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# Alternated phenol and trichloroethylene biodegradation in an aerobic granular sludge reactor



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#### ABSTRACT

Co-metabolic removal of a synthetic solvent, trichloroethylene (TCE) was conducted. Phenol was used as the primary substrate, and aerobic granules as the biocatalyst. An airtight reactor was designed and constructed with glass, and the configuration was verified of its applicability with volatile solvent. Phenol and TCE were fed into the reactors alternately, and both were efficiently removed during 6 weeks of operation. Compared with the control reactor receiving no TCE, biomass in the TCE-fed reactors had lower phenol-dependent specific oxygen utilization rate, but higher concentration, bigger size, and better settling ability. The morphology of the biomass in TCE and control reactors exhibited distinct characteristics. Granules in the TCE reactors slightly broke up early in the operation, but generally retained their shape and structure throughout. The biomass in the control reactor, however, lost its granular structure and totally disintegrated into flocs. Therefore, TCE co-metabolism likely improved the structural integrity of aerobic granular sludge, and co-metabolic degradation of TCE by phenol-grown aerobic granules showed long term stability.

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#### 1. Introduction

Aerobic granule is a relatively new form of immobilized cells, usually cultivated in fully aerated column reactors operated under sequencing batch mode [1]. Aerobic granular sludge (AGS) is characterized by its near spherical shape, compact structure, high density and settling speed [2]. AGS reactors can retain abundant biomass within the reactors. In addition, granules' size and structure provide diffusion barrier to the inner layer cells [3]. As a result, AGS reactor can withstand high concentrations and stressful loadings of toxic substances [4]. For example, various researchers have successfully used AGS reactors to treat toxic compounds like phenol [5], *p*-nitro phenol [6], phthalic acid [7], and halogenated phenols [8–10].

These substances were all treated as the sole or partial carbon and energy sources. However some pollutants, usually synthetic, cannot sustain microbial growth. Sometimes they can be removed via non-growth linked mechanisms, such as co-metabolism [11]. Trichloroethylene (TCE), a synthetic solvent and major groundwater pollutant, is often studied as the model compound for aerobic co-metabolism. It can hardly support aerobic microbial growth,

http://dx.doi.org/10.1016/j.bej.2015.10.026 1369-703X/© 2015 Elsevier B.V. All rights reserved. but can be effectively transformed by cultures grown on methane [12], ammonia [13], toluene [14] and phenol [15]. The non-specific enzymes synthesized in the degradation of these primary substrates, e.g. methane monooxyganse, ammonia monooxygenase, toluene mono- and di-oxygenases, and phenol hydroxylase, can also fortuitously catalyze TCE epoxidation, which would lead to its further degradation and mineralization [16].

However, several obstacles hinder the application of aerobic co-metabolism in TCE removal. One is the exhaustion of reducing power, as TCE catalysis exerts a net drain on intracellular NAD(P)H pool [17]. Another factor is the TCE product toxicity (TPT). Aerobic co-metabolism of TCE generates highly reactive intermediates or byproducts that can bind to the non-specific enzymes and other cellular materials, causing structural change and function loss [18]. The worst scenario resulted in cell death without potential of recovery. Therefore the ratio of the amount of TCE transformed to the biomass before total inactivation is defined as "transformation capacity" (T<sub>C</sub>) [18], the determination of which could take several hours to several days. To overcome these obstacles, it is necessary that an energy generating substrate is provided to the microorganisms, to replenish the reducing power and to regenerate the damaged cellular materials. However, if the primary substrate and TCE are present together, competitive inhibition might occur, as they are catalyzed by the same enzyme. Therefore it is proposed that physical or temporal separation should be imposed on TCE



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and the primary substrate, that they be degraded in two different reactors, or in one reactor operated in a sequential mode [19]. For example, Segar et al. used phenol as the growth substrate, and tested various combinations of rejuvenation frequency, rejuvenation duration, hydraulic retention time and phenol concentrations in a sequencing batch biofilm reactor [20]. They found that appropriate strategies were necessary to maintain enzyme activity and active biomass profile.

As AGS has shown good potential in toxic substance removal, it is of practical interest to explore its application in aerobic TCE cometabolism. AGS reactor has the advantages of small footprint, easy operation and good biomass-liquid separation [21]. In addition, they have mostly been operated in SBR (sequencing batch reactor) modes. By adding a phase of non-aerated TCE co-metabolism, alternative primary substrate and TCE degradation could easily be achieved. In previous studies, AGS was formed on phenol as the growth substrate [22], and the kinetics, rate limiting factors and the toxic effect of TCE co-metabolism on the phenol-grown AGS were systematically studied [23–24]. The objective of this study is, therefore, to further explore the applicability of AGS in TCE co-metabolism. An AGS reactor was designed and constructed, and operated in a mode where phenol and TCE are alternatively degraded. The feasibility, limitations, and performance of the reactor, and characteristics of the granules were studied to evaluate the efficiency and stability of the system.

#### 2. Materials and methods

#### 2.1. Reactor setup

Several concerns need to be addressed when designing an AGS reactor with phenol as the growth substrate and TCE as the cosubstrate. TCE is highly volatile and dissolves plastic materials, therefore the reactor and any materials directly in contact with its content should ensure air tightness and solvent compatibility. Out of potential materials, glass was chosen for its light weight, low cost, transparency, and ease to make into different shapes. A glass column was therefore made, with an inner diameter of 40 mm, and a height of 600 mm, giving it a max. working volume of 750 mL. A lid was fitted at the top, with the contact surface grounded to



Fig. 1. Schematic of reactor setup. = Viton tubing, - silica tubing.

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