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2,4-Dinitrotoluene removal in aerobic granular biomass sequencing batch reactors

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ABSTRACT

Aerobic granules were cultivated in sequencing batch reactor (SBR) by feeding 2,4-dinitrotoluene (2,4-DNT) along with acetate. Aerobic granules with an SVI_{10} of 34.57 ± 2.6 mL g^{-1} and average diameter of 0.78 ± 0.3 mm were formed during 30 d of SBR start-up period. In an alternative approach, aerobic granules cultivated using acetate as carbon source were acclimatized and evaluated for 2,4-DNT removal. In both the approaches, the aerobic granules exhibited rapid 2,4-DNT removal wherein >90% of 10 mg L^{-1} 2,4-DNT was removed within 24 h cycle period. The aerobic granules also exhibited ammonium-nitrogen and phosphorus removal in addition to organic carbon removal, indicating that presence of 2,4-DNT did not negatively affect nutrient removal. In aerobic granular biomass reactors, most of the organic carbon was consumed within the first 6 h while, majority of the 2,4-DNT was removed during the 24 h cycle period. HPLC analysis detected smaller amounts of 2-amino-4-nitrotoluene, a biotransformation product of 2,4-DNT. 2,4-DNT removal by granules under anaerobic conditions was observed to be much smaller compared to the aerobic SBR. Thus, 2,4-DNT removal by aerobic granules was likely mediated by combination of both oxidative and reductive pathways. Although, the mechanisms of 2,4-DNT removal requires further investigations, effective and stable removal of 2,4-DNT in aerobic granular biomass reactors offers practical possibilities for treatment of wastewaters from ammunition factories.

1. Introduction

Activated sludge process (ASP) is the most widely employed aerobic wastewater treatment wherein microbial community grows in the form of suspended 'flocs' (or bioflocs) (van Loosdrecht and Brdjanovic, 2014). Besides aeration tank, ASP requires a settling tank for separation of flocculent sludge from the treated waters. Morgenroth et al. (1997) reported formation of aerobic granular biomass when column-type reactors were inoculated with activated sludge and operated in sequencing batch reactor (SBR) mode with bubbled-aeration and short settling times. Since then, aerobic granular biomass has attracted increased attention because of its great potential in transforming the future of municipal and industrial wastewater treatment plants (Sarma et al., 2016). The dense microbial granules formed in these reactors can be rapidly separated from the treated water in the same reactor tank by gravity settling. Thus, the need for a separate settling tank becomes almost obsolete, therefore this new technology significantly minimizes plant footprint. In addition, aerobic granular biomass systems allow retention of high amount of biosolids in the reactor. Moreover, the granular biofilm structure of aerobic granules maintains different redox conditions i.e. aerobic, anoxic and anaerobic microenvironment in the granules which allows desired biological processes like organic carbon removal, ammonium oxidation, denitrification, and phosphorus removal to take place in wastewater treatment (de Kreuk et al., 2005). Although the mechanisms of formation of aerobic granules are not yet fully understood, environmental biotechnological applications of this novel microbial community are constantly evolving (Zhang et al., 2016; Sarma et al., 2016).

Biodegradation of numerous xenobiotic compounds such as phenol (Tay et al., 2005a), *p*-nitrophenol (Suja et al., 2012), chlorinated phenols (Khan et al., 2011), pyridine (Adav et al., 2007), phthalic acids and esters (Zeng et al., 2008), *tert*-butyl alcohol (Tay

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et al., 2005b), chloroanilines (Zhu et al., 2011), metal chelating agents (Nancharaiah et al., 2006b), dyes (Kolekar et al., 2012), and organophosphorous esters (Kiran Kumar Reddy et al., 2014; Nancharaiah et al., 2015) by aerobic granular biomass has been demonstrated in laboratory scale reactors. Generally, xenobiotic compounds which support microbial growth have been used as the sole carbon source for cultivating aerobic granules (Adav et al., 2007; Tay et al., 2005b; Zeng et al., 2008). In the case of other xenobiotic compounds that did not support microbial growth, cultivation of aerobic granules can be achieved if they are supplied along with glucose or acetate (Khan et al., 2011; Kiran Kumar Reddy et al., 2014; Kolekar et al., 2012; Nancharaiah et al., 2006b, 2015; Suja et al., 2012; Zhu et al., 2011). Often, aerobic granules pre-cultivated by feeding acetate are adapted for establishment of xenobiotic biodegradation (Carucci et al., 2008; Nancharaiah et al., 2008; Tay et al., 2005a). Removal of metal ions (Nancharaiah et al., 2010) and radionuclides (Nancharaiah et al., 2006a) has been demonstrated using native or chemically modified aerobic granules (Wang et al., 2015; Suja et al., 2014). Denitrification of nitrate was also significant in aerobic granular biomass reactors (Nancharaiah and Venugopalan, 2011; Suja et al., 2015). Reductive precipitation of soluble and toxic Cr(VI) to less soluble Cr(III) was demonstrated for remediation of chromate contaminated waters (Nancharaiah et al., 2010). Dynamic community and diverse metabolic capabilities of microbial granules allows the treatment of various xenobiotic compounds.

Occurrence of nitroaromatic compounds in the atmosphere, terrestrial and aquatic environments is attributed to agricultural, military and industrial activities (Boopathy et al., 1998). Nitroaromatic compounds are widely used in chemical synthesis, manufacturing of explosives, herbicides, fungicides, insecticides, polyurethane foam and dyes (Boopathy et al., 1994; Hughes et al., 1999; Podlipná et al., 2015; Vanderloop et al., 1999). Nitrotoluenes (2- and 4-nitrotoluenes) and dinitrotoluenes (2,4-dinitrotoluene (2,4-DNT); 2,6-dinitrotoluene (2,6-DNT)) are the by-products in manufacturing explosives i.e. 2,4,6-trinitrotoluene (TNT) (Hughes et al., 1999; Anand and Celin, 2017). Due to toxicity, carcinogenicity and persistence in the environment, 2,4-DNT, 2,6-DNT and TNT are categorized as priority pollutants by the US EPA. Thus, technologies for effective removal of these compounds from effluents originating from manufacturing plants and polluted sites is required to avoid release, transport and toxicity to biota in the environment (Clark and Boopathy, 2007; Anand and Celin, 2017). Use of ineffective technologies and improper disposal practices has led to the release of these nitroaromatics and contamination of soil and groundwater near ammunition manufacturing facilities (Boopathy, 2000). Adsorption by activated carbon and incineration of exhausted carbon is currently practised for treating 2,4-DNT contaminated wastewater (Vanderloop et al., 1999). However, this method is expensive and also causes air pollution (Snellinx et al., 2002).

Microbial technologies are promising for remediation because of the effective biotransformation and biodegradation capabilities. Several bacterial strains such as *Arthrobacter* sp. (Küce et al., 2015), *Rhodococcus pyridinivorans* NT2 (Kundu et al., 2015), *Burkholderia* sp. (Nishino et al., 2000), and *Pseudomonas* sp. (Spanggord et al., 1991) have been reported to be capable of 2,4-DNT biodegradation. 2,4-DNT removal by microorganisms was observed under aerobic, anoxic and anaerobic conditions (Huang et al., 2015; Kundu et al., 2015; Noguera and Freedman, 1996; Vanderloop et al., 1999). Coupled aerobic and anaerobic systems have been also studied for effective 2,4—DNT removal (Wang et al., 2011). Removal mechanisms of 2,4-DNT by microorganisms involves biodegradation (Kundu et al., 2015), biotransformation (Huang et al., 2015) or both (Freedman et al., 1996; Wang et al., 2011).

Previous studies on 2,4-DNT removal were carried out using axenic bacterial cultures, activated sludge and biofilms (Wang et al., 2011). Though aerobic granules have been reported to be superior to activated sludge for biodegradation of toxic and recalcitrant compounds (Zhang et al., 2016), no studies pertaining to use of aerobic granules in treating wastewater containing explosives have been reported. In this study, the effectiveness of aerobic granular biomass SBRs for removal of 2,4-DNT was investigated for the first time. Experiments were carried out in one litre volume bubble column SBRs containing aerobic granules cultivated under 2,4-DNT enrichment conditions in the presence of acetate or lactate. In addition, 2,4-DNT removal was studied in serum bottles to determine the biotransformation potential of aerobic granules under anaerobic conditions.

2. Materials and methods

2.1. Cultivation of aerobic microbial granules

A bubble-column glass reactor with a total volume of 1.6 L (total height: 42 cm, diameter: 6.5 cm) was used with 1 L working volume (working height: 30 cm, diameter: 6.5 cm) for the cultivation of microbial granules (Fig. 1A). The effective height to diameter (H/D) ratio of the reactor was 4.6. Activated sludge collected from the aeration tank of an operating domestic wastewater treatment plant located at Kalpakkam, India was used as the seed sludge. Reactor was inoculated with 0.25 L activated sludge and operated in SBR mode. The SBR (hereafter, SBR-I) was fed with simulated wastewater (SWW) prepared in deionised water containing the following constituents (in mM): sodium acetate (6.3), $MgSO_4 \cdot 7H_2O$ (0.36), KCl (0.47), NH_4Cl (3.54), K_2HPO_4 (0.42), KH_2PO_4 (0.21), and $CaCl_2 \cdot 2H_2O$ (0.25). 1 mL of trace elements mix (Nancharaiah et al., 2008) was added to 1 L SWW. 2,4-DNT was added from a stock solution (200 mg L^{-1} 2,4-DNT) to obtain a final concentration in the range of 5—10 mg L^{-1} 2,4-DNT in the SBR (Table 1). The SBR was operated in 24 h cycle period which consisted of 10 min fill, 23 h aeration, 5 min settle, 10 min decant, and 45 min idle periods. During aeration period, the reactor was supplied with compressed air at the bottom of SBR through a porous stone at a superficial air velocity of 1.2 cm s^{-1}. The SBR was operated with 70% volumetric exchange ratio in a temperature (~30 °C) controlled room. A port located at 9 cm from the bottom was used for decanting the treated water at the end of the cycle period.

2.2. Operating performance of SBR fed with acetate and 2,4-DNT

After formation of microbial granules, reactor performance was monitored. The SBR-I was fed with SWW containing acetate and 2,4-DNT (10 mg L^{-1}). Samples were collected at the beginning and end of the cycle periods for monitoring reactor performance. Occasionally, samples were also collected at regular time intervals during the cycle period. Samples were centrifuged at 8000 rpm for 10 min to remove the suspended cells and stored at 4 °C until further analysis. The samples were appropriately diluted and analysed for total organic carbon (TOC), 2,4-DNT, NH_4^+-N, NO_3^--N, NO_2^--N and PO_3^{3-}-P. Samples were also analysed for putative 2,4-DNT intermediates such as aminonitrotoluene isomers and diaminotoluenes. The substrate utilisation pattern was indirectly monitored online using a dissolved oxygen (DO) probe (Hach, USA).

2.3. Removal of 2,4-DNT by aerobic microbial granules cultivated by feeding only acetate

Aerobic microbial granules were harvested from an operating laboratory scale SBR treating acetate-containing SWW (without