract required to produce 1 μmol NADH per minute under the specified conditions.

According to the literature (Sasaki et al., 2015; Zamir et al., 2015), an experimental correlation for estimating specific activity values (U mg^{-1} protein) was demonstrated by Eq. (2):

$$\text{Specific activity} = \frac{\Delta A/min \times (T_V/S_V) \times 1000}{\varepsilon \times L \times (M/S_V)} \quad (2)$$

where ($\Delta A/min$) represents the rate of the OD 340 nm values, as the slope of the fitted trend line shown in Fig. S1; T_V and S_V represent total