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Anaerobic degradation of benzoate: Batch studies

Uma Upadhyay *, Pradeep Kumar, Indu Mehrotra

Department of Civil Engineering, Indian Institute of Technology Roorkee, Roorkee 247667, India

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Abstract

Response of benzoate along with phenol to different anaerobic inocula has been investigated in batch reactors. In Phase I, the anaerobic biodegradability of benzoate and phenol were evaluated using (a) washed acclimatized granular sludge (WAGS) collected from a passive phenol fed bench-scale up-flow anaerobic sludge blanket reactor (UASBR) and (b) unacclimatized flocculent sludge (UFS) from a UASB based sewage treatment plant (STP). The effect of varying concentrations of benzoate has been investigated in Phase II using acclimatized granular sludge (AGS) from a bench-scale UASBR. Extent of degradation of benzoate was more than the phenol. Increasing benzoate COD from 2500 to 11,700 mg/L, resulted in decrease in (i) rate constant, k from 0.79 to 0.11/d and (ii) ultimate biochemical methane potential (μ_b, g CH_4–COD formed/g benzoate COD) from 84% to 60%. Temporal trend conforming to logistic S-curve indicated stressed conditions at higher benzoate concentration. Benzoate degradation was found to be sensitive to nature as well as quantity i.e. food to microorganism (F/M) of inocula used.
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Keywords: Benzoate; Anaerobic biodegradability; Degradation rate constant (k); Biochemical methane potential (μ_b)

1. Introduction

Benzoate, a key product in anaerobic degradation of phenol and many complex aromatic chemicals is a common pollutant in the wastewaters from chemical industries. Benzoate is an appropriate substrate for studying the feasibility of anaerobic treatment of wastewater containing phenol and other aromatic pollutants. Li et al. (1995) demonstrated that a laboratory scale UASB reactor was capable of removing 91–95% of total COD at 37 °C, pH 7.5 and hydraulic retention time (HRT) of 9.8 h for loading rates up to 30.6 g COD/(L)(d). Highly settleable granules of 1–3 mm size with a layered microstructure, were developed. Fang et al. (1995) investigated the toxic effect of phenolic pollutants on anaerobic benzoate degrading granules. Phenol has been recognized as being either toxic or lethal to fish at concentrations of 5–25 mg/L (Razo-Flore et al., 2003). The permissible limit of phenol is 1 mg/L for industrial effluent to be discharged into inland surface waters (IS: 2490, 1981) and 5 mg/L into public sewers (IS: 2296, 1974). Degradation of phenolic wastewaters has been reviewed by Veeresh et al. (2005). Phenol at concentration more than 500 mg/L can either be treated with a co-substrate or by recirculating the effluent. Biotransformation of phenol to methane proceeds via rate limiting benzoate formation, dearomatization, ring fission, β-oxidation, acetogenesis and methanogenesis.

Effects of temperature and sulfates on benzoate degradation have been investigated by Leven and Schnurer (2005) and Fang et al. (1997), respectively. Biotransformation of substrates to methane is sensitive to the nature of inoculum (Razo-Flores et al., 1996). It is established that the structural characteristics of bacterial aggregates improve the tolerance of anaerobic bacteria to toxic compounds and also allow the bacteria to adopt to inhibitory compounds. Since benzoate is a key intermediate in the degradation of phenol and other aromatic compounds, a study has been undertaken to investigate the effect of

* Corresponding author. Tel.: +91 9899249943.
E-mail addresses: upadhyay_uma_iitr@yahoo.com (U. Upadhyay), pkumafce@iitr.ernet.in (P. Kumar).

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nature of inoculum on the degradation of benzoate. Effects of increasing ratio of substrate to microorganism (F/M) on benzoate degradation have also been investigated. The observation would emphasize the importance of seed sludge source used during start-up of UASB reactors treating aromatic compounds.

2. Methods

Batch experiments were performed in two phases. Operating conditions of each phase are briefly summarized in Table 1. In Phase I, effect of the nature of inocula on anaerobic biodegradability of both benzoate and phenol was studied. Ratio of food to microorganism (F/M) was maintained at 0.75. In Phase II, biodegradability of benzoate COD in the range from 2500 to 11,700 mg/L was evaluated using another kind of inoculum. Amount of inocula used in different reactors was kept same. This resulted in variation of F/M from 0.75 to 3.75. Studies in each phase (I and II) were carried over a period ranging from 30 to 90 days. Oxitop bottles (WTW, Germany) of 312 mL with working volume of 250 mL were used as anaerobic batch reactors. Oxitop bottles are equipped with pressure sensors. The biogas is measured through increase in pressure. Biogas is collected to release the pressure. Methane is analyzed in the collected gas. Either benzoate or phenol was used as source of carbon while NH_4Cl and K_2HPO_4 were used as sources of nitrogen and phosphorous respectively. In a study conducted in the Environmental Engineering Laboratory, Department of Civil Engineering, Indian Institute of Technology Roorkee, Roorkee (29° 52′ N, 77° 53′ 52″ E), India, COD:N:P of 300:1:0.1 and F/M of 0.75 were found to be optimum for anaerobic degradation of phenol. Accordingly, in all the reactors during Phase I and II, COD:N:P was maintained at 300:1:0.1 by adding appropriate amounts of substrate (either benzoate or phenol), NH_4Cl and K_2HPO_4 respectively. Defined media containing nutrients for mixed anaerobic culture was prepared as per Owen et al. (1979).

Table 1
Operating variables

Phase	Types of inocula used	Substrate	Substrate COD (mg/L)	F/M	COD:N:P
I	Washed acclimatized granular sludge (WAGS) from UASBR	Phenol Benzoate	1180 1080	0.75	300:1:0.1
	Unacclimatized flocculent sludge (UFS) from UASBR	Phenol Benzoate	1130 840		
II	Acclimatized granular sludge (AGS) from UASBR	Benzoate	2500 4380 6770 8830 11,700	0.75 1.5 2.25 3.0 3.75	300:1:0.1

UASBR – upflow anaerobic sludge blanket reactor.

Two different types of sludges having widely different characteristics were used in Phase I. Phenol was fed to a bench scale UASB reactor for nearly 3 yr in the Environmental Engineering Laboratory, Department of Civil Engineering, Indian Institute of Technology Roorkee, Roorkee, India. Sludge stored in the passive reactor was washed with tap water and used as inoculum. This inoculum has, therefore, been named as washed acclimatized granular sludge (WAGS). Secondly, flocculent sludge, procured from a nearby full-scale 38 ML/d (million litre per day) UASB based sewage treatment plant (STP) located at Saharanpur (29° 58′ N, 77° 23′ E), India was used as inoculum without acclimatization (for phenol) or washing. It is referred as unacclimatized flocculent sludge (UFS). The two sludges i.e. WAGS and UFS were analyzed for different parameters.

Sludge for Phase II was grown in a 10 L capacity bench-scale continuously fed UASB reactor. Influent COD was maintained at 1000 ± 100 mg/L. Phenol and dilute molasses were used as the sources of phenolic and non-phenolic CODs respectively. Macro and micro nutrients were added as per guidelines given by Gonzalez et al. (1998). Initially the reactor was started using synthetic feed containing (a) non phenolic, molasses COD $\approx$ 75%, and (b) phenolic COD $\approx$ 25%. Phenolic COD was increased from 25 to 50, 75 and 100% with concomitant decrease in non phenolic COD from 75 to 0% in three steps. At pseudo steady state (PSS), organic and sludge loading rates (OLR and SLR) ranged from 2.18 to 2.06 kg phenol COD/(m^3)(d) and from 0.073 to 0.096 kg phenol COD/(kg VSS)(d) respectively. COD removal was 85 ± 5%. Percent methane in biogas was found to be around 65%. Phenol acclimatized granular sludge (AGS) was withdrawn at PSS for the study of Phase II as per the requirement. UASB was operated only to generate the sludge to be used in experiments of Phase II. The performance of the reactor, therefore, has not been discussed in Section 3.

The procedure adopted by Owen et al. (1979) was followed to anaerobically transfer (a) substrate (either benzoate or phenol), (b) nitrogen, (c) phosphorous, (d) inoculum, and (e) defined media to different reactors. Blanks (i.e. reactors without substrate) were run parallel in both phases of the study. Bottles were incubated in a temperature controlled chamber maintained at 30 ± 2 °C. Each bottle was prepared in triplicate. Inocula VSS (Volatile Suspended Solids) was determined before the start of every phase. Biomass was also digested initially to determine its N and P. Benzoate and phenol CODs were measured before adding benzoate or phenol as substrate to different reactors. Contents (mixture of benzoate or phenol, N, P, seed and defined media) of each reactor were initially analyzed for total COD and soluble COD. Gas produced in reactors was recorded through Oxitop controller measuring system. Biogas collected at room temperature was normalized to standard temperature and pressure (0 °C and 1 atm [101.25 kPa]). Methane content in the biogas was monitored by passing total biogas through NaOH solution

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