DENITRIFICATION OF PHENOLIC WASTEWATER BY IMMOBILIZED SLUDGE

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ABSTRACT

Wastewater containing NO_3^--N (223 mg l^{-1}), phenol (100 mg l^{-1}) and m-cresol (50 mg l^{-1}) was treated at 30°C in an upflow reactor packed with polyvinyl-alcohol (PVA) beads entrapped with acclimated sludge. An additional 500 mg l^{-1} of sucrose was added as co-substrate, keeping the COD/NO_3^--N ratio at 4.3/1. The experiment was conducted by lowering the hydraulic retention time (HRT) stepwise from 12.2 to 0.52 hour. Results showed that nitrate was completely denitrified to less than 0.1 mg l^{-1} and all the phenol, m-cresol and sucrose were degraded to less than 1 mg l^{-1} even at the HRT of 0.52 hour. At this HRT, the degradation rates were 4.3 $g \cdot l^{-1} \cdot d^{-1}$ for phenol and 2.1 $g \cdot l^{-1} \cdot d^{-1}$ for m-cresol, and the denitrification rate was 10.1 g-NO_3^--N l^{-1} d^{-1}. Throughout the experiment, there was no noticeable disintegration of PVA beads, and the total solid content in the effluent never exceeded 112 mg l^{-1}. Each gram of NO_3^--N reduction required an average of 4.14 grams of COD, and the average sludge yield was estimated as 0.22 g-VSS$\cdot$ g-COD^{-1}. Operated at a COD/NO_3^--N ratio of 5.0/1, part of the excess COD was converted to methane; whereas at 3.1/1, the excess NO_3^--N was converted to N_2O. Scanning electron microscopic examination showed that bacteria were mostly populated on the immobilized beads surface due to the availability of nitrate and substrate.

INTRODUCTION

Many surface and ground waters are often polluted by nitrate, a pollutant of mostly agriculture origin, and aromatic chemicals, which are mainly from the various chemical-related industries. Although chemical and physicochemical methods have been developed for the removal of nitrate, such as chlorination, ion-exchange, air-stripping, etc., biological denitrification is in most cases the most cost-effective treatment process [1]. In this process, heterotrophic bacteria convert nitrate into nitrogen, using other organic pollutants for supply of carbon and electrons.

Recent studies have found that many simple aromatic pollutants, such as phenol [2] and benzoate [3], could be biologically degraded in anaerobic reactors. Another study [4] also showed that m-cresol could be degraded in a batch reactor under anoxic condition. Although the feasibility of denitrification using aromatic pollutants as substrate has been demonstrated [5, 6, 7], information related to the effect of operational parameters on the denitrification efficiency using aromatic substrate in a continuous reactor is still limited. On the other hand, immobilized sludge exhibited better resistance than the suspended sludge towards pH fluctuations and the presence of toxic pollutants in wastewater [8]. Furthermore, denitrification using immobilized sludge appeared to be able to prevent the biomass from being washed out [9, 10, 11, 12, 13]. It is thus of great interest to conduct an experimental study on the denitrification of phenolic wastewater using immobilized sludge, particularly at low hydraulic retention time (HRT).

MATERIALS AND METHODS

Sludge Immobilization

Seed sludge were obtained from a 2.8-l reactor conducted for another study treating high-strength wastewater containing nitrate, phenol and m-cresol. With a 1-day HRT (hydraulic retention time), the reactor was able to reduce 100% of nitrate and remove over 90% of COD (chemical oxygen demand). The seed sludge was immobilized in PVA (Sigma, MW70,000-100,000) beads, using a method modified from a previous study [9]. A solution was prepared by dissolving 7.5 g of PVA into 30 ml of water at 80°C. The solution was then cooled to 40°C followed by the addition of 60 ml of sludge, which contained 1.2 g of volatile suspended solids (VSS), and 2.0 g of portland cement (China Cement Ltd., Class 52.5-N), which was used to enhance the mechanical strength of the beads. The final slurry was forced through a 10 ml syringe dropwise into a saturated boric acid

solution. After 1 hour of stirring, the drops were developed into gel beads, which were then transferred to a 1M potassium dihydrogenphosphate solution for another hour for further hardening. The beads averaged 3 mm in diameter.

Batch Tests

Batch tests were conducted in a 157-ml serum bottle. Four grams of wet PVA beads (containing an estimated 50 mg of VSS) were added to the serum bottle along with 100 ml of feed solution containing phenol (100 mg l^{-1}), m-cresol (50 mg l^{-1}), NO_3^--N (265 mg l^{-1}), and sucrose (500 mg l^{-1}). Nutrient, vitamins and trace metals were also added to the feed solution, using the formulation developed by Owen, *et al.* [14] and Dolfing and Mulder [15] for a methanogenic-activity test, except excluding sodium sulfide. The pH of the feed solution was buffered at 7.2~7.6 by bicarbonate.

The bottle was purged with a gas mixture of nitrogen (N_2) and carbon dioxide (CO_2) at 3:1 ratio to ensure the absence of oxygen, and then placed in a 37°C shaking waterbath. The mixed liquor and the biogas were monitored regularly. The biogas production was measured using a syringe and its composition was analyzed by a gas chromatograph (GC). The concentrations of phenol, m-cresol and fatty acids in the mixed liquor were analyzed by another GC. The test was terminated after 425 hours when the biogas production was ceased and all the substrates in the mixed liquor were below the detectable levels. Subsequently, two additional batches were repeated in the same bottle. In these two batches, nitrogen production was closely monitored, as in the first batch basing upon the increase of biogas volume and its nitrogen composition, so that the reproducibility of denitrification could be confirmed.

Continuous Experiments

The 148-day experiment was conducted at 30°C in a 150-ml upflow reactor [16] packed with PVA beads containing an estimate of 910 mg of seed sludge. The synthetic wastewater contained NO_3^--N (223 mg l^{-1}), phenol (100 mg l^{-1}), m-cresol (50 mg l^{-1}), and sucrose (500 mg l^{-1}). The COD in the wastewater averaged 960 mg l^{-1}, corresponding to an average COD/NO_3^--N ratio of 4.3/1. Initially, the HRT was kept at 12.2 hour. It was then step-decreased reaching 0.52 hour at the end. At 0.52 hour of HRT, the loading rate was 10.1 g-NO_3^--N l^{-1} d^{-1} or 43.4 g-COD l^{-1} d^{-1}. There were two periods of perturbation (days 88-98 and days 137-148) during which nitrate concentration was deviated from the normal level so that the effect of COD/NO_3^--N ratio could be examined.

Analytical Methods

Gas composition, including methane (CH_4), N_2, CO_2 and nitrous oxide (N_2O), were analyzed by a gas chromatograph (GC, Hewlett-Packard, Model 5890 Series II)

equipped with a thermal conductivity detector and a 10 m stainless steel column packed with HayeSepQ (80/100 mesh). The content of nitric oxide (NO) was not measured, because it is not a significant intermediate product of denitrification [17]. Helium was used as the carrier gas at a flow rate of 22 ml min^{-1}. The column was operated at a temperature program of 90°C for 1.2 minutes followed by 2 minutes at 110°C. The respective temperatures of injector and detector were 130°C and 200°C

Phenol, m-cresol, and probable metabolic intermediates, such as benzoate and VFAs (from acetic to heptanoic acids), were determined by a second gas chromatograph (Hewlett-Packard, Model 5890 Series II) equipped with a 10 m x 0.53 mm HP-FFAP fused-silica capillary column and a flame ionization detector (FID), using helium as the carrier gas. Injector and detector temperatures were 200°C and 250°C, respectively. The fluid sample was filtered through a 0.45 μm membrane filter and acidified to pH 3 with concentrated phosphoric acid prior to injecting into the column using the fast injection technique. The initial temperature of the column was 70°C for 4 min followed with a first ramp of 10°C·min^{-1} to the temperature of 140°C for 2 min and a second ramp of 10°C·min^{-1} and a final temperature of 170°C for 2 min. Nitrate and nitrite were analyzed by an ion chromatograph (Shimadzu Model LC-10) with the Shim-pack IC-A3 column. Sucrose was analyzed by a spectrophotometer at 625 nm wavelength (UV-160A, Shimadzu). The detectable levels were less than 1 mg l^{-1} for phenol, m-cresol, benzoate, VFA and sucrose, and less than 0.1 mg l^{-1} for nitrate and nitrite. Other parameters such as total suspended solids (TSS), VSS and soluble COD were measured according to the *Standard Methods* [18].

Scanning Electron Microscopic Examination

After continuous experiment, PVA beads were sampled for microscopic examination using scanning electron microscope (SEM, Cambridge Steroscan 360). The sample preparation procedures were as reported previously [19].

RESULTS AND DISCUSSIONS

Batch Tests

Figure 1a illustrates that both phenol and m-cresol were depleted rapidly from the mixed liquor in the first 100 hours, and their concentrations in the mixed liquor became undetectable (<1 mg l^{-1}) by hour 205. At the end of the test, the mixed liquor did not contain any residual sucrose and nitrate. On the other hand, Figure 1b illustrates that, while the CH_4 production was levelled off after hour 100, production of N_2 continued and did not level off until hour 348. This shows that the sludge retained its bioactivity of degrading aromatic pollutants and denitrifying nitrate after the immobilization.
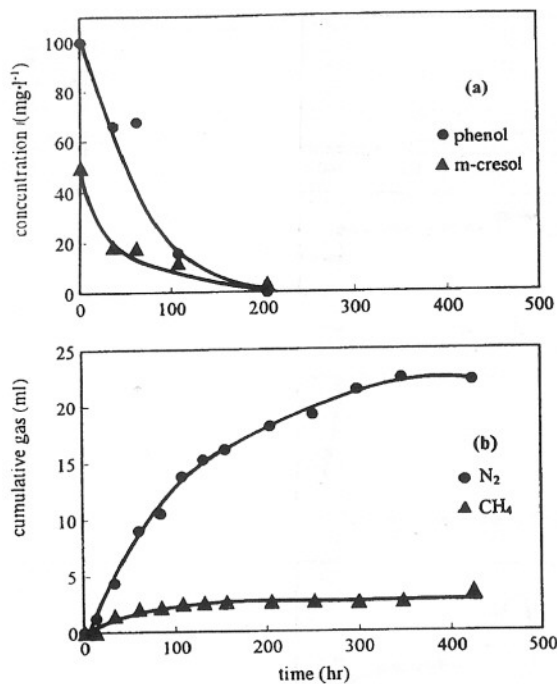
Figure 1. Results of batch test: (a) depletion of phenol and m-cresol in mixed liquor, and (b) cumulation of methane and nitrogen in the biogas.

Furthermore, it also shows that even after phenol/m-cresol were depleted from the mixed liquor, denitrification continued for 140 more hours. It is likely some of these organic pollutants were removed from the mixed liquor by adsorption by the beads, and provided carbon and electrons for the subsequent denitrification. Figure 2 illustrates that the reproducibility of the three repeated batches was satisfactory, judging from the N_2 production data. At hour 425 when the tests were terminated, the average N_2 production in the repeated batches was 21.01 ml with a standard deviation of 1.34 ml.
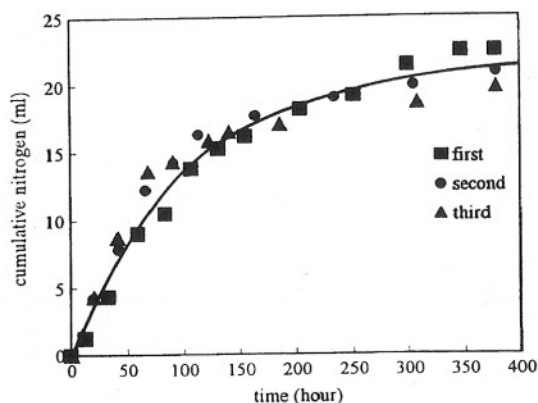

Figure 2. Nitrogen production in three batch tests.

For comparison, fully denitrification of all the NO_3^- would have produced 22.68 ml of N_2. Judging from visual observations, all beads remained unchanged physically without signs of disintegration after the three repeated batches.

Continuous Studies

The continuous experiment was conducted at 30°C. The pH of the wastewater was kept at pH8.1, while that of effluent averaged pH7.5. All PVA beads remained physically stable, without any noticeable sign of disintegration at the end. There was little sludge washout throughout the experiment; even at the HRT of 0.52 hour, the effluent contained less than 112 mg l^{-1} of TSS and 85 mg l^{-1} of VSS. Throughout the experiment, there was no nitrite and benzoate residues detected in the effluent. Figure 3 illustrates the two major operational parameters, (a) HRT and (b) COD/NO_3^--N ratio, and concentrations of residual (c) NO_3^--N, (d) soluble COD, (e) phenol, and (f) m-creosol in the effluent throughout the experiment. The two shaded areas represent the perturbation periods during which the nitrate concentration in wastewater was deviated from the normal 223 mg l^{-1}. During days 88-98, the nitrate was lowered to 192 mg l^{-1} by accident, increasing the COD/NO_3^--N ratio to 5.0/1. While during days 137-148, the nitrate concentration was purposely increased to 310 mg l^{-1} to see the effect of a low COD/NO_3^--N ratio.

Denitrification and degradations of phenol/m-cresol:

Residual nitrate and sucrose in the effluent were lowered rapidly and became undetectable after day 18 despite the continuing decrease of HRT. The depletion of nitrate is illustrated in Figure 3c. Similar pattern was also followed by the depletion of COD, as illustrated in Figure 3d, except there was a COD residue of about 36 mg l^{-1} in the effluent. Figures 3e and 3f respectively illustrate that phenol and m-cresol were depleted from mixed liquor in a rate even faster than those of nitrate and COD; both became undetectable (< 1 mg l^{-1}) by day 10. This indicates that phenol and m-cresol were able to be completely removed from the mixed liquor, even when the HRT was as short as 0.52 hour. Since the effluent did not contain sucrose, aromatic pollutants, and VFA intermediates during these periods, the residual COD in the effluent could likely be due to the soluble microbial products resulted from decay of bacterial cells [20, 21] and/or a small fraction of PVA which might be leached out from the beads.

At the beginning of the continuous experiment, the reactor produced CH_4 at a rate of 0.75 ml l^{-1} d^{-1}. The production rate was gradually reduced, and eventually it was totally ceased after day 28. On the other hand, the N_2 production rate increased in proportion to the nitrate loading rate, and was thus inversely proportional to the decrease of HRT. At 0.52 hour of HRT, the production rate reached 8.8 l-N_2 l^{-1} d^{-1}.
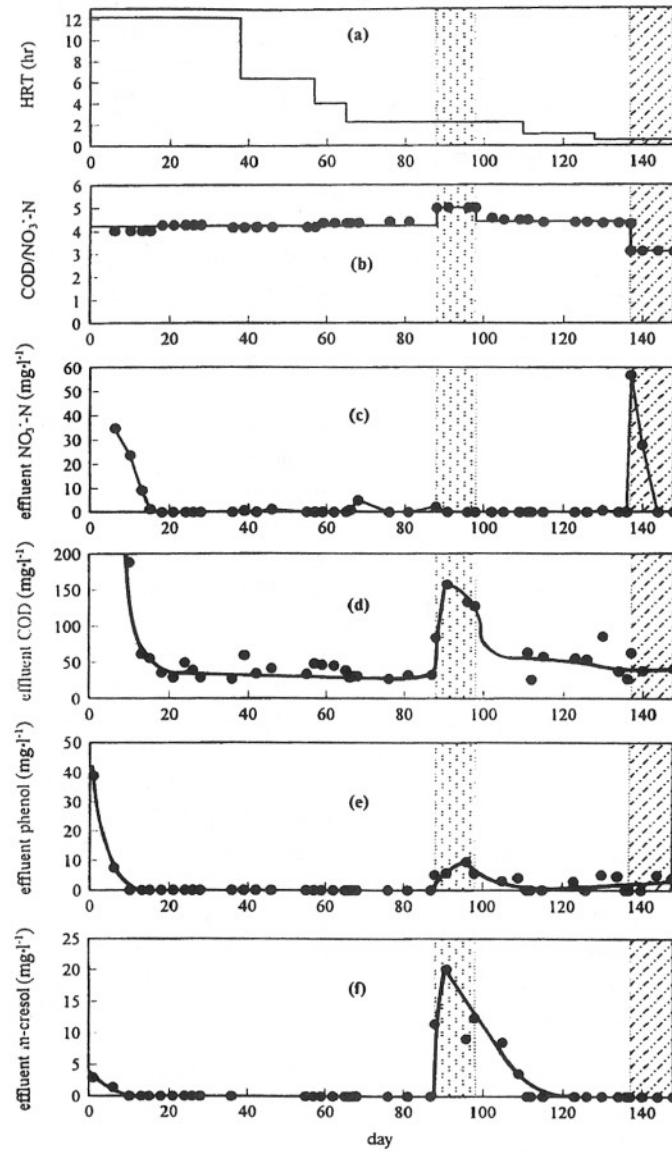
Figure 3. Operational parameters and results of continuous test: (a) HRT, (b) COD/NO_3^--N ratio, plus residual (c) NO_3^--N, (d) COD, (e) phenol and (f) m-cresol in the effluent.

These results show that methanogens were unable to compete with heterotrophic denitrifiers for available substrates. Meanwhile, N_2O, an intermediate of nitrate incomplete denitrification, was not produced in all HRTs, except when HRT was lowered to 0.52 hour N_2O was produced at an average rate of 0.31 l l^{-1} d^{-1}.

Overall, results in Figure 3 show that the immobilized sludge was able to completely degrade phenol/m-cresol and denitrify nitrate even at a HRT as low as 0.52 hour. Figures 4 and 5 illustrate that, over a wide range of loading rate (up to 43 g-COD l^{-1} d^{-1} and 10 g-NO_3^--N l^{-1} d^{-1}) the sludge was able to consistently remove 96.4% of COD and reduce 100% of NO_3^--N.
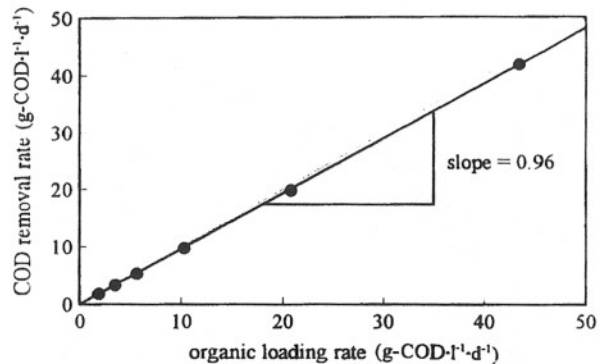

Figure 4. Volumetric COD removal rate at various volumetric COD loading rate.
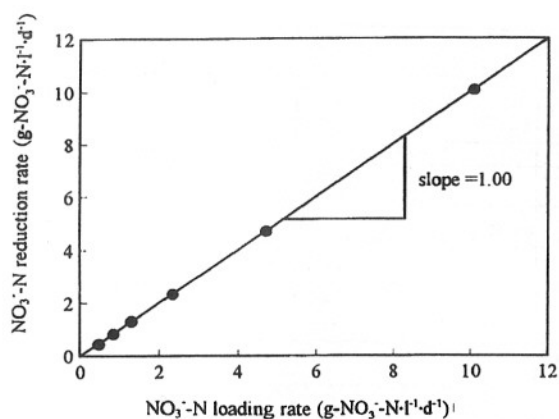
Figure 5. Volumetric NO_3^--N reduction rate at various volumetric NO_3^--N loading rate.

At 0.52 hour of HRT, the reactor with immobilized sludge was able to degrade 4.3 g l^{-1} d^{-1} of phenol and 2.1 g l^{-1} d^{-1} of m-cresol, both rates were considerably higher than the literature data. Fang et al. [2] reported a maximum phenol degradation rate of 2.44 g l^{-1} d^{-1} in an anaerobic UASB reactor at 12 hours of HRT, while Ramanand and Suflita [4] reported a m-cresol degradation rate of 0.004 g l^{-1} d^{-1} in a batch test of anoxic aquifer sludge. Since the experiment was terminated without further lowering the HRT, it is likely that the degradation rates of phenol and m-cresol may be even higher.

COD/NO_3^--N ratio:

Heterotrophic denitrifiers require organics for the supply of carbon and electrons. McCarty et al. [22] estimated that each gram of NO_3^--N would require 3.7 grams of COD for denitrification. Throughout most of the experiment the COD/NO_3^--N ratio was kept at 4.3/1 to ensure sufficient supply of COD for denitrification. The COD/NO_3^--N ratio was increased to 5.0/1 during days 88-98, but lowered to 3.1/1 during days 137-148. During the first perturbation, there was an excess of electron donors for denitrification. Figure 3 illustrates that, although nitrate was completely denitrified on day 91, the effluent COD was drastically increased to 157 mg l^{-1} with corresponding increases of phenol and m-cresol concentrations. However, the perturbation had more impact on m-cresol (went up to 20.1 mg l^{-1} on day 91) than on phenol (went up to 8 mg l^{-1}). This seems to suggest that m-cresol is more refractory than phenol for anoxic degradation. In addition, CH_4 production became noticeable (up to 13 ml l^{-1} d^{-1}) during this period, indicating that the sludge retained certain methanogenic activity even after being operated at an extended period of anoxic condition. This could be due to the nature of immobilized sludge. Methanogens entrapped in the bead interior were protected from the exterior anoxic environment, and they could be re-activated when the mixed liquor became nitrate-free and contained residual COD. After the COD/NO_3^--N ratio was returned to the normal level of 4.3/1 on day 99, it took about 15 days for the residual phenol and m-cresol to return to the pre-perturbation levels.

On the other hand, during the second perturbation period when there was insufficient electron donors for denitrification, effluent COD level was increased slightly from 32.6 to 54.5 mg l^{-1}. Effluent NO_3^--N was increased drastically to 56.9 mg l^{-1} within one day, but it was again decreased to below the detectable level in 7 days. The lowering of nitrate in the effluent was accompanied by the increased production of N_2O, of which the composition in the biogas went up to as much as 23%, as illustrated in Figure 6.
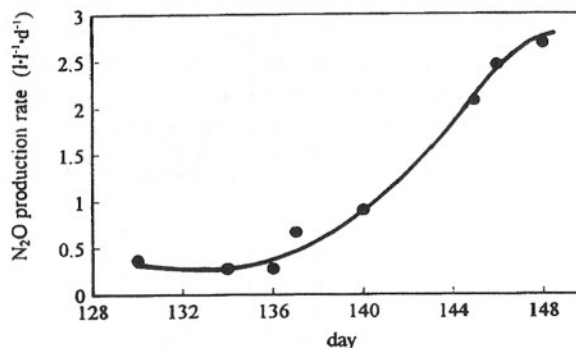

Figure 6. Production of N_2O at COD/NO_3^--N ratio of 3.1/1.

This indicated that when electron donors were in short supply, bacteria conducted partial denitrification, converting nitrate to the intermediate N_2O. On day 148, the N_2O production rate was 2.7 l l^{-1} d^{-1}, indicating 21.3% of NO_3^--N in the wastewater was reduced to N_2O-N. Schulthess et al. [17] reported that the accumulation of partially reduced nitrogen, including N_2O, NO and nitrite, would cause severe inhibition of enzymes along the denitrification respiratory chain. But, in this study, the production of N_2O did not seem to have adverse effect on the degradation of phenol/m-cresol, nor on nitrate reduction.

Figure 7 illustrates that over a wide range of loading rate, denitrifying one gram of NO_3^--N in this study consumed an average of 4.14 grams of COD. This is slightly higher than the 3.62-3.68 reported by Yang et al. [13] using methanol as substrate, and the 3.73 reported by Delanghe et al. [23] using ethanol as substrate.
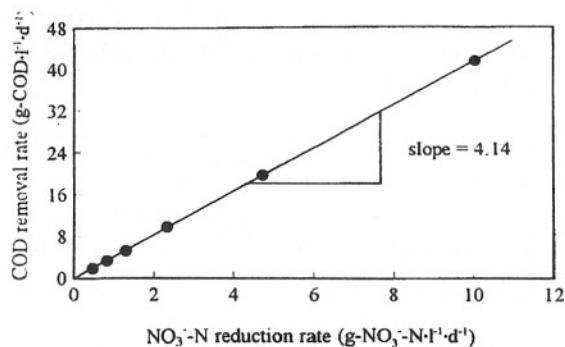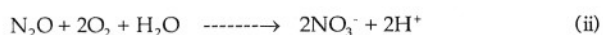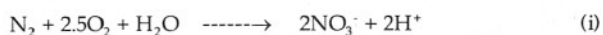

Figure 7. Relationship between COD removal rate and NO_3^--N reduction rate.

COD balance and sludge yield:

Conventionally, COD is meant to be the organic-COD alone. However, strictly speaking, COD by definition is a measurement of reducing capacity, namely electrons available for chemical oxidation. The electrons are available in the organic as well as in the nitrogenous chemicals. In a strict anaerobic process, the amount of available electrons remains constant, and thus the total COD remains unchanged. The original COD in the pollutants are transformed into the COD of the reduced products, plus those in the sludge. A balance of COD, therefore, shows the transformation of these available electrons.

According to the stoichiometry of the four following reactions, each gram of N_2, N_2O, NO_2^- and CH_4 are, respectively, equivalent to 2.86, 2.29, 1.14 and 4.00 grams of COD:

$$N_2 + 2.5O_2 + H_2O \quad \text{------} \rightarrow \quad 2NO_3^- + 2H^+ \qquad \text{(i)}$$

$$N_2O + 2O_2 + H_2O \quad \text{------} \rightarrow \quad 2NO_3^- + 2H^+ \qquad \text{(ii)}$$

$$NO_2^- + 0.5O_2 \qquad \text{------} \rightarrow \quad NO_3^- \qquad \text{(iii)}$$

$$CH_4 + 2O_2 \qquad \text{------} \rightarrow \quad CO_2 + 2H_2O \qquad \text{(iv)}$$

Thus the stoichiometric amount of COD utilized for denitrification can be calculated, based on the measured data of various reduced products. Figure 8 illustrates that the rate of COD stoichiometrically needed for denitrification was consistently 68.6% of the actual COD removal rate. The remaining 31.4% was presumably utilized for cell synthesis. Assuming the microbial cell has the chemical composition of $C_5H_7O_2N$, each gram of biomass has a COD-equivalent of 1.42 grams. Based on these results, the sludge yield is thus estimated as 0.22 g-VSS $\cdot$ g-COD^{-1}. This is higher than 0.11 g-VSS $\cdot$ g-COD^{-1} reported by Yang *et al.* [13], but lower than the 0.30-0.36 g-VSS $\cdot$ g-COD^{-1} reported by Sison *et al.* [24]. The reason for the differences in sludge yield was unclear, but it could be resulted from the differences in the use of substrate and in the COD/NO_3^--N ratio.

SEM observations:

After 148 days of continuous operation, PVA beads entrapped with immobilized sludge were sampled for SEM observations. A rod-shaped bacteria were predominant on the bead surface (Figure 9a). Chains of cocci bacteria were found both on the surface (Figure 9b) and in the interior (Figure 9c) of PVA beads.

Furthermore, it seems that there was a much higher density of bacterial population on the surface (Figure 9a) than in the interior (Figure 9c). This was probably due to the
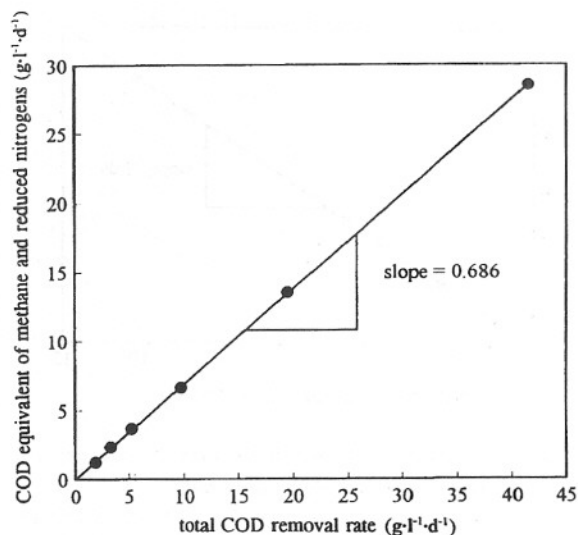

Figure 8. Relationship between CH_4- and N_2-COD production rate and total-COD removal rate.

availability of substrate. Most of the substrate were used up by the bacteria near the surface, leaving very little substrate for those bacteria in the interior. As a result, the PVA beads gradually became a biofilm support.

CONCLUSIONS

Results of this study showed that a reactor packed with immobilized sludge was very effective for denitrification using phenolic pollutants as substrate. Operated at a COD/NO_3^--N ratio of 4.3/1 and HRTs as low as 0.52 hour, all nitrate were denitrified to less than 0.1 mg l^{-1} and the residual phenol and m-cresol in the effluent were both below the detectable level of 1 mg l^{-1}. Operated at a higher COD/NO_3^--N ratio of 5.0/1 resulted in an increase of residual COD, phenol and m-cresol in the effluent, whereas operating at a lower COD/NO_3^--N ratio of 3.1/1 resulted in the production of N_2O. Denitrifying one gram of NO_3^--N consumed an average of 4.14 gram of COD, and sludge yield was estimated as 0.22 g-VSS·g-COD^{-1}. There was no noticeable disintegration of immobilized beads after 148 days operation. Scanning electron microscopic examination showed that bacteria were mostly populated on the bead surface, probably due to the availability of nitrate and substrate.
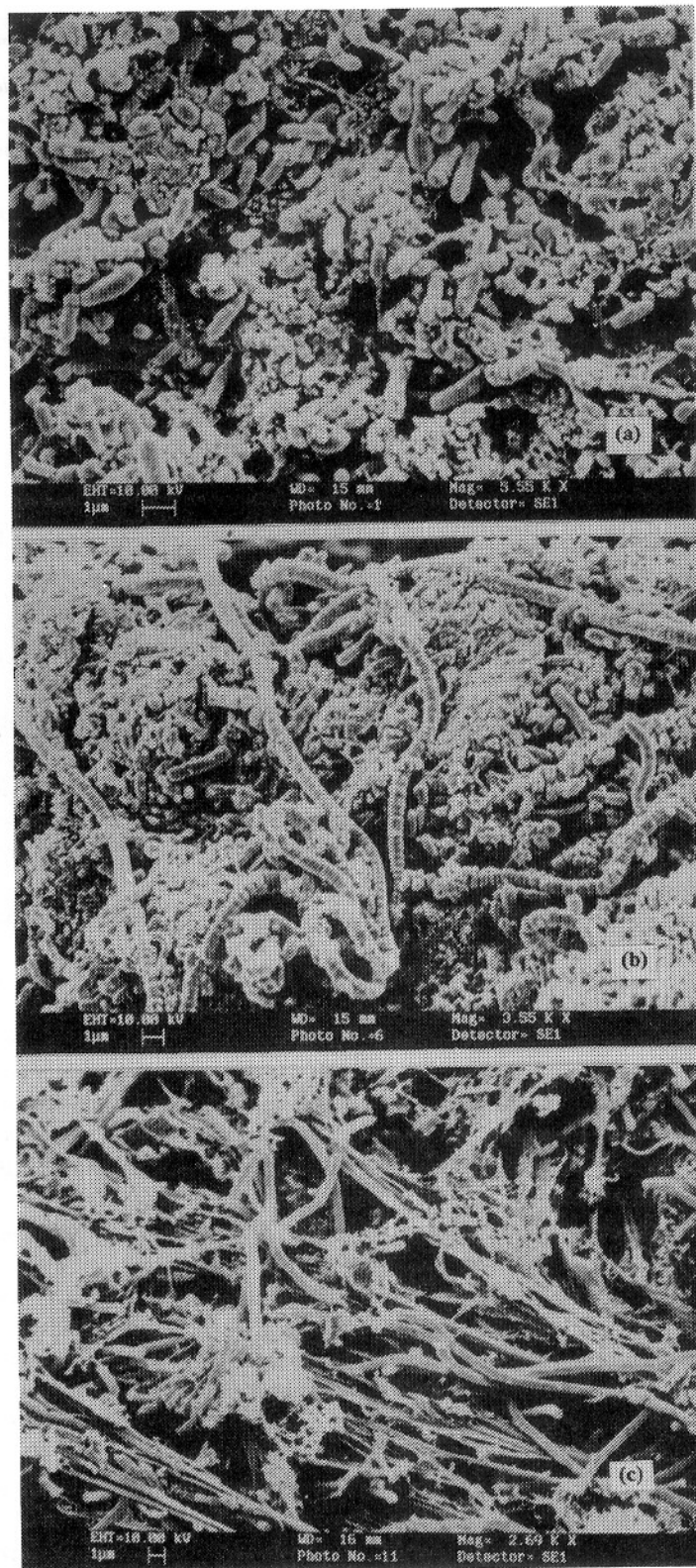
ACKNOWLEDGMENTS

Figure 9. Scanning electron micrographs: (a) a dense population of bacteria on the bead surface, plus chains of cocci (b) on the surface and (c) in the interior.

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