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Journal of Environmental Management 88 (2008) 939–946

Journal of Environmental Management

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Studies on the toxic effects of pentachlorophenol on the biological activity of anaerobic granular sludge

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Received 8 June 2006; received in revised form 4 April 2007; accepted 30 April 2007
Available online 27 June 2007

Abstract

In order to explore the pathway of the anaerobic biotreatment of the wastewater containing pentachlorophenol (PCP) and ensure the normal operation of Upflow Anaerobic Sludge Blanket (UASB) reactor, the anaerobic sludge under different acclimation conditions were selected to seed and start up UASB reactors. Anaerobic toxicity assays were employed to study the biological activity, the tolerance and the capacity to degrade PCP of different anaerobic granular sludge from UASB reactors. Results showed that the anaerobic granular sludge acclimated to chlorophenols (CPs) could degrade PCP more quickly (up to 9.50 mg-PCP g^{-1} TVS d^{-1}). And the anaerobic granular sludge without acclimation to CPs had only a little activity of degrading PCP (less than 0.07 mg-PCP g^{-1} TVS d^{-1}). Different PCP concentrations (2, 4, 6, 8 mg L^{-1}) had different inhibition effects on glucose utilization, volatile fatted acidity (VFA)-degrading and methanogens activity of PCP degradation anaerobic granular sludge, and the biological activity declined with the increase in PCP concentration. The methanogens activity suffered inhibition from PCP more easily. The different acclimation patterns of seeded sludge had distinctly different effects on biological activity of the degradation of PCP of anaerobic granular sludge from UASB reactors. The biological activity of the anaerobic granular sludge acclimated to PCP only was also inhibited. This inhibition was weak compared to that of anaerobic granular sludge acclimated to CPs, further, the activity could recover more quickly in this case. In the same reactor, the anaerobic granular sludge from the mid and base layers showed higher tolerance to PCP than that from super layer or if the sludge is unacclimated to CPs, and the corresponding recovery time of the biological activity in the mid and base layers were short. Acetate-utilizing methanogens and syntrophic propinate degraders were sensitive to PCP, compared to syntrophic butyrate degraders.

Keywords: Pentachlorophenol; Anaerobic granular sludge; Methanogens; Anaerobic toxicity assay

1. Introduction

Pentachlorophenol (PCP) was widely used in the past as pesticide, herbicide, antifungal agent, bactericide and wood preservative (Kaoa et al., 2004). PCP, quite a bit, is used in the chlorine bleaching process of paper manufacturing industry. PCP is acutely toxic to a variety of microorganisms and mammals, and is thought to inhibit oxidative phosphorylation (Shen et al., 2005). Besides, PCP could disrupt the proton gradient across membranes in cells (Escher et al., 1996; Ye et al., 2004), accumulates within the food chains and is considered to be mutagenic or at least comutagenic (Lu et al., 1997). It is of significant risk to health of human beings (Dougherty, 1997). Therefore, it has been designated as a priority pollutant by the Environmental Protection Agencies in many countries, along with other chlorophenols (CPs) (Chang et al., 1996).

Currently, focus of research community is on possible biotreatment of PCP (Larsson et al., 1988; Bolaños et al., 2001; Montenegro et al., 2002; Dudal et al., 2004; Kaoa et al., 2004; Pizzigallo et al., 2004) and many works have indicated that PCP endures much microbial degradation due to its highly chlorinated organic nature (Moos et al., 1983; Melcer and Bedford, 1988). However, recent studies also showed that microbial degradation of PCP is possible,

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0301-4797/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.jenvman.2007.04.021

but at a very slow rate (Edgelhill and Finn, 1982; Saber and Crawford, 1985; Apajalahti and Salkinoja-Salonen, 1986). In contrast, reductive dechlorination of CPs has been shown to occur under anaerobic conditions (Mohn, 1992; Togna et al., 1995). The more chlorines the compounds contain, the greater of the realized dechlorination rate. Several pathways for microbial degradation of PCP have already been found, e.g., PCP methylation, reductive or oxidative dehalogenation and ring cleavage.

For these reasons and environmental influences of PCP, the anaerobic degradation of PCP was investigated extensively in the literature (Chang et al., 1995; Duff et al., 1995; Hiroshi et al., 1996; Wilson et al., 1997; Piringer and Bhattacharya, 1999; Pieper et al., 2004). During anaerobic biodegradation, chlorines of PCP are removed from the aromatic ring via reductive dechlorination (Mikesell and Boyd, 1985, 1986; Bryant et al., 1991) and the position of chlorine atom on the aromatic rings of PCP and CPs is an important factor that affects reductive dechlorination. Many studies on anaerobic biotreatment have focused on conventional anaerobic systems, methanogenic systems and anaerobic acidogenesis in two-phase digestion systems (Ghosh et al., 1975; Fox and Pohland, 1994). Two-phase digestion physically separates acidogens from methanogens, allowing for higher loading rate and shorter retention time, as well as enhancement of effluent quality (Ghosh et al., 1975). Biodegradation of PCP can be a promising treatment for accumulation of excess reducing equivalents, in the form of hydrogen, which is characteristic of acidogenic systems that should stimulate reductive dechlorination. Anaerobic/aerobic biodegradation of PCP has also been regarded as an effective part for the integrated system (Wilson et al., 1997). Both anaerobic and aerobic biodegradation pathways for PCP and CPs have been researched deeply in the past several decades. Chlorinated phenols are anaerobically biodegraded through reductive chlorination. Researchers have found that PCP and other chlorinated compounds were degraded by both acclimated and unacclimated cultures that are obtained from the sediments and sewage in batch tests. Furthermore, both the culture's source and the acclimation process influence biodegradation pathways (Wilson et al., 1997). Fixed film bioreactors are able to remove PCP up to 90% from the mineral solution in the presence of glucose and upto 60% without glucose addition (Duff et al., 1995). Possibility of anaerobic biodegradation of PCP was demonstrated by the observation that the less chlorinated phenols were accumulated with PCP disappearance in the anaerobic sewage sludge. Reductive dechlorination, or direct removal of chlorinate atoms from the ring of aromatic compounds, is a significant process, because dechlorinated products are usually less toxic and more readily degradable under both anaerobic and aerobic conditions.

Studies developing on both the metabolic characteristics and physiological and biochemical mechanisms of anaerobic microbes and some on anaerobic microbial activities of detoxifications and degradation of poisonous organic pollutants are available in the literature and in this process a certain number of activities not discovered in the aerobic system so far, e.g. the reductive dechlorination of polychloroalkane and aromatic hydrocarbon (Fox and Pohland, 1994) came to be noticed and understood. The environmental engineers all over the world are attempting to adopt the metabolic characteristics of the anaerobic microbes to remove the difficultly degradable chlorinated organic compounds (such as PCP). Researchers also find that, however, if the concentration of toxic substrate is too high in the wastewater, the acidification phenomenon would appear in Upflow Anaerobic Sludge Blanket (UASB) reactor, and the biological activity of anaerobic microbes would weaken, even the anaerobic granular sludge would break in UASB reactor.

It is essential to grope the effect of toxic organic pollutants on the anaerobic microbes to improve treatments and to ensure successful anaerobic biotreatment. Hence, we focus on the effects of PCP and other CPs on the metabolic activity of anaerobic microbes in this study, to, optimize the anaerobic treatment techniques of removing toxic organic pollutants. Authors conducted the following investigation on the anaerobic biotreatment of wastewater containing CPs.

2. Materials and methods

2.1. Chemicals and instruments

PCP of 99.5% purity was from QingPu New Products Institute. 4-CP of 96.0% purity was from the third Shanghai chemical agent factory. 3-CP of 96.0% purity was from Fluka AG. Chem. Fabrik CH-9470 Buchs. 2-CP with the purity of 95–98% was from Shanghai Laizhe Fine Chemical Institute.

We used the following instruments: High-performance liquid chromatography instruments, SP-502 gas chromatography instruments (from Shandong Ludong Chemical Instruments Factory), 102-G gas chromatography instruments (from Shanghai Analyzer Instruments Factory), chemical oxygen demand (COD) measuring equipments.

2.2. UASB reactor, seeding, start-up and operation

A sketch of UASB reactor and experiment process is illustrated in Fig. 1. Experiments were performed in the constant-temperature room, at $28 \pm 1\ ^{\circ}C$. The total volume of each reactor was 1100 mL and the efficient volume was 866 mL. Reactor 1# was seeded with the anaerobic activated sludge that was acclimated to PCP for half a year, and Reactor 2# was seeded with an equal amount of anaerobic activated sludge that was a mixture of equal scale sludge acclimated to PCP, 4-CP, 3-CP and 2-CP for half a year, separately. The amount of seeded sludge was 20,100 mg-VSS. The control UASB reactor was seeded with the same amount of anaerobic sludge but without acclimation.