Journal of Hazardous Materials 286 (2015) 204–210

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Toxic and inhibitory effects of trichloroethylene aerobic co-metabolism on phenol-grown aerobic granules

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HIGHLIGHTS

- The inhibitory and toxic effects of TCE on aerobic granules were studied in detail.
- TCE up to 70 mg L^{-1} in liquid did not harm granules or induce phenol hydroxylase.
- TCE degradation reduced endogenous respiration rate, indicating product toxicity.
- Lower degree of TCE transformation stimulated subsequent phenol uptake.
- Granular structure probably provided protection against TCE product toxicity.

ARTICLE INFO

Article history:
Received 13 September 2014
Received in revised form
10 December 2014
Accepted 1 January 2015
Available online 3 January 2015

Keywords:
Aerobic granules
Co-metabolism
Trichloroethylene
Phenol
Toxicity

ABSTRACT

Aerobic granule, a form of microbial aggregate, exhibits good potential in degrading toxic and recalcitrant substances. In this study, the inhibitory and toxic effects of trichloroethylene (TCE), a model compound for aerobic co-metabolism, on phenol-grown aerobic granules were systematically studied, using respiratory activities after exposure to TCE as indicators. High TCE concentration did not exert positive or negative effects on the subsequent endogenous respiration rate or phenol dependent specific oxygen utilization rate (SOUR), indicating the absence of solvent stress and induction effect on phenol-hydroxylase. Phenol-grown aerobic granules exhibited a unique response to TCE transformation product toxicity, that small amount of TCE transformation enhanced the subsequent phenol SOUR. Granules that had transformed between 1.3 and 3.7 mg TCE g SS^{-1} showed at most 53% increase in the subsequent phenol SOUR, and only when the transformation exceeded 6.6 mg TCE g SS^{-1} did the SOUR dropped below that of the control. This enhancing effect was found to sustain throughout several phenol dosages, and TCE transformation below the toxicity threshold also lessened the granules' sensitivity to higher phenol concentration. The unique toxic effect was possibly caused by the granule's compact structure as a protection barrier against the diffusive transformation product(s) of TCE co-metabolism.

1. Introduction

Aerobic granule is a relatively new form of microbial self-aggregation, which is usually obtained in fully aerated column type reactors operated under sequencing batch mode (sequencing batch reactor, SBR in short) [1]. The harsh conditions applied in these reactors facilitate the formation of granular sludge of near spherical shape, compact structure, high density and settling speed [2]. As the result of the abundant biomass retained within the reactors, and the diffusion barrier provided by granules' large size and compact structure [3], aerobic granular sludge blanket (AGSB) reactors can withstand high concentration and stressful loading of toxic substances [4].

Various researchers have applied toxic substrates with AGSB reactors, e.g., phenol [5], *p*-nitro phenol [6], phthalic acid [7], and halogenated phenols [8–10]. The mechanisms of toxicity by these substances on microorganisms are differed. For example, phenol affects the metabolic activities of cells by decreasing the integrity and function of cell membrane [11], while *p*-nitro phenol is an uncoupling agent for oxidative phosphorylation [6], which affects the energy generation of microorganisms. Despite their toxicity, these substrates have been successfully and efficiently degraded by aerobic granules as the sole or partial carbon and energy source for the biomass. However, some organic compounds cannot sustain

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http://dx.doi.org/10.1016/j.jhazmat.2015.01.003
0304-3894/

microbial growth as carbon or energy source, but can sometimes be transformed via non-growth linked mechanisms, such as co-metabolism.

Co-metabolism is the biotransformation catalyzed by a non-specific enzyme or cofactor, which requires the concurrent metabolism of a growth-supporting substrate or another transformable compound [12]. In the degradation of the growth substrates, non-specific enzymes are synthesized which also catalyze certain crucial steps in the transformation of the co-substrates. One example of such co-substrates is trichloroethylene (TCE), a synthetic solvent and a major pollutant in groundwater [13]. Substrates supporting TCE aerobic co-metabolism mainly include methane [14], ammonia [15], toluene [16], and phenol [17]. The corresponding non-specific enzymes involved are methane monooxygenase, ammonia monooxygenase, toluene mono- and dioxygenases, and phenol hydroxylase, which catalyze the first steps in the degradation of both the growth substrates and TCE (TCE to TCE epoxide) [18].

Due to its resistance to growth-linked metabolism, TCE has been extensively studied as a model compound for aerobic co-metabolism. Another factor why TCE is of particular interest is its unique toxic effect(s). As an organic solvent, TCE can exert solvent stress on microorganisms if present at high level [19]. Furthermore, aerobic co-metabolic transformation of TCE can generate highly reactive intermediates or byproducts that are severely detrimental on the cells carrying out the degradation [20]. These substances could bind to the non-specific enzymes and other cellular materials, causing structural change and function loss [21]. As a result, cells transforming TCE exhibited lowered cell viability [16], respiratory activities [22] and growth substrate degradation rates [15] after the co-metabolism. As the toxicity is caused by the product(s), not TCE itself, it is defined as transformation product toxicity (TPT). The degree of compromise was found to be positively correlated with the amounts of TCE transformed [23], and a certain amount of biomass can only co-metabolize a finite amount of TCE before it totally loses its activity. The ratio of TCE to biomass is defined as the transformation capacity (T_C) [24].

As AGSB reactors have demonstrated enhanced ability in treating toxic and recalcitrant substances, it is of practical interest to explore its application in co-metabolic removal of non-growth supporting substrates. In previous studies, aerobic granules were formed on phenol as the growth substrate [25]. The rate limiting factors for the granules were further investigated [26], and both diffusion limitation and TCE TPT were found to play roles in the finite TCE transformation by aerobic granules. In this study, it was intended that the toxic effect(s) of TCE transformation on phenol-grown aerobic granules be investigated in a more detailed and systematic fashion. The toxicity studied would include the direct TCE solvent stress, the turn-over dependent toxicity, and the toxicity associated with phenol, as it is the growth substrate used in biomass recovery. TCE toxicity on attached-grown biomass has seldom been studied before, therefore pertinent knowledge can be obtained on the sustainability of a new system, i.e., AGSB. In addition, the multiple and complex toxic effect(s) of TCE and its degradation can be a tool to study the mechanism of AGSB's resistance to certain toxins, and its applicability in the removal of recalcitrant and inhibitory substances.

2. 2. Materials and methods

2.1. Reagents and medium

Phenol stock solution was prepared by dissolving phenol crystal (Merck, Germany) to the concentration of 50 g L^{-1}. TCE stock solution was made by thoroughly mixing 2 mL pure TCE (J.T. Baker) with 60 mL Milli Q water to result in the saturation concentration of 1100 mg L^{-1} (25 °C). In the operation of the phenol-fed AGSB reactor, the influent contained (in mg L^{-1}) NH_4Cl 405; KH_2PO_4 81; Na_2HPO_4 81; $MgSO_4 \cdot 7H_2O$ 48 and $FeSO_4 \cdot 7H_2O$ 30, into which 500 mg L^{-1} phenol was supplied as the sole carbon and energy source, and the pH was adjusted to over 7.5 by 1 M $NaHCO_3$. A phosphate-buffered mineral medium was used in all batch studies with the composition of (in g L^{-1}) KH_2PO_4 0.694, K_2HPO_4 0.854, $(NH_4)_2SO_4$ 1.234, $MgSO_4 \cdot 7H_2O$ 0.860, $CaSO_4 \cdot 2H_2O$ 0.176, and $FeSO_4 \cdot 7H_2O$ 0.001. 1 or 5 mL of trace elements solution as described previously [26] was supplemented to the reactor influent or the mineral medium, respectively. Technical grade chemicals and tap water were used in reactor operation, and analytical grade ingredients and Milli Q water were applied in batch studies.

2.2. Aerobic granules

Two kinds of granules were used in this study, cultivated with sodium acetate and phenol as the sole carbon and energy sources, respectively. The formation of aerobic granules on sodium acetate was as described by Tay et al. [27], and the granules obtained had a mean diameter (M.D.) of approximately 600 μm. Phenol-grown aerobic granules were formed in a column type reactor, according to the procedure depicted in previous work [25]. After four months of granulation, the reactor was stabilized at a suspended solid (SS) concentration of 5–6 g L^{-1} and volatile suspended solid (VSS) approx. 83% of SS. The stable phenol-grown granules also had a M.D. of 600 μm, a sludge volume index (SVI) of 20–30 mL g SS^{-1} and specific oxygen unitlization rate (SOUR) around 200 mg O_2 g VSS^{-1} h^{-1} (with 250 mg L^{-1} phenol). Haldane's equation was found to simulate phenol kinetics adequately, with the parameters V_{max} = 1.6 mg phenol g SS^{-1} min^{-1}, K_S = 37 mg L^{-1}, K_I = 462 mg L^{-1}. Michaelis–Menton kinetics could describe TCE transformation with the parameters of V_{max} = 0.0142 mg TCE g SS^{-1} min^{-1}, K_S = 1.02 g L^{-1}. In addition, the granules had T_C of approx. 11 mg TCE g SS^{-1}, and 2.5 mol chloride ions was released per mol TCE transformed, indicating nearly total mineralization.

2.3. Preparation of biomass

Aerobic granules were harvested from the stably operating reactor, centrifuged twice at 3000 g for 15 min, washed with the mineral medium, and resuspended in the same medium. 20 mL of the washed sludge was dispensed in a 60 mL serum bottle, which was sealed with a teflon faced septum (Wheaton) and an aluminum crimp. A sub-fraction was used to determine the biomass concentration in the bottles. TCE was injected through a gas tight syringe (SGE) to give the intended concentrations, and the bottles were shaken at 250 rpm. After TCE transformation, the bottles were opened and the content purged with air. The biomass therein was then washed again with fresh mineral medium and resuspened for further study. Abiotic loss of TCE was determined concurrently and found to be negligible.

2.4. Toxicity study

This study used a respiratory method to evaluate the extent of TCE toxicity and granule's response to it, as the method is sensitive, easily automated and labor saving [28]. After exposure to and/or transformation of TCE, the granules were then washed and subjected to various substances of different concentrations. The resultant respiratory activities were measured, mainly with a respirometer (MicroOxymax, Columbus instruments), which was capable of monitoring the respiratory activities continuously for hours. 20 mL of medium containing biomass was placed in 100 mL flasks, which were sealed and connected to the instrument via gas