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Degradation of phenol in an upflow anaerobic sludge blanket (UASB) reactor at ambient temperature

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Abstract: A synthetic wastewater containing phenol as sole substrate was treated in a 2.8 L upflow anaerobic sludge blanket (UASB) reactor at ambient temperature. The operation conditions and phenol removal efficiency were discussed, microbial population in the UASB sludge was identified based on DNA cloning, and pathway of anaerobic phenol degradation was proposed. Phenol in wastewater was degraded in an UASB reactor at loading rate up to 18 gCOD/(L·d), with a 1:1 recycle ratio, at 26 ± 1℃, pH 7.0—7.5. An UASB reactor was able to remove 99% of phenol up to 1226 mg/L in wastewater with 24 h of hydraulic retention time (HRT). For HRT below 24 h, phenol degradation efficiency decreased with HRT, from 95.4% at 16 h to 93.8% at 12 h. It further deteriorated to 88.5% when HRT reached 8 h. When the concentration of influent phenol of the reactor was 1260 mg/L (corresponding COD 3000 mg/L), with the HRT decreasing (from 40 h to 4 h, corresponding COD loading increasing), the biomass yields tended to increase from 0.265 to 3.08 g/(L·d). While at 12 h of HRT, the biomass yield was lower. When HRT was 12 h, the methane yield was 0.308 L/(gCOD removed), which was the highest. Throughout the study, phenol was the sole organic substrate. The effluent contained only residual phenol without any detectable intermediates, such as benzoate, 4-hydrobenzoate or volatile fatty acids (VFAs). Based on DNA cloning analysis, the sludge was composed of five groups of microorganisms. *Desulfotomaculum* and *Clostridium* were likely responsible for the conversion of phenol to benzoate, which was further degraded by *Syntrophus* to acetate and H_2/CO_2. *Methanogens* lastly converted acetate and H_2/CO_2 to methane. The role of *epsilon-Proteobacteria* was, however, unsure.

Keywords: ambient temperature; anaerobic degradation; phenol; UASB; DNA; wastewater

Introduction

Phenol is the raw material for the commercial production of a wide variety of resins, including phenolic resins as construction materials for automobiles and appliances, expoxy resins as adhesives, and polyamide for various applications. In addition, phenols are often found in wastewaters from coal gasification, coke-oven batteries, refinery and petrochemical plants. Phenol and its derivatives are also found in wastewaters from other industries, such as synthetic chemicals, herbicides, pesticides, antioxidants, pulp-and-paper, photo-developing chemicals, and so on. The level of phenol in some of the effluents from these industries can be as high as 6000 mg/L (Cross, 1982).

Phenol is also a biocide and disinfectant. Therefore, it is often perceived as inhibitory to the bioactivity of microorganisms. Studies have been conducted to investigate the inhibition effect of phenol on the conversion of acetate to methane (Patel, 1991; Sierra-Alvarez, 1991; Wang, 1991). Young and Rivera (Young, 1985) found that phenol itself could be stoichiometrically converted to methane and carbon dioxide by anaerobic sludge from a municipal digester. Kobayashi *et al*. (Kobayashi, 1989) also found that phenol was biodegradable under anaerobic condition, but requiring dosing peptone as a co-substrate for the anaerobes. On the other hand, Wang *et al*. (Wang, 1986) demonstrated that phenol could be removed in an expanded-bed reactor, but requiring activated carbon served as adsorbent for phenol as well as carrier for the anaerobes.

Anaerobic treatment of industrial wastewater has become a viable technology in recent years due to the rapid development of high-rate reactors, such as anaerobic filter (AF) and upflow anaerobic sludge blanket (UASB) reactor. The UASB reactor has been most commonly used for wastewaters from food/beverage/agricultural industries, in which the pollutants are mostly carbohydrates. However, recent studies have demonstrated that the UASB technology is applicable to treating wastewaters containing concentrated proteins, corn starch (Fang, 1994) and aromatic chemicals, such as benzoate (Li, 1995). It is thus warranted to investigate the feasibility of removing phenol in wastewater using the UASB process. Furthermore, it is also of engineering and scientific interest to investigate the effect of operational parameters on the reactor performance and the characteristics of the phenol-degrading granules.

Fang *et al*. (Fang, 1996) investigated performance and characteristics of the UASB reactor degrading phenol at 37℃ (mesophilic). Fang and Chan (Fang, 1997) studied toxicity of phenol towards anaerobic biogranules. Fang and Zhou (Fang, 2000) reported degradation of phenol and *p*-cresol in UASB reactors. Tay *et al*. (Tay, 2001) studied phenol degradation with and without glucose as a co-substrate in

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UASB reactor at 37℃ (mesophilic). Anaerobic degradation of phenol was performed almost at mesophilic conditions. However, so far, anaerobic phenol degradation at the ambient temperature has not been reported.

1 Materials and methods

1.1 Reactor startup and operation

The experiment was conducted in the 2.8 L UASB reactor (Fang, 1996) which had an internal diameter of 84 mm and a height of 500 mm. Five evenly distributed sampling ports were installed over the height of the reactor. Total biomass in the reactor was estimated based on the profile of the volatile suspended solids (VSS) of the samples taken from these ports. On top of each reactor was a 2.0 L gas-liquid-solid separator with an internal diameter of 144 mm and a height of 250 mm. Volumetric loading rates were estimated, based on the reactor volume alone, excluding volume of the gas-liquid-solid separator. The reactor was operated at ambient temperature (26 ± 1℃) and at pH 7.0—7.5.

Prior to the experiment, the reactor was seeded with 1.0 L of methanogenic sludge (flocculent sludge and partially granulated sludge) from phenol methanogenesis at 37℃ in 1995 (Fang, 1996), which was kept at refrigerator of 4℃. The reactor was fed with wastewater containing phenol as substrate and operated at a constant temperature of 37℃ during the startup. Glucose was added as a co-substrate. Initial influent phenol and glucose concentrations were 100 mg/L and 1000 mg/L, respectively. The concentration of phenol was gradually increased and reached 1260 mg/L at the end. Glucose concentration, on the other hand, was decreased stepwise in correspondence to increase of phenol, from an initial 1000 mg/L to become completely absent from the wastewater in the later stage of the startup. At last, the temperature of the reactor was changed and maintained at 26 ± 1℃. Synthetic wastewater comprising phenol as the sole substrate, plus balanced nutrient, trace elements and buffer chemicals, was used to feed the reactor using a peristaltic pump. For each gram of COD, the wastewater was supplemented with 1 g of $NaHCO_3$, 260 mg of NH_4Cl, 42.5—64.4 mg of $MgSO_4 \cdot 7H_2O$, 24.8—37.5 mg of K_2HPO_4, 9.9—15.0 mg of KH_2PO_4, 13.0—17.2 mg of $CaCl_2$, 22.4—34.0 mg of sodium citrate, 5.3—8.0 mg of $NiSO_4 \cdot 7H_2O$, 4.1—6.2 mg of $FeCl_3 \cdot 6H_2O$, 1.1 mg of $MnCl_2 \cdot 4H_2O$, 0.6 mg of $ZnCl_2$, 0.6 mg of $CoCl_2 \cdot 2H_2O$, 0.4 mg of $(NH_4)_2MoO_4 \cdot 4H_2O$, 0.3 mg of $CuCl_2 \cdot 2H_2O$, and 0.2 mg of $NaBO_2 \cdot 10H_2O$.

Throughout this experiment the phenol was as sole substrate and the reactor was maintained at ambient temperature (26 ± 1℃). A fraction of the effluent was recirculated to the bottom of the reactor to dilute the phenol concentration of incoming wastewater, the recycle flowrate equalled to that of the incoming wastewater. During days 1—60, the influent phenol concentration of the reactor was increased gradually from initial of 300 mg/L to 840 mg/L. During days 61—170, the phenol concentration was raised to 1260 mg/L and kept at this levels throughout the experiment, while the HRT was lowed stepwise from 40 h to 4 h, corresponding to an increase of COD loading rates from 1.8 to 18 gCOD/(L·d).

1.2 Analytical techniques

Compositions of phenol, benzoate, 4-hydrobenzoate, and volatile fatty acids (VFA; from acetic acid to heptoic acid) in the effluent were measured by a gas chromatograph (GC; Hewlett Packard Modle 5890 Ⅱ). The GC was equipped with a flame ionization detector (FID), a 30 m × 0.53 mm Alltech (AT-1) column (used to measure phenol, benzoate, and 4-hydrobenzoate), and a 30 m × 0.53 mm HP-FFAP fused-silica capillary column (used to analyze VFA), using helium as the carrier gas at a flow rate of 25 ml/min. The fluid sample was filtered through a 0.45 μm membrane filter and acidified with formic acid prior to injecting into the column using the fast injection technique. VFA standards (Supeico, Inc., Bellefonte, PA) and reagent grade sodium benzoate, 4-hydrobezoate, and phenol (Merck) were used for the calibration of the FID. For measuring phenol, benzoate, and 4-hydrobenzoate, the initial temperature of the column was 70℃ for 2 min followed with a ramp of 5℃/min and a final temperature of 160℃ for 2 min, the temperatures of injector and detector were 250℃ and 275℃, respectively. For measuring VFA, the initial temperature of the column was 70℃ for 3 min followed with a ramp of 10℃/min and a final temperature of 180℃ for 4.5 min, the temperature of injector and detector were 200℃ and 250℃, respectively.

The amount of biogas produced was recorded daily using the water displacement method. The content of hydrogen, nitrogen, methane, and carbon dioxide in the biogas were analyzed by a second GC of the same model equipped with a thermal conductivity detector (TCD) and a 2 m × 2 mm (inside diameter) stainless-steel column packed with Porapak N (80-100 mesh). The temperatures of column, injector and detector were kept at 50℃, 57℃ and 180℃, respectively. Argon was used as the carrier gas with a flow rate of 30 ml/min.

The UASB sludge was sampled at HRT of 12 h for DNA extraction. The extracted DNA was amplified by polymerase chain reaction (PCR), and cloned as described previously (Fang, 2002). The inserted DNA of the major clones was sequenced. The obtained DNA sequences were aligned and used to construct phylogenetic trees by ARB 2.5 (Strunk, 1996).

2 Results and discussion

2.1 Phenol degradation

In general, most anaerobic processes are operated at either mesophilic or thermophlic temperature, low temperature typically decrease the methanogenic kinetics and lead to the accumulation of substance and/or intermediates in anaerobic systems. However, in this study, phenol degradation in a UASB reactor is at ambient temperature (26 ± 1℃) throughout, the reactor pH remained stable, ranging

7.0—7.5(the influent pH kept 8.1—8.7, the effluent pH remained 7.5—8.2). Experimental results indicated the efficiencies of phenol degradation are more than 95% when the COD loading rates are not exceed 6.0 gCOD/(L·d). The COD removal efficiency of this study is very closed to that of mesophilic conditions(Fang, 1996; Fig.1).

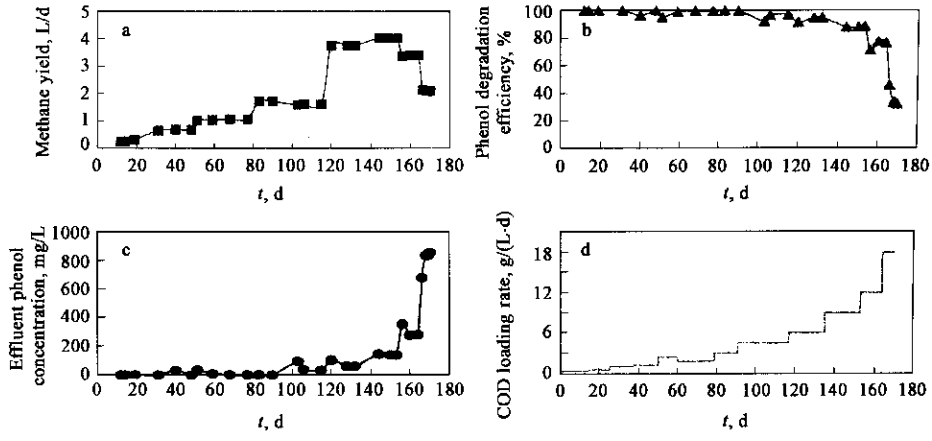

Fig.1 COD loading rate, effluent phenol concentration, phenol degradation efficiency and methane yield of this study

a. methane yield; b. phenol degradation efficiency; c. phenol concentration of the filtered effluent; d. the corresponding COD loading rates throughout this study

2.2 Effect of COD loading rate

Fig.2 illustrates the relationship between COD loading rate and efficiency of phenol degradation. When COD loading rate is from 0.36 to 1.2, the phenol of effluent is below detectable level; as the loading rate is from 1.8 to 3.0, effluent phenol degradation efficiency is 99%—99.9%; when the loading rate is 4.5 and 6.0, the efficiency is 97.1% and 95% respectively; however, when the loading rate is 9.0 and exceeded 9.0 to 18.0, the efficiency is decreased violently from 88.8% to 32.5%. The optimum COD loading rate of UASB reactor degrading phenol at ambient temperature is 4.5 to 6.0.

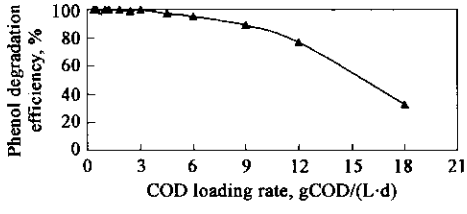

Fig. 2 Relationship between COD loading rate and phenol degradation efficiency

2.3 Effect of HRT

Fig. 3 illustrates the relationship between phenol degradation efficiency and HRT. Starting on day 61 and the day after, the influent phenol concentration of the reactor was maintained 1260 mg/L while HRT was ranging 40—4 h. During days 61—91 when HRT was ranging 40—24, the efficiency of phenol degradation was 99.8%—99.9%. During days 92—170, as HRT continued to decrease, the degradation efficiency decreased. At 16 h of HRT, the degradation efficiency was 97.1%; at 12 h of HRT, the degradation efficiency was 95%; at 8 h of HRT, the degradation efficiency was decreased to 88.8%; when the HRT was further lowered to 6 and 4 h, the degradation efficiency was decreased to 76.7% and 32.5%, respectively. During days 171—198, at 7 h of HRT, the degradation efficiency was recovered and attained to 70%. The degradation efficiency was lower than that of 6 h of HRT, because before the reactor was operated at 7 h of HRT, it was operated at 4 h of HRT, the degradation ability was not recovered entirely. The optimum HRT of UASB reactor degrading phenol at ambient temperature is 16 to 12 h.

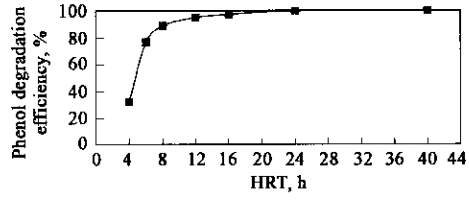

Fig. 3 Relationship between phenol degradation efficiency and HRT
(influent phenol concentration = 1260 mg/L)

2.4 COD balance and biomass yield

In this study, benzoate, 4-hydrobenzoate, and VFA were not detected in effluent. When the concentration of influent phenol of the reactor was 1260 mg/L(corresponding COD 3000 mg/L), with the HRT decreasing(from 40 h to 4 h, corresponding COD loading increasing), the biomass yields tended to increase from 0.265 to 3.08 g/(L·d). While at 12 h of HRT, the biomass yield was lower, but at 12 h of HRT, the methane yield was higher. When HRT was 12 h, the methane yield was 0.308 L/(gCOD removed), which was the highest.

2.5 Phylogenetic tree of microbial groups

Fig. 4 illustrates the phylogenetic tree of microbial groups found in UASB granular sludges of this study and those reported previously(Sekiguchi, 1998; Wu, 2001; Liu, 2002). It shows that four groups had been identified in other UASB granular sludges were also found in this study.

Desulfotomaculum and *Clostridium* were likely responsible for the conversion of phenol to benzoate (Letowski, 2001), which was then further degraded by *Syntrophus* (Li, 1995) to acetate and H_2/CO_2. *Methanogens* lastly converted acetate and H_2/CO_2 to methane. However, the role of *epsilon-Proteobacteria*, which was only found in the phenol-degrading granular sludge of this study, was unclear.

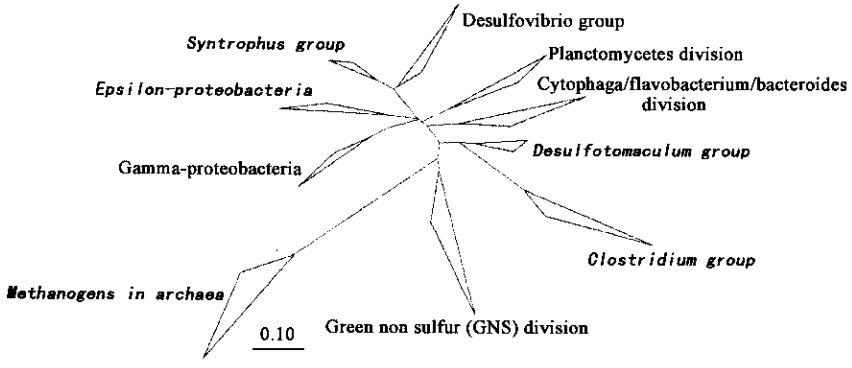

Fig. 4 Phylogenetic tree of microbial groups found in various UASB granular sludges
Those in bold-ital. are detected in the phenol-degrading granules of this study

3 Conclusions

Phenol in wastewater was degraded in an UASB reactor at loading rate up to 18 gCOD/(L·d), with a 1∶1 recycle ratio, at 26 ± 1℃, pH 7.0—7.5. An UASB reactor was able to remove 99% of phenol up to 1226 mg/L in wastewater with 24 h of hydraulic retention time (HRT). For HRT below 24 h, phenol degradation efficiency decreased with HRT, from 95.4% at 16 h to 93.8% at 12 h. It further deteriorated to 88.5% when HRT reached 8 h. When the concentration of influent phenol of the reactor was 1260 mg/L (corresponding COD 3000 mg/L), with the HRT decreasing (from 40 h to 4 h, corresponding COD loading increasing), the biomass yields tended to increase from 0.265 to 3.08 g/(L·d). While at 12 h of HRT, the biomass yield was lower. When HRT was 12 h, the methane yield was 0.308 L/(gCOD removed), it was the highest. Throughout the study, phenol was the sole organic substrate. The effluent contained only residual phenol without any detectable intermediates, such as benzoate, 4-hydrobenzoate or volatile fatty acids (VFAs). Based on DNA cloning analysis, the sludge was composed of five groups of microorganisms. *Desulfotomaculum* and *Clostridium* were likely responsible for the conversion of phenol to benzoate, which was further degraded by *Syntrophus* to acetate and H_2/CO_2. *Methanogens* lastly converted acetate and H_2/CO_2 to methane. The role of *epsilon-Proteobacteria* was, however, unsure.

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