INTERACTIONS OF METHANOGENS AND DENITRIFIERS IN DEGRADATION OF PHENOLS

By Herbert H. P. Fang,[1] Member, ASCE, and Gong-Ming Zhou[2]

ABSTRACT: Experiments were conducted at 37°C in an upflow anaerobic sludge blanket reactor treating wastewater containing phenol (200 mg/L), *m*-cresol (100 mg/L), and nitrate at various concentrations. Results show that anaerobic sludge was able to conduct denitrification without much acclimation. Denitrifiers outcompeted methanogens for substrates for carbon and electron supplies. They were able to use phenol and *m*-cresol as substrate without a carbohydrate cosubstrate. Denitrifying 1 g of NO_3^--N required 3.34 g of chemical oxygen demand. Methanogenesis occurred only at chemical oxygen demand/NO_3^--N ratios greater than 3.34. At the ratio of 5.23, over 98% of phenol but only 60% of *m*-cresol were degraded jointly by denitrifiers and methanogens with 1 day of hydraulic retention. At ratios less than 3.34, methanogenesis ceased to take place and denitrification became incomplete because of insufficient supply of substrate. Batch tests further confirmed that degradation of *m*-cresol was enhanced not only by the presence of nitrate, but also by the presence of either sucrose or phenol as cosubstrate.

INTRODUCTION

Surface and ground waters in China have been polluted heavily by phenols and nitrate in recent years. Phenolic pollutants originate mainly from coal gasification, coke-oven batteries, oil refineries, and petrochemical industries, whereas nitrate is mostly from agricultural and food-related industries. Phenolic pollutants are highly toxic and potentially carcinogenic. Several processes have been used for the removal of phenols from wastewater, including activated carbon adsorption (Nakhla et al. 1990), solvent extraction (Patterson 1975), chemical oxidization (Wang 1992), and biological degradation. Among them, biodegradation is often the least expensive. Simple phenol could be removed either aerobically (Ganczarczyk and Elion 1979; Sack and Bokey 1979) or anaerobically using expanded-bed reactors (Wang et al. 1986; Nakhla et al. 1990) or upflow anaerobic sludge blanket (UASB) reactors (Lettinga et al. 1980; Fang et al. 1996). In general, anaerobic processes are preferred for the treatment of high-strength wastewaters, because of the intrinsic advantage in energy saving and low sludge yield.

However, most of the studies on the anaerobic degradation of complex phenols in wastewater were conducted only in batch tests. Cresols were slowly biodegradable in anaerobic batch reactors (Tschech and Fuchs 1987; Roberts et al. 1988; Suflita et al. 1989; Ramanand and Suflita 1991). The degradation rate was enhanced in the presence of either a more biodegradable cosubstrate, such as peptone (Bisailon et al. 1991) and toluene (Flyvbjerg et al. 1993), or under sulfate-reducing or nitrate-reducing conditions. Little is known on the efficiency of continuous treatment of these complex phenols in wastewater.

Under strict anaerobic condition, phenols are first converted to benzoate, which is further converted by acidogens into intermediate fatty acids, such as propionate and butyrate (Kobayashi et al. 1989). Propionate and butyrate then are converted by acetogens to acetate and hydrogen; both are lastly converted by respecti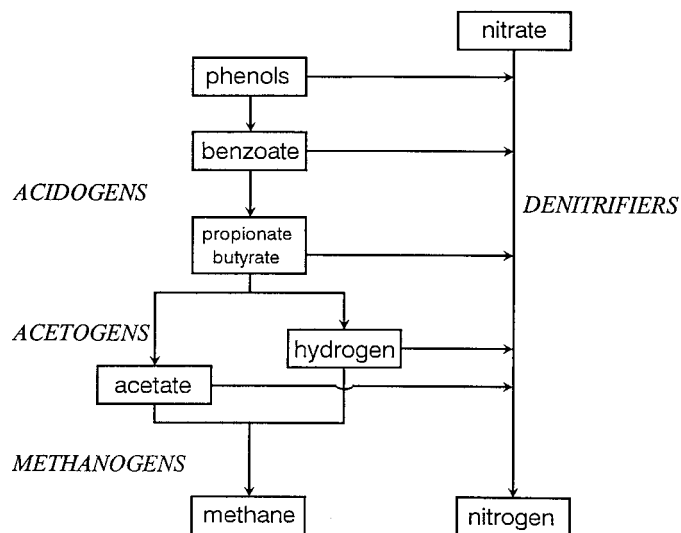ve methanogens into methane (Gujer and Zehnder 1983). During biodegradation, some carbons in the substrate are oxidized to carbon dioxide, in the process releasing electrons for reduction or forming hydrogen. Under strict anaerobic conditions, methanogens utilize the released electrons and hydrogen to produce methane. In the presence of nitrate, denitrifying bacteria reduce nitrate to nitrogen using a wide variety of substrates for supplies of carbon and electrons. Thus, denitrifiers compete with methanogens for acetate and hydrogen, as illustrated in Fig. 1.

This study was conducted to investigate the efficacy of treating wastewater containing medium-strength phenolic pollutants and nitrate in both continuous and batch reactors. The influence of a carbohydrate cosubstrate and the interaction between methanogens and denitrifiers were examined. Information developed in this study should be of value for the treatment of wastewaters and surface/ground waters containing these pollutants.

MATERIALS AND METHODS

Continuous Experiment

The continuous experiment was conducted in a 2.8-L UASB reactor, which had an internal diameter of 84 mm and a height of 500 mm (Fang et al. 1996), as illustrated in Fig. 2. On the

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carbon dioxide and water not shown

FIG. 1. Interactions between Methane-Forming Bacteria and Denitrifiers in Anaerobic Degradation of Phenols

top of the reactor was a 2.0 L gas-liquid-solid separator. The reactor was water-jacketed and operated at a constant temperature of 37°C. Wastewater was synthesized containing constant concentrations of phenol (200 mg/L) and *m*-cresol (100 mg/L), plus balanced nutrient, trace elements, and buffer chemicals. The experiment was divided into two phases. In phase 1 (days 1–232), sucrose (1,000 mg/L) was added to the wastewater as a carbohydrate cosubstrate. Sucrose then was removed from the wastewater in phase 2 (days 233–318), after a 13-day transition period (days 233–245) during which sucrose concentration was lowered to 500 mg/L. For each gram of chemical oxygen demand (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 the experiment, the hydraulic retention time was kept at 1 day, the temperature at 37°C, and the mixed liquor pH at 7.5–8.0. In phase 1, the COD averaged 1,938 mg/L, whereas the concentration of NO_3^--N was increased stepwise from the initial 252 mg/L to 595 mg/L. At high COD/NO_3^--N ratios, there was an abundant supply of substrate for both denitrification and methanogenesis. As the ratio

lowered, competition between denitrification and methanogenesis intensified. In phase 2, the wastewater COD was reduced to an average of 857 mg/L because of the absence of sucrose. The wastewater nitrate concentration also was adjusted to further examine the effect of low COD/NO_3^--N ratios. Details of the key operational parameters at various stages of the experiment are summarized in Table 1.

The reactor was seeded with a 1.51 L mixture of anaerobic sludge containing 27.0 g of volatile suspended solids (VSS), of which 13.0 g was obtained from the sludge digester of a local wastewater-treatment plant and 14.0 g was from a 65-L UASB reactor treating carbohydrate-rich wastewater. Both sludges had not been in contact with nitrate and phenols.

Batch Experiments

Sludge samples were taken from the UASB reactor on days 182 and 282 for two series of batch tests. The former sludge was sampled when the wastewater being treated in the UASB reactor had a COD/NO_3^--N ratio of 5.23, whereas the second sludge was sampled when the ratio was 2.51. All batch tests were conducted at 37°C in 157-mL serum vials. The sludge content in each vial was equivalent to 1,000 mg/L of VSS. The day-182 sludge was tested for the effect of nitrate on the degradation of phenol and *m*-cresol. Experiments were conducted in two batches treating identical wastewater except one was nitrate-free and the other containing nitrate at the same COD/NO_3^--N ratio of 5.23 as in the UASB reactor. The day-282 sludge was tested in three batches for the effect of phenol as cosubstrate for the degradation of *m*-cresol. All three feed solutions had a COD/NO_3^--N ratio of 2.0, but contained the following different substrates: (1) Phenol as sole substrate; (b) *m*-cresol as sole substrate; and (c) phenol and *m*-cresol jointly as cosubstrate.

Analytical Methods

Biogas composition, including methane, nitrogen gas, carbon dioxide, and nitrous oxide, was analyzed by a gas chromatograph (Hewlett-Packard, model 5890 series II). Phenol, *m*-cresol, and possible metabolic intermediates, such as benzoate and volatile fatty acids (from acetic to heptanoic acids) were analyzed by a second gas chromatograph of the same model; the detectable levels for these organic chemicals were 1 mg/L. Details of operational conditions followed those described in a previous study (Fang et al. 1996). Nitrate and nitrite were analyzed by an ion chromatograph (Shimadzu model LC-10) with the Shim-pack IC-A3 column with a detectable level of 0.1 mg/L. Sucrose was measured by a spectrophotometer at 625 nm (UV-160A, Shimadzu). Other parameters, such as VSS and soluble COD were measured according to the *Standard Methods* (American Public Health Association 1985).

RESULTS AND ANALYSIS

Continuous Experiment

Throughout the 318-day continuous experiment, concentrations of phenol and *m*-cresol in the wastewater were 200 and 100 mg/L, respectively. Fig. 3 illustrates the reactor performance by comparing the following concentrations: (1) Sucrose; (2) NO_3^--N; (3) phenol; and (4) *m*-cresol in both the incoming wastewater and the effluent. Figs. 3(e and f) illustrate the COD/NO_3^--N ratio of the wastewater and the methane production rate, respectively.

During anaerobic degradation, phenols are first converted to benzoate (Kobayashi et al. 1989), which is further converted by acidogens into intermediate fatty acids, such as propionate
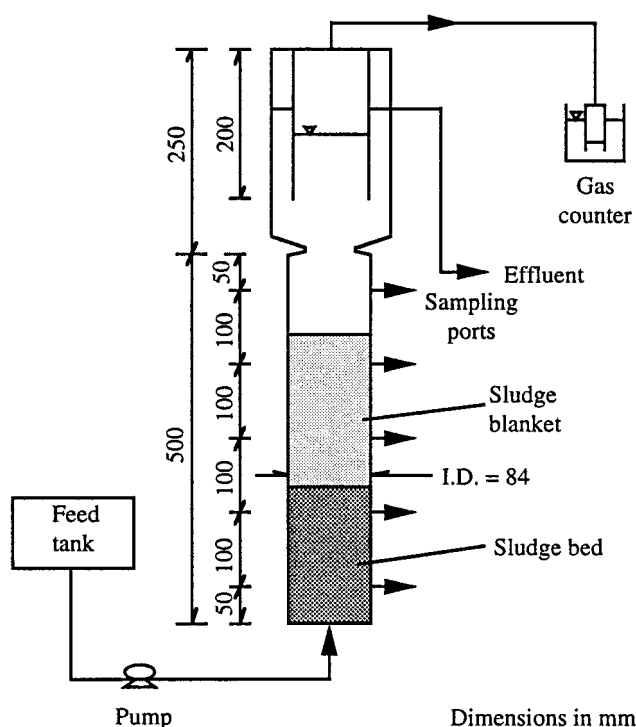

FIG. 2. Schematic Diagram of UASB Reactor Setup

TABLE 1. Parameters of Wastewater Treated in UASB Reactor

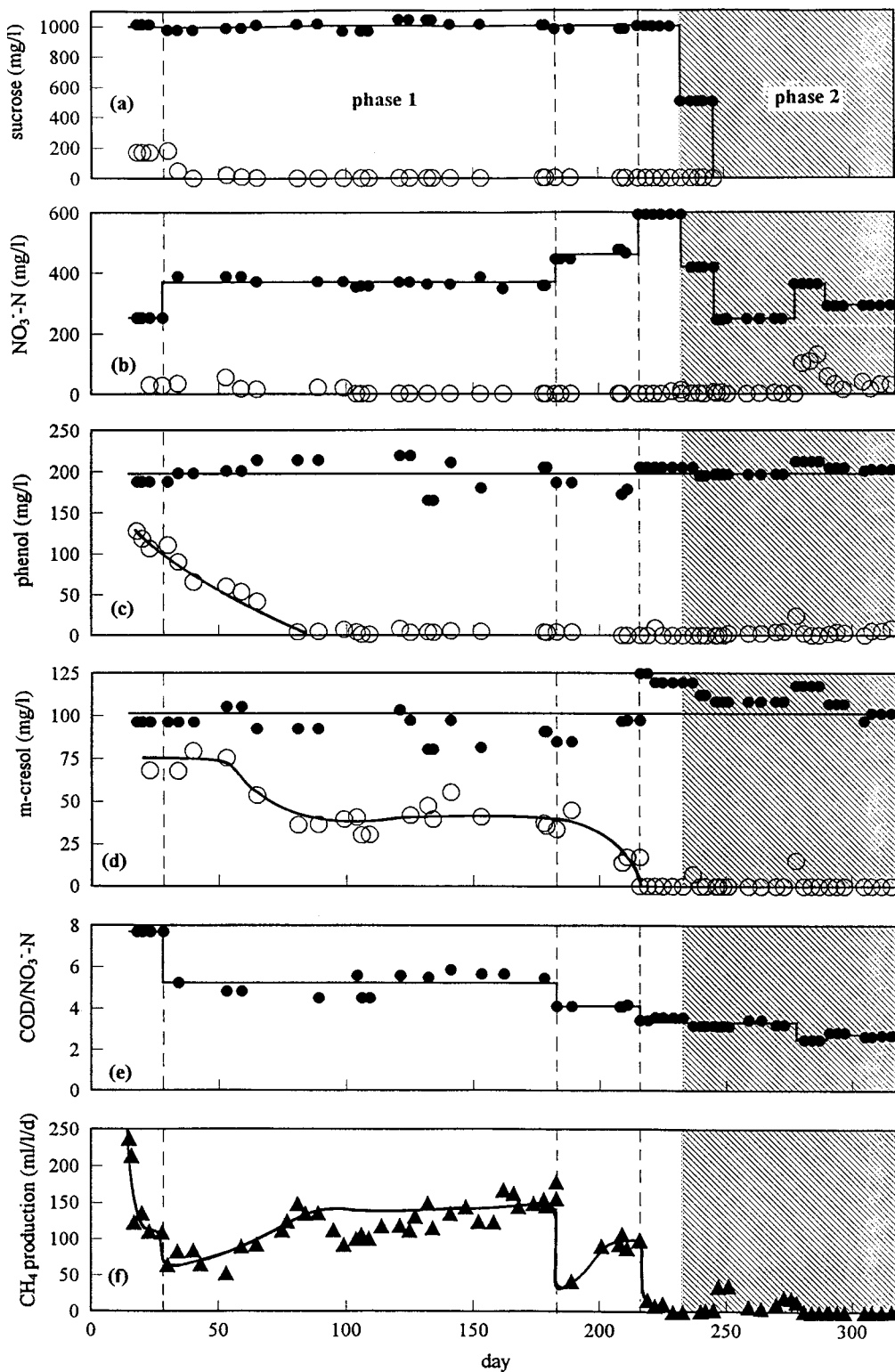| Phase (1)	Days (2)	Sucrose (mg/L) (3)	COD (mg/L) (4)	NO_3^--N (mg/L) (5)	COD/NO_3^--N (6)
1	1–27	1,000	1,943	253	*
1	28–182	1,000	1,925	368	5.23
1	183–215	1,000	1,895	460	4.12
1	216–232	1,000	1,987	595	3.34
2	233–245	500	1,400	419	3.34
2	246–277	0	828	248	3.34
2	278–289	0	909	362	2.51
2	290–318	0	835	292	2.86
*Not meaningful during this acclimation period.					

FIG. 3. Concentrations in Wastewater and Effluent of: (a) Sucrose; (b) NO_3^--N; (c) Phenol; (d) *m*-Cresol; (e) Influent COD/NO_3^--N Ratio; (f) Methane Production Rate, in Continuous Experiment

and butyrate. Both propionate and butyrate then are converted by acetogens to acetate and hydrogen; both reactions require syntrophic association between the hydrogen-producing acetogens and hydrogenotrophic methanogens (Fang et al. 1995a,b). Last, acetate and hydrogen are converted to methane by methanogens.

However, there were no detectable intermediate products such as nitrite, benzoate, propionate, and butyrate in the effluent. The absence of nitrite suggests that conversion of

nitrate to nitrite was likely the rate-limiting step in denitrification; nitrite produced by *Nitrosomonas* was denitrified readily by *Nitrobacter*. The absence of benzoate appears to suggest that the subsequent degradation of benzoate had a higher rate than the conversion of phenol and *m*-cresol to benzoate. The absence of propionate and butyrate in the effluent concur with the observations of two previous studies treating simple phenol (Fang et al. 1996) and benzoate (Li et al. 1995).

Denitrification and Methanogenesis in Presence of Carbohydrate Cosubstrate

Phase 1 (days 1–232) was conducted for the treatment of wastewater containing constant influent concentrations of phenol (200 mg/L) and *m*-cresol (100 mg/L) in the presence of a carbohydrate cosubstrate (sucrose, 1,000 mg/L) at various concentrations of NO_3^--N (averaging from 252 to 595 mg/L). Because the seed sludge had no prior exposure to nitrate, the NO_3^--N in the wastewater initially was kept at a moderate average concentration of 250 mg/L, allowing the sludge to adapt to the presence of nitrate. However, it did not take long for the sludge to develop denitrification capability. When the first measurement of nitrate was taken on day 20, the residual NO_3^--N in the effluent was already below 30 mg/L. The NO_3^--N concentration in the wastewater thus was increased to 370 mg/L on day 28, and then to 460 mg/L on day 183 and last to 600 mg/L on 216. The corresponding COD/NO_3^--N ratios in these three stages were 5.23, 4.12, and 3.34, respectively (Fig. 4).

After day 20, nearly all NO_3^--N in the wastewater was denitrified regardless of the concentration of residual substrates and the degrees of methanogenesis; the residual NO_3^--N in the rest of phase 1 effluent averaged below 10 mg/L.

On the other hand, methanogenic activity was affected by the presence of nitrate in wastewater. Fig. 3(f) illustrates that the biomass initially had considerable methanogenic activity. The methane production rate was over 230 mL/(L·day) on day 15. This was because the biomass had not yet fully developed its denitrification capability, and thus most of the substrate was utilized by methanogens. But, the rate rapidly decreased to the 100-mL/(L·day) level during days 15–27 when nitrate became fully denitrified. On day 28 when nitrate concentration was increased to 370 mg/L, the methane production rate dropped immediately to 60 mL/(L·day). This is likely because the denitrifiers outcompeted methanogens for substrate at increased concentrations of nitrate. The methane production rate gradually increased after day 28 with the increase of phenol and *m*-cresol degradations. The methane production rate was leveled off at 140 mL/(L·day) level by day 80.

Degradations of Phenol and m-Cresol in Presence of Carbohydrate Cosubstrate

Fig. 3(a) illustrates that all the sucrose was degraded after day 34; the residual sucrose in the effluent was below the detectable level of 1 mg/L. On the other hand, biomass took time to develop phenol- and *m*-cresol-degrading capability. Fig. 3(c) illustrates that when nitrate was denitrified completely on day 20, the residual phenol and *m*-cresol concentrations in the effluent were 130 and 68 mg/L, respectively.
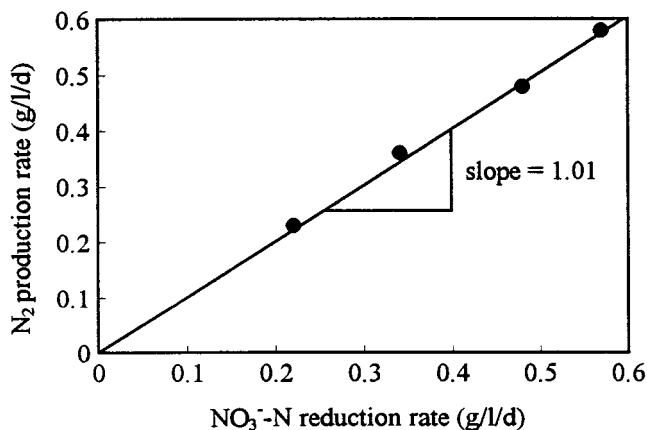

FIG. 4. (a) Percentages of Electron Flow; (b) Degradation Efficiency of *m*-Cresol at Various COD/NO_3^--N Ratios

Most of the phenol and *m*-cresol presumably were degraded at this stage by denitrifiers using sucrose as cosubstrate.

The phenol and *m*-cresol-degrading capability of biomass was improved after day 20 because of increased methanogenesis, because complete denitrification had already taken place. This also was evidenced by the increased production of methane during days 28–80. The reactor was operated under a steady-state condition during days 80–182 when the wastewater contained 368 mg/L of NO_3^--N at the COD/NO_3^--N ratio of 5.23. Fig. 3(c) illustrates that, under this condition, 98.5% of phenol was removed when NO_3^--N levels were 368 mg/L or higher.

However, the *m*-cresol-degrading capability of methanogens was still limited. The biomass was only capable of degrading 60% of *m*-cresol with 1 day of hydraulic retention during days 80–182. Fig. 3(d) illustrates that the residual *m*-cresol concentration was further decreased with the increase of NO_3^--N concentration in wastewater. When the NO_3^--N was increased to 595 mg/L during days 216–232, 98% of *m*-cresol was removed. This was because at this condition, nearly all the substrate was used by denitrifiers, as evidenced by the cease of methane production. These results clearly indicate that denitrifiers had superior *m*-cresol-degrading capability than methanogens.

Denitrification in Absence of Carbohydrate Cosubstrate

In phase 2, the sucrose was removed completely from the wastewater after a 13-day transition period (days 233–245), while concentrations of phenol and *m*-cresol in wastewater remained unchanged. The NO_3^--N concentration then was adjusted to 248 mg/L (days 246–277) so that the COD/NO_3^--N ratio remained at 3.34. Subsequently, it was adjusted to 362 mg/L (days 278–289) and 292 mg/L (days 290–318); the corresponding COD/NO_3^--N ratios were 2.51 and 2.86, respectively. Figs. 3(c and d) illustrate that, when COD/NO_3^--N ratio was 3.34 or lower, denitrification was the predominant reaction with little methanogenesis taking place. Denitrifiers were able to degrade nearly 100% of phenol and *m*-cresol with 1 day of hydraulic retention even in the absence of sucrose.

Fig. 3(b) illustrates that, during days 278–289 at the COD/NO_3^--N ratio of 2.51, there was a significant amount of residual NO_3^--N, averaging 120 mg/L, accumulated in the effluent. This was because the wastewater did not have sufficient quantities of substrate for complete denitrification. In addition, nitrous oxide, a key denitrification intermediate, also was found in the biogas, accounting for 7.6% of NO_3^--N in the wastewater. However, nitrite, the other key intermediate, was not found in the effluent. When the NO_3^--N concentration was lowered to 292 mg/L during days 290–318, the denitrification remained incomplete. At a COD/NO_3^--N ratio of 2.86, the residual NO_3^--N averaged 32 mg/L and nitrous oxide remained detectable in the biogas.

Nitrogen Balance

The wastewater contained NH_4Cl as a nutrient supplement throughout the experiment. The NH_4^+-N/COD ratio was kept at 0.068, which was adequate for bacterial growth. Fig. 4 illustrates the relationship between the quantities of nitrogen gas production and nitrate reduction during phase 1. The slope of 1.01 indicates that nearly all the nitrate in the incoming wastewater was denitrified. This agrees with the analytical results that there was not detectable intermediates of nitrate reduction, such as nitrite and nitrous oxide, in both the mixed liquor and the biogas.
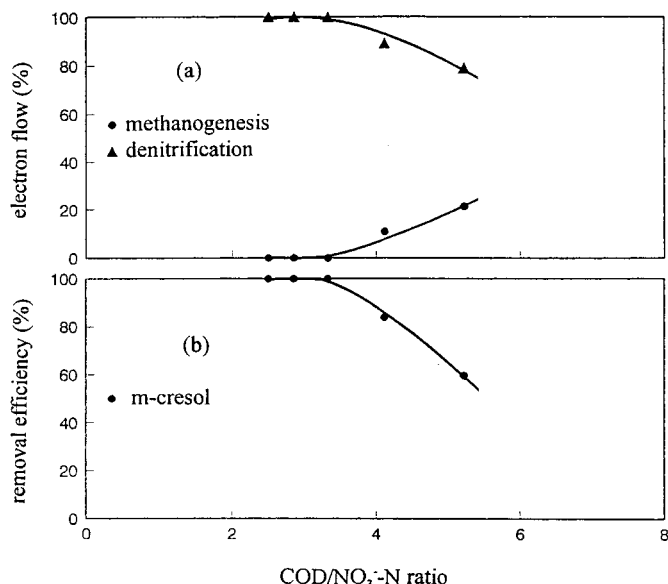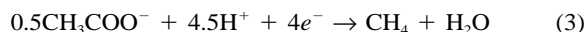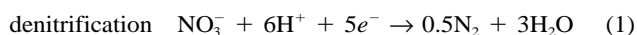
FIG. 5.   Conversion of Nitrate to Nitrogen Gas during Phase 1 of Continuous Experiment

Competition for Electrons between Denitrification and Methanogenesis

Gujer and Zehnder (1983) estimated that in the strict anaerobic degradation of organic matters 30% of the carbon source for methanogenesis was from bicarbonate and 70% from acetate. In the reactor treating nitrate-containing wastewater, denitrifiers and methanogens compete for electrons producing nitrogen and methane, respectively, according to the following reduction half-equations:

$$\text{denitrification} \quad NO_3^- + 6H^+ + 5e^- \rightarrow 0.5N_2 + 3H_2O \quad (1)$$

$$\text{methanogenesis} \quad HCO_3^- + 9H^+ + 8e^- \rightarrow CH_4 + 3H_2O \quad (2)$$

$$0.5CH_3COO^- + 4.5H^+ + 4e^- \rightarrow CH_4 + H_2O \quad (3)$$

where electrons e^- are obtained from the oxidation half-reactions, in which hydrogen and organic substrate, such as acetate, are oxidized to H^+ and CO_2, as follows:

$$H_2 \rightarrow 2H^+ + 2e^- \quad (4)$$
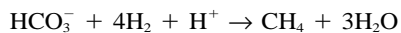
$$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^- \quad (5)$$

The reaction in (1) shows that denitrifying each mole of NO_3^- consumes $5e^-$. Based on the estimation by Gujer and Zehnder (1983), producing each mole of CH_4 would consume $5.2e^-$ $(0.3 \times 8 + 0.7 \times 4 = 5.2)$. Therefore, fractions of electron flow to methanogenesis and denitrification can be estimated from the amount of nitrate denitrified and methane produced.

Fig. 5(a) illustrates that electron flows to methanogenesis and denitrification were dependent on the COD/NO_3^--N ratio. At the COD/NO_3^--N ratio of 3.34, all electrons were utilized by denitrification, as evidenced by the cease of methane production. The fraction of electron flow to methanogenesis increased with the COD/NO_3^--N ratio, from nil at the ratio of 3.34 to 21.2% at 5.23. Strictly speaking, COD is a measurement of electrons in the pollutants available for oxidation. These results clearly indicate that the denitrifiers outcompeted methanogens for electrons. When COD/NO_3^--N ratios were low, meaning a shortage of electron supply, denitrifiers would utilize all the electrons available, and only the leftovers would be utilized by methanogens. This is because denitrification is thermodynamically more favorable than methanogenesis. Using hydrogen as a reference electron donor, the two reactions can be written as follows:

$$NO_3^- + 2.5H_2 + H^+ \rightarrow 0.5N_2 + 3H_2O$$

$$\Delta G^{0\prime} = -560.3 \text{ kJ/reaction} \quad (6)$$

$$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$$

$$\Delta G^{0\prime} = -135.6 \text{ kJ/reaction} \quad (7)$$

where $\Delta G^{0\prime}$ = change of standard Gibbs free energy at pH 7 (Thauer et al. 1977). The $\Delta G^{0\prime}$ value of denitrification is considerably lower than that of methanogenesis, indicating the former is a more favorable reaction.

Effect of COD/NO_3^--N Ratio

According to the reaction in (6), denitrifying 1 g of NO_3^--N chemically requires 2.86 g of COD, because each mole of hydrogen is equivalent to 16 g of COD $(2.5 \times 16/14 = 2.86)$. The actual required COD/NO_3^--N ratio for complete denitrification would be greater than 2.86 because extra COD is required for cell growth. In this study, complete denitrification took place when the COD/NO_3^--N ratio was as low as 3.34. There was no methane production at this ratio, indicating that all the COD was consumed for denitrification alone. The ratio of 3.34 is comparable with the wide range of reported data in literature, varying from 3.45 to 5.34 (Narkis et al. 1979; Hanaki and Polprasert 1989; Chen and Lin 1993; Liessens et al. 1993). The variation could partly be caused by the difference in substrate. Using methanol as the sole substrate, a ratio of 3.70 was reported by McCarty et al. (1969), while Yang et al. (1995) reported 3.62–3.68. Using ethanol, Delanghe et al. (1994) reported a ratio of 3.73, but Christensson et al. (1994) reported values ranging from 3.85 to 6.1. Using acetate, Narkis et al. (1979) reported a ratio of 3.6.

When COD/NO_3^--N ratios were greater than 3.34, as in phase 1, denitrifiers were unable to consume all the substrate. The excess substrates then were consumed by methanogens. After 80 days of acclimation, the methanogens were able to degrade fully phenols at COD/NO_3^--N ratios as high as 5.23. However, the methanogens' *m*-cresol-degrading capability was still limited. Fig. 5(b) illustrates that only 84% of *m*-cresol was degraded at the COD/NO_3^--N ratio of 4.12 and 60% at the ratio of 5.23.

Batch Experiments

Degradations of Phenol and m-Cresol in Nitrate-Rich and Nitrate-Free Feed Solutions

Sludge was sampled from the UASB reactor on day 182 when it was treating wastewater at a COD/NO_3^--N ratio of 5.23 using sucrose as cosubstrate. Two batch tests were conducted in parallel. The feed solutions were of the same composition as in the UASB reactor, except nitrate. Both feed solutions contained sucrose, balanced nutrient, trace element, and buffer chemicals; but in one reactor, it contained nitrate keeping the COD/NO_3^--N ratio at 5.23, whereas in the other reactor it was nitrate free. Results of the two parallel tests are illustrated in Fig. 6.

Fig. 6(a) illustrates that, in the reactor treating nitrate-rich feed solution, NO_3^--N in the mixed liquor was depleted rapidly by hour 200. The rate of NO_3^--N depletion in the mixed liquor matched the rates of nitrogen production [as illustrated in Fig. 6(a)] and sucrose depletion (not shown). Fig. 6(b) illustrates that methane was not produced until nitrate had been depleted from the mixed liquor. This shows once more that denitrifiers outcompeted methanogens for substrate/electrons. Fig. 6(b) also illustrates that methane was produced instantly in the nitrate-free solution. The difference in methane production in the two reactors was caused by the competition for substrate by denitrifiers.
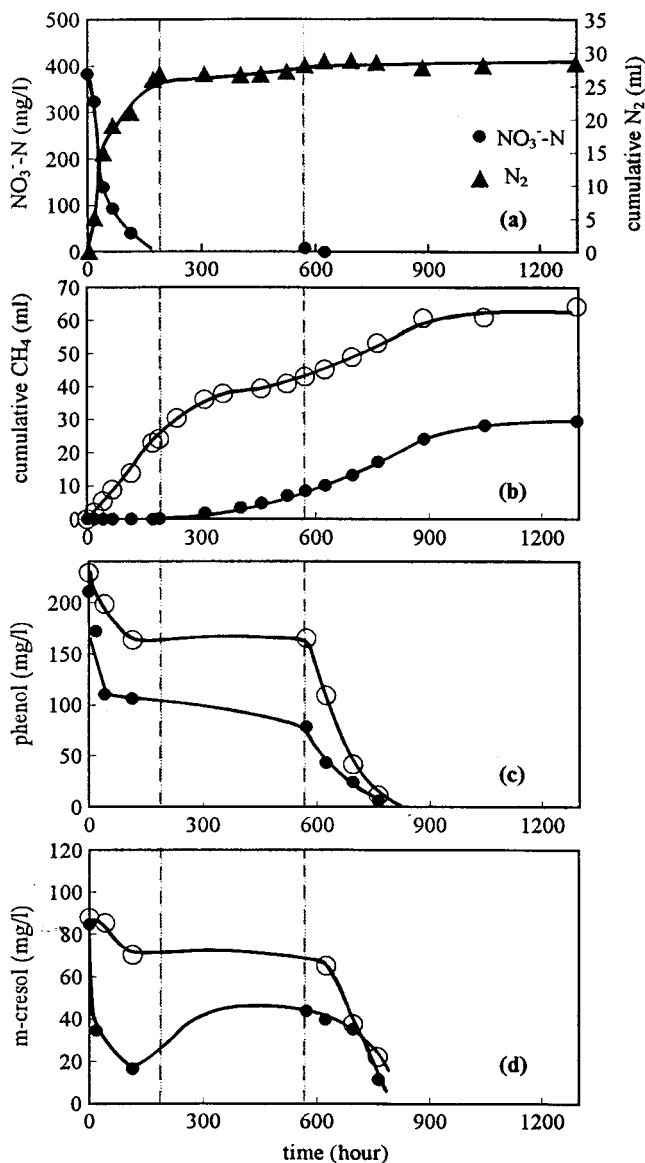
FIG. 6. Comparisons between Batch Treatment of Nitrate-Rich (● and ▲) and Nitrate-Free (○) Wastewaters: (a) Nitrate Depletion and Nitrogen Production; (b) Cumulative Methane Production; (c) Phenol; (d) m-Cresol Concentrations in Mixed Liquor

Figs. 6(c and d) illustrate that, in the presence of sucrose during the first 120 h, both denitrifiers and methane formers were able to degrade phenol and m-cresol. However, the degradation rate of denitrifiers was much higher than that of methane-forming bacteria, as observed in the continuous experiment. In the presence of sucrose as cosubstrate, the average specific degradation rates of phenol and m-cresol in the initial 120 h were 24.0 and 15.9 mg/(g-VSS·h), respectively, in the nitrate-rich solution. The corresponding rates in nitrate-free solution were only 15.6 and 4.3 mg/(g-VSS·h).

Figs. 6(c and d) further illustrate that methanogens were unable to degrade both pollutants immediately after the depletion of nitrate and sucrose. However, after an extended period of adjustment of approximately 500 h, they were able to degrade both phenol and m-cresol without cosubstrate.

Degradation of Phenol and m-Cresol as Individual Substrates and as Cosubstrate

Sludge was sampled from the UASB reactor on day 282 when it was treating sucrose-free wastewater at a COD/NO_3^--N ratio of 2.51 for three parallel batch tests. The feed
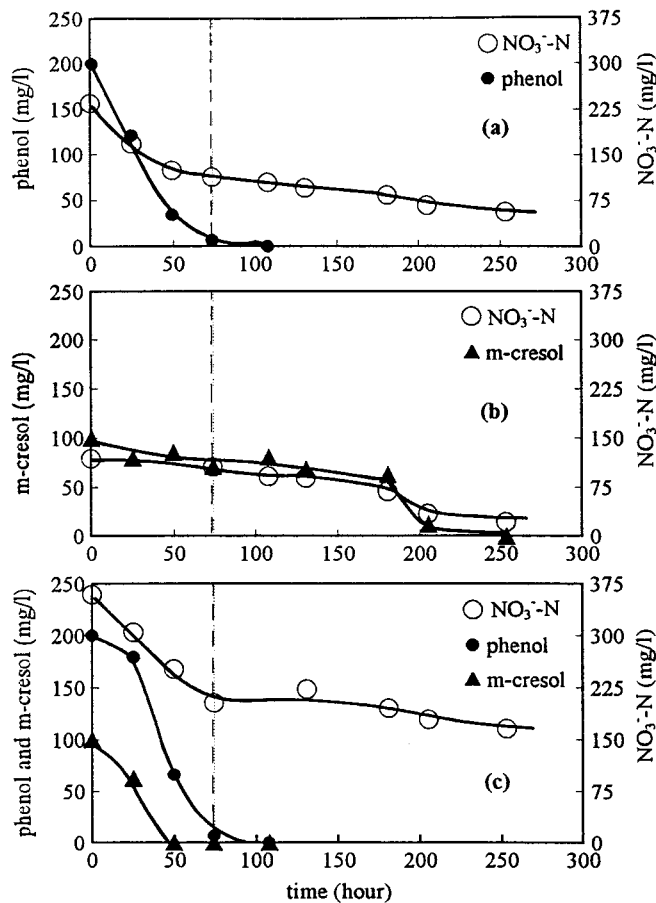
FIG. 7. Reduction of Nitrate and Degradations of: (a) Phenol as Sole Substrate; (b) m-Cresol as Sole Substrate; (c) Phenol and m-Cresol as Cosubstrate

solution for the two reactors contained phenol (200 mg/L) and m-cresol (100 mg/L), respectively, as individual substrates. The feed solution for the third reactor contained both substrates. Nitrate was added to all reactors to keep the COD/NO_3^--N ratio at 2.0. Results are illustrated in Fig. 7.

Without a cosubstrate, phenol was degraded in 74 h at an average rate of 33.6 mg/(g-VSS·h) [Fig. 7(a)]. The presence of m-cresol lowered the rate only slightly to 28.1 mg/(g-VSS·h) [Fig. 7(c)]. However, the degradation of m-cresol was very sensitive to the presence of phenol. The degradation rate of m-cresol was only 3.3 mg/(g-VSS·h) [Fig. 7(b)] without phenol as a cosubstrate; but, with phenol, it increased drastically to 20.2 mg/(g-VSS·h) [Fig. 7(c)].

CONCLUSIONS

Anaerobic sludge was able to conduct denitrification without much acclimation. Denitrifiers out-competed methanogens for substrates for carbon and electron supplies. The former were able to use phenol and m-cresol as substrate even in the absence of a carbohydrate cosubstrate. Denitrifying 1 g of NO_3^--N required 3.34 g of COD. Methanogenesis occurred only at COD/NO_3^--N ratios greater than 3.34. At 37°C and 1 day of hydraulic retention, nearly all the phenol, but only a fraction of m-cresol, were degraded in the presence of a carbohydrate cosubstrate. At COD/NO_3^--N ratios less than 3.34, methanogenesis ceased to take place and denitrification became incomplete. Degradation of m-cresol was enhanced by the presence of either sucrose or phenol as a cosubstrate.

APPENDIX. REFERENCES

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