ELSEVIER

Bioresource Technology 80 (2001) 179–186

BIORESOURCE TECHNOLOGY

Biotransformation of nitrophenols in upflow anaerobic sludge blanket reactors

Khursheed Karim, S.K. Gupta *

Centre for Environmental Science and Engineering, Indian Institute of Technology, Bombay 400 076, India

Received 5 March 2001; received in revised form 14 May 2001; accepted 16 May 2001

Abstract

Four identical bench-scale upflow anaerobic sludge blanket (UASB) reactors, R1, R2, R3 and R4, were used to assess nitrophenols degradation at four different hydraulic retention times (HRT). Reactor R1 was used as control, whereas R2, R3, and R4 were fed with 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), and 2,4-dinitrophenol (2,4-DNP), respectively. The concentration of each nitrophenol was gradually varied from 2 to 30 mg/l during acclimation. After acclimation reactors were operated under steady-state conditions at four different HRTs – 30, 24, 18, and 12 h, to study its effect on the removal of nitrophenols. Overall removal of 2-NP and 4-NP was always more than 99% but 2,4-DNP removal decreased from 96% to 89.7% as HRT was lowered from 30 to 12 h. 2-Aminophenol (2-AP), 4-aminophenol (4-AP) and 2-amino,4-nitrophenol (2-A,4-NP) were found to be the major intermediates during the degradation of 2-NP, 4-NP and 2,4-DNP, respectively. Out of the total input of nitrophenolic concentration (30 mg/l), on molar basis, about 41.2–48.4% of 2-NP, 59.4–68% of 4-NP, 30–26.6% of 2,4-DNP was recovered in the form of their respective amino derivatives at 30–12 h HRT. COD removal was 98–89%, 97–56%, 97–52%, and 94–46% at 30–12 h HRT for R1, R2, R3 and R4, respectively. Average cell growth was observed to be 0.15 g volatile suspended solid (VSS) per g COD consumed. Methanogenic inhibition was observed at lower HRTs (18 and 12 h), however denitrification was always more than 99% with non-detectable level of nitrite. The granules developed inside the reactors were black in color and their average size varied between 1.9 and 2.1 mm.

Keywords: Acetate; Aminophenol; Biodegradation; Denitrification; Detoxification; Granule; Methanogenesis; Nitrate; Nitrophenol; UASB

1. Introduction

Nitrophenols are among the most widely used industrial organic compounds. They are frequently used as intermediates in the production of explosives, pharmaceuticals, pesticides, pigments, dye, wood preservatives and rubber chemicals (Uberoi and Bhattacharya, 1997). Nitrophenols also result from microbial activity and natural processes in the biosphere. They are produced by microbial hydrolysis of several organophosphorous pesticides, such as parathion or by photodegradation of pesticides that contain nitrophenol moiety (Haghighi-Podeh et al., 1995). 4-Nitrophenol may be produced in the atmosphere through the photochemical reaction between benzene and nitrogen monoxide and has been detected in rainwater in Japan (EPA, 1980).

The annual production of 4-nitrophenol alone is 20 million kg (Donlon et al., 1996). Since these chemicals are frequently used for industrial, agricultural and defense purposes, usually they find their way into the effluents from these sources. These compounds pose significant health risks since they are carcinogenic (Uberoi and Bhattacharya, 1997). 2-Nitrophenol, 4-nitrophenol and 2,4-dinitrophenol are listed on the US Environmental Protection Agency's (USEPA's) ''Priority Pollutants List''. The USEPA recommends restricting their concentrations in natural waters to below 10 ng/l (Haghighi-Podeh et al., 1995).

Although physical and chemical changes, such as volatilization, photodegradation, occur in nature and are the eventual fate of many organic pollutants, biodegradation is perhaps the ultimate degradation mechanism. Both aerobic as well as anaerobic biodegradation processes have been used to treat nitrophenolic wastewater (Michael et al., 1990; Thomas et al., 1993; Donlon et al., 1996; Tseng and Lin, 1994; Gorontzy et al., 1993). However, electron-withdrawing groups inherent to

* Corresponding author. Tel.: +91-22-5767853; fax: +91-22-5723-3480.

E-mail addresses: karim@cc.iitb.ac.in (K. Karim), skgupta@cc.iitb.ac.in (S.K. Gupta).

0960-8524/01/$ - see front matter
PII: S0960-8524(01)00092-X

nitroaromatics reduce the electron density of the aromatic ring and inhibit oxidative attack by the electrophilic oxygenases (Shelley et al., 1996). Furthermore, the conjugation of unstable nitroso and hydroxyamino intermediate results in the formation of complex azo or azoxy compounds under aerobic conditions. However, under anaerobic conditions these compounds readily get transformed to aromatic amines. On the average aromatic amines are 500-fold less toxic than their corresponding nitroaromatics (Donlon et al., 1996). This suggests that anaerobic treatment will at least detoxify nitrophenolic wastewaters, if not completely mineralize them.

Boopathy et al. (1993) found significant removal of trinitrotoluene (TNT) under nitrate reducing conditions. They reported that the nitrite reductase enzyme, present in nitrate reducing conditions, could have acted on the nitrite group of TNT and reduced it to amino group. Since many a time nitrophenols exist in industrial effluents along with high nitrate nitrogen concentration, use of nitrate as an electron acceptor will be a more appropriate combination for treatment.

Application of high rate anaerobic wastewater treatment processes for the treatment of aromatic compounds can offer significant improvement in process stability and reliability. Upflow anaerobic sludge blanket (UASB) reactors have been used successfully for the treatment of wastewaters containing refractory organics by several researchers (Krumme and Boyd, 1988; Duff et al., 1995; Donlon et al., 1996; Fang and Zhou, 1999; Prakash and Gupta, 2000).

The present study was conducted to investigate the ability of granular sludge to degrade nitrophenols under denitrifying conditions in bench-scale UASB reactors.

2. Methods

The continuous experiments were conducted in four identical bench-scale UASB reactors (R1, R2, R3 and R4) having 12.5 l volume. Inner dimension of the reactors was 10 cm × 10 cm and a height of 1.2 m with 15 cm long hopper bottom. On the top of the reactor there was a 2 l gas–liquid–solid separator. The reactors were made of transparent acrylic plastic sheet of 6.0 mm thickness and maintained at room temperature (27 ± 4°C).

Reactors were inoculated with 5 l anaerobic granular sludge (VSS = 25 g/l, granule size = 0.25–4 mm, sludge volume index = 40 ml/g) collected from a bench-scale UASB reactor treating chlorinated aliphatic compounds. In the present study reactor R1 was kept as control, whereas R2, R3 and R4 were fed with 2-NP, 4-NP, and 2,4-DNP, respectively, along with substrate and nutrients. Sodium acetate was used as electron donor (substrate) and sodium nitrate was used as electron acceptor.

During acclimation the reactors R2, R3 and R4 received 2 mg/l concentration of 2-NP, 4-NP and 2,4-DNP at 24 h hydraulic retention times (HRT), respectively. Subsequently nitrophenols concentration was increased in steps to 5, 10, 20 and 30 mg/l. At each increment the reactors were acclimated for 30–53 days to achieve nitrophenols removal of more than 75%. Initially influent COD was of 1000 mg/l. After 45 days of acclimation, influent COD was increased to 1500 mg/l and after next 40 days influent COD was further increased to 2000 mg/l. COD/NO_3^-–N ratio was kept constant as 20. Influent and effluent samples were analyzed for pH, COD, respective nitrophenols and NO_3^-–N.

After acclimation of the granular sludge, the reactors were operated at four different HRTs 30, 24, 18 and 12 h. The composition of the synthetic wastewater used in this study is given in Table 1. Trace metals solution was prepared in distilled water by dissolving per liter 5 g $MgSO_4 \cdot 7H_2O$, 6 g $FeCl_2 \cdot 4H_2O$, 0.88 g $CoCl_2 \cdot 4H_2O$, 0.1 g H_3BO_3, 0.1 g $ZnSO_4 \cdot 7H_2O$, 0.05 g $CuSO_4 \cdot 5H_2O$, 1 g $NiSO_4 \cdot 8H_2O$, 5 g $MnCl_2 \cdot 4H_2O$ and 0.64 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Prakash and Gupta, 2000). 1 ml of this solution was added per liter of the feed solution.

Table 1
Composition of the synthetic feed for HRT study

Compounds	Concentration (mg/l)			
	R1	R2	R3	R4
Sodium acetate (trihydrated)	4000	4000	4000	4000
Sodium nitrate	667	667	667	667
Ammonium chloride	120	120	120	120
KH_2PO_4	200	200	200	200
K_2HPO_4	49.8	49.8	49.8	49.8
$NaHCO_3$	267	267	267	267
$CaCl_2 \cdot 2H_2O$	200	200	200	200
$MgCl_2$	250	250	250	250
KCl	300	300	300	300
2-Nitrophenol	–	30	–	–
4-Nitrophenol	–	–	30	–
2,4-Dinitrophenol	–	–	–	30

Throughout the HRT study influent COD and nitrophenol concentration were kept constant as 2000 and 30 mg/l, respectively. COD/NO_3^-–N ratio was kept constant as 20, which is favorable for complete denitrification (Hendriksen and Ahring, 1996). Influent pH and alkalinity were 7.2 ± 0.3 and 1000 ± 50 mg/l, respectively. At every HRT the reactors were operated for about 20–25 days under steady-state conditions. Steady state was arbitrarily considered as variation of nitrophenol and COD concentration in the effluent, and biogas production within 15% of the average value (Haghighi-Podeh et al., 1995).

The analytical procedures for all tests were as outlined in the Standard Methods for the Examination of Water and Wastewater (APHA, 1985 & 1989), unless specified otherwise. Daily measurements were taken for the rate of gas production, influent and effluent pH, COD, NO_3^-–N. Influent and effluent samples were analyzed for nitrophenols and aminophenols on alternate days. Volatile fatty acids (VFAs), alkalinity and gas composition were analyzed once a week. The sludge samples were analyzed biweekly for suspended solids (SS) and volatile suspended solids (VSS).

The specific gravity of the sludge was determined by comparing the mass of a known volume of a homogenous sludge sample at a specific temperature to the mass of the same volume of distilled water at 4°C (Ghangrekar, 1997). Temperature correction factor was applied for the measured temperature as per standard methods (APHA, 1985 & 1989).

Nitrophenols and aminophenols were analyzed by injecting 25 μl filtered liquid samples to high pressure liquid chromatograph (Shimadzu, LC 6A, Japan) equipped with UV–VIS detector (SPD 6AV) and C_{18} reverse-phase column (250 mm × 4.6 mm, 5 μ ODS, Hypersil, UK). The detection wavelengths used were 254 nm (for 2-NP and 2-AP) and 280 nm (for 4-NP, 4-AP, 2,4-DNP and 2-A,4-NP). Mobile phase was 1:1 de-ionized water and HPLC grade methanol at a flow rate of 1 ml/min (Uberoi and Bhattacharya, 1997).

VFAs were analyzed by injecting 2 μl of filtered and acidified samples to GC equipped with a flame ionization detector (FID). The analysis was done at an oven temperature of 150°C, injector temperature of 180°C and detector temperature of 250°C, using a 10% free fatty acid phase (FFAP) on 60/80 Chromosorb WHP/ 0.1% H_3PO_4 SS column. The carrier gas was nitrogen applied at a flow rate of 30 ml/min. Hydrogen and air mixture was used to fuel the flame.

Methane and nitrogen percentage in the biogas was analyzed by injecting 1 ml headspace sample to gas chromatograph (GC) equipped with thermal conductivity detector (TCD). The analysis was done at an oven temperature of 40°C, injector temperature of 100°C and detector temperature of 180°C, using SS molecular sieve M16, $3 \times 1/8$ in.^{2} column. The carrier gas was helium applied at a flow rate of 30 ml/min. TCD filament current was kept around 300 mV.

Microscopic examination of the granular sludge was done using a scanning electron microscope (SEM). The granular sludge samples were washed with 0.1 M phosphate buffer three times and fixed in phosphate buffered glutaraldehyde (6%) overnight. Then fixed samples were washed with 0.2 M sodiumcacodylate buffer five times. Subsequently samples were dehydrated in graded series of acetone–distilled water solution including 30%, 50%, 70%, 90%, 95% and 100% acetone, followed by critical point drying as mentioned by Prakash and Gupta (2000). The dried samples were sputter-coated with gold–palladium and viewed under a Cameca SEM, model SU-30.

3. Results and discussion

3.1. Acclimation

Four bench-scale UASB reactors were operated to investigate their ability to degrade nitrophenols under denitrifying conditions. Initially, the influent COD was about 1000 mg/l with essential nutrients and trace elements. After 45 days of operation, influent COD was increased to 1500 mg/l and further increased to 2000 mg/l after next 40 days. HRT was kept constant at 24 h throughout this period. On the third day of startup nitrophenols were introduced at 2 mg/l concentration and increased periodically to 5, 10, 20 and 30 mg/l when more than 75% removal of nitrophenols have been achieved. After every change in influent nitrophenol concentration reactors stability (biogas production and COD removal efficiency) got disturbed. Similar observations have been reported by Haghighi-Podeh et al. (1995). Figs. 1–3 show the performance of nitrophenols-fed reactors in comparison with control reactor (R1) during acclimation.

After 45 days the granulation was found to be about 50% in all the four reactors. However, 75% granulation was achieved in the reactors after 80 days of continuous operation. Visually granules were black in color and size was varying between 0.1 and 2 mm.

3.2. HRT study

After acclimation of the granular sludge the HRT study was carried out to see its effect on the degradation of nitrophenols. The performance of the reactors R1, R2, R3 and R4 at different HRT is given in Tables 2–5, respectively. Whenever the reactors were switched over to a new HRT, their performance became unstable for 2–8 days and gradually stabilized. The reactors were operated at steady state for 20–25 days at every HRT. 2-Nitrophenol and 4-nitrophenol removal was more than

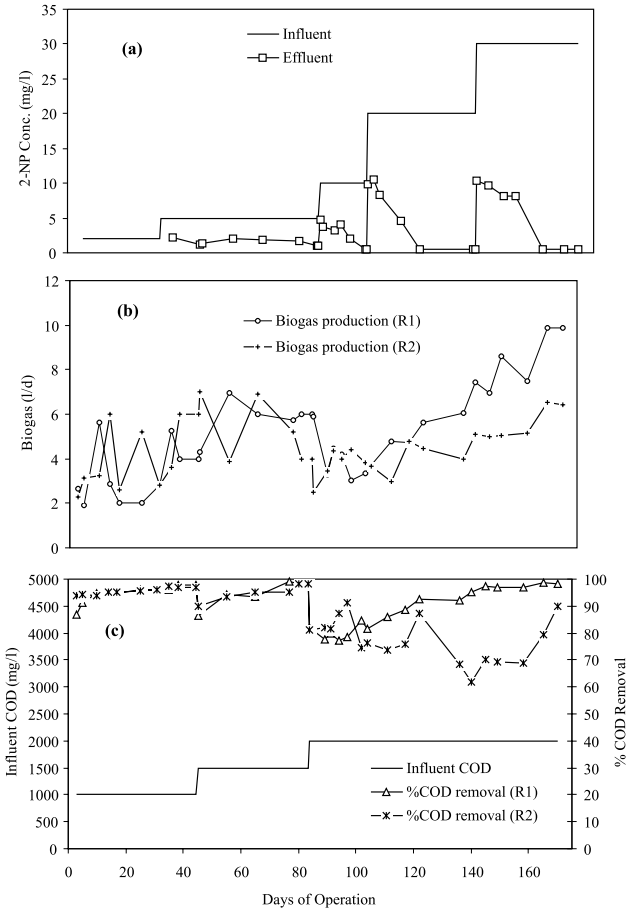

Fig. 1. Plot showing performance of reactor R2 and control R1 during acclimation: (a) 2-NP concentration; (b) biogas production; (c) % COD removal.

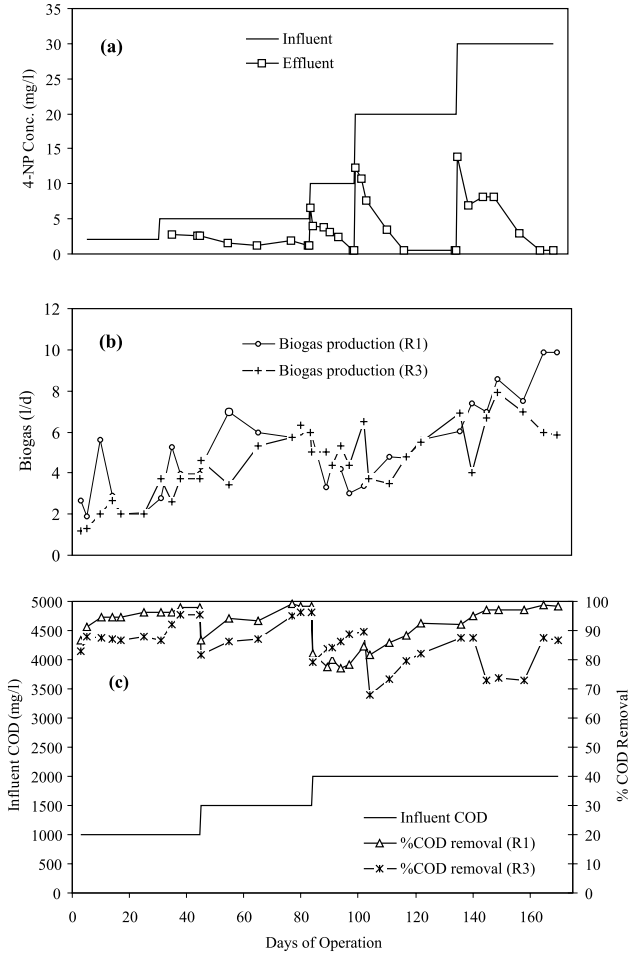

Fig. 2. Plot showing performance of reactor R3 and control R1 during acclimation: (a) 4-NP concentration; (b) biogas production; (c) % COD removal.

99% at all HRTs. However, 2,4-DNP removal was 96–89.7% at 30–12 h HRT. Nitrophenols readily got reduced to their respective amino derivatives. This reduction brought about detoxification of the parent compounds to less toxic aromatic amines. On molar basis about 41.2–48.4% 2-aminophenol (2-AP) recovery, 59.4–68% 4-aminophenol (4-AP) recovery and 30–26.6% 2-amino,4-nitrophenol (2-A,4-NP) recovery was observed at 30–12 h HRT, in the reactors R2, R3 and R4, respectively.

COD removal efficiency of the control reactor decreased from 98% to 89.1% with the lowering of HRT from 30 to 12 h. However, other reactors fed with nitrophenols showed significant decrease in COD removal efficiency and the methane content of the biogas with the lowering of HRT (Tables 2–5). This suggests methanogenic inhibition due to increase in nitrophenolic loading corresponding to shortening of HRT. Out of the three nitrophenols, 2,4-DNP caused the most severe inhibition. Nitrophenolic inhibition towards acetate using methanogens has been reported by many researchers (Haghighi-Podeh et al., 1995; Uberoi and Bhattacharya, 1997). Fig. 4 shows methane production rate versus nitrophenolic loading, which yields a linear relationship with regression coefficient (R^2) = 0.90, 0.95 and 0.96.

Nitrophenols did not cause any inhibition to the denitrification process throughout the study. Denitrification was almost cent percent in all of the four reactors, with non-detectable level of NO_3^-–N and NO_2^-–N in the effluent. The absence of nitrite suggests that the conversion of nitrate to nitrite was likely the rate-limiting step in denitrification. Similar observations were reported by Fang and Zhou (1999), and Tarre et al. (1994). Fig. 5 illustrates the relationship between the nitrogen gas production rate, at room temperature and atmospheric pressure per unit volume of the reactor, and NO_3^-–N reduction during HRT study. The slope of the linear regression lines (1.29, 1.28, 1.04 and 1.10 for R1, R2, R3 and R4, respectively) is designated as nitrogen gas production per unit nitrate nitrogen consumed. The amount of nitrogen contributed by nitrophenols was relatively insignificant as compared to the NO_3^-–N added to the feed in the form of sodium nitrate ($\leqslant$3%), hence was not considered in the calculation. No inhibi-

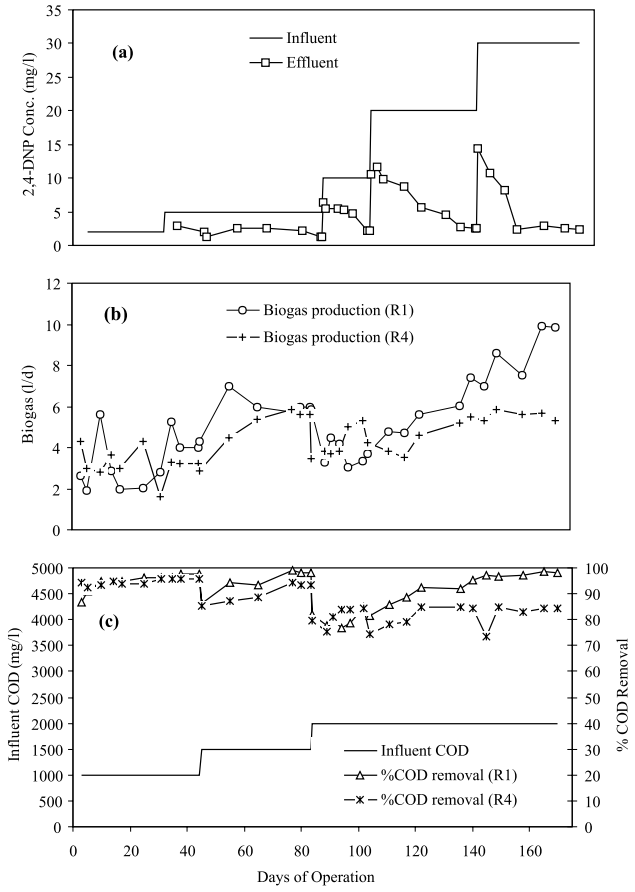

Fig. 3. Plots showing performance of reactor R4 and control R1 during acclimation: (a) 2,4-DNP concentration; (b) biogas production; (c) % COD removal.

tion due to nitrophenols was observed on denitrification and there was almost 100% denitrification in all the four reactors. Theoretically there should not be any difference in nitrogen gas production in the reactors. However, Fig. 5 shows some variation in nitrogen gas production rate among the reactors, which was probably caused by contamination by atmospheric nitrogen while sampling and analysis.

Denitrification resulted in the generation of alkalinity and high pH inside the reactors. Average alkalinity generation per g of NO_3^-–N denitrification was calculated as 5.5, which is on higher side of the theoretical value 3.57. Higher alkalinity generation can be attributed to CO_2 (by-product) conversion to bicarbonate due to high pH condition in the reactors (Chui et al., 1994). Effluent pH of the reactors ranged between 8.2 and 8.7 (Tables 2–5).

In the present study it was observed that lowering of HRT did not have significant effect on the nitrophenol removal, but at lower HRTs (18 and 12 h) high concentrations of VFA accumulated in the nitrophenols-fed reactors (Tables 3–5). Another very important observation at these two HRTs was the expansion of sludge bed in the reactors fed with nitrophenols (11–74% of the initial height). Frequent rising of the complete sludge bed was also observed at 12 h HRT. Rising of sludge cannot be attributed to high gas production as at these HRTs accumulation of VFA was observed and there was no enhancement in the biogas production. Under the present set of conditions 24 h was found to be the optimum HRT.

Table 2
Performance of R1 (blank reactor) at different HRT

HRT (h)	Sludge loading rate (kg COD/kg VSS d)	Organic loading rate (kg COD/m^3 d)	COD removal (%)	Eff. VFAs (mg/l)	Eff. pH	Biogas			
						l/d	CH_4 (%)	N_2 (%)	CO_2 (%)
30	0.136	1.60 ± 0.07	98	44	8.4	7.3	70.6	9.4	20
24	0.185	2.04 ± 0.04	97	52	8.6	7.9	68.6	9.4	22
18	0.254	3.10 ± 0.07	93	141	8.7	9.9	64.8	17.2	18
12	0.318	4.02 ± 0.17	89	203	8.6	11.6	61.5	20.5	18

Table 3
Performance of R2 at different HRTs

HRT (h)	Sludge loading rate (kg COD/kg VSS d)	Organic loading rate (kg COD/m^3 d)	COD removal (%)	2-NP (mg/l)		Eff. 2-AP (mg/l)	Eff. VFAs (mg/l)	Eff. pH	Biogas			
				Infl.	Effl.				l/d	CH_4 (%)	N_2 (%)	CO_2 (%)
30	0.13	1.6 ± 0.07	97	30.6	<0.5	10.1	47	8.3	6.0	69	8.5	22.5
24	0.168	2.0 ± 0.02	89	30.0	<0.5	10.2	228	8.5	6.4	64	11.3	24.7
18	0.247	3.1 ± 0.08	77	30.7	<0.5	10.8	442	8.8	6.5	59	21	20
12	0.332	4.2 ± 0.18	56	31.0	<0.5	11.9	1049	8.5	6.2	47.6	34.4	17

Infl. – Influent, Effl. – Effluent.

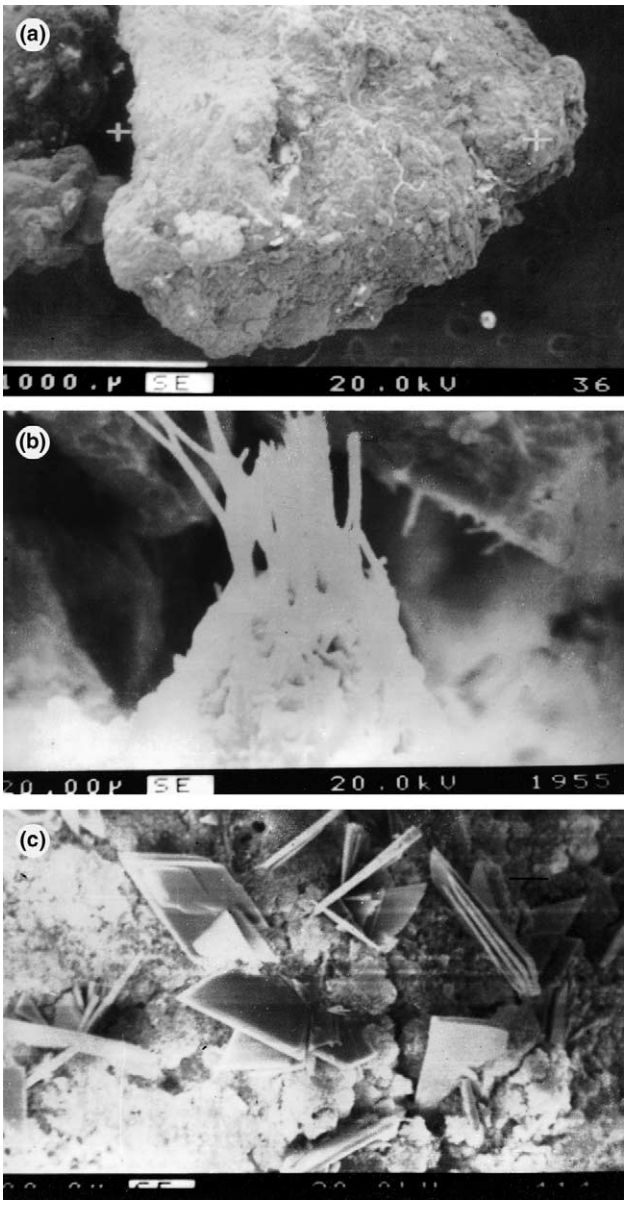

Fig. 6. SEM photographs of granular sludge: (a) cavity on the surface of a typical granule; (b) filamentous bacteria; (c) precipitated crystals inside the granular biomass.

have also reported low VSS/SS ratio (0.27) due to precipitation of calcium salt. Since the pH inside the denitrifying biofilm remains higher than that of the bulk liquid (Liehr, 1995), salts precipitation is more likely to take place inside the granules.

Biomass yield was calculated as net sludge production by considering biomass wasted, biomass lost in the effluent and increase in biomass inside reactor, for a particular time period per unit weight of COD removed. Average biomass yield was observed to be 0.15 g VSS/g COD. This is similar to the observed cell yield of 0.16 g VSS/g COD for treating synthetic hydrolyses of RDX (explosive) using biological denitrification (Zoh et al., 1999).

Granules were viewed under SEM. SEM pictures showed that the overall surfaces of the granules were uneven. Large cavities were present on the surface (Fig. 6). A heterogeneous bacterial population was observed on the surface. Both filamentous and cocci-shaped microbes were found. Inside the granules cocci-shaped microbes were predominant. SEM pictures also showed the presence of some inorganic crystals inside the granules. Average size of the granule was found to be about 2.1 mm for R1, R2 and R3 and about 1.9 mm for R4. The size of the granules was comparable to other type of granules developed, where average diameter was found to be ranging from 0.92 to 2.1 mm (Visser et al., 1993).

4. Conclusions

Anaerobic granular sludge was able to detoxify and degrade nitrophenols under nitrate reducing conditions. Based on the present study the following broad conclusions can be drawn:

(i) 2-Nitrophenol, 4-nitrophenol and 2,4-dinitrophenol can be detoxified and degraded in a UASB reactor using acetate and nitrate as electron donor and electron acceptor, respectively. 2-Aminophenol, 4-aminophenol and 2-amino,4-nitrophenol were found as the main intermediate metabolites in case of 2-NP, 4-NP and 2,4-DNP, respectively.

(ii) All the three nitrophenols were toxic to methanogens. VFA accumulated in the nitrophenols-fed reactors at lower HRT, this suggests increasing methanogenic inhibition with the lowering of HRT.

(iii) Nitrophenols did not cause inhibition to denitrifiers as complete denitrification was observed throughout the study with non-detectable level of nitrite nitrogen concentration in the effluents.

(iv) The characteristics of granules developed inside the reactors were comparable to the other type of granules reported in the literature. Calcium precipitation inside the granules resulted in better settling, as sludge wash out was never observed. SEM pictures of the granular biomass showed that the granules consisted of both filamentous and cocci-shaped microbes, but cocci-shaped microbes were dominating.

References

APHA, 1985 & 1989. Standard methods for the examination of water and wastewater, sixteenth and seventeenth ed. American Public Health Association, Washington, DC.

Arvin, E., Kristensen, G.H., 1982. Effect of denitrification on the pH in biofilms. Water Sci. Technol. 14 (8), 833–848.

Boopathy, R., Wilson, M., Kulph, C.F., 1993. Anaerobic removal of 2,4,6-trinitrotoluene (TNT) under different electron accepting conditions: laboratory study. Water Environ. Res. 65 (3), 271.

Chui, H.K., Fang, H.H.P., Li, Y.Y., 1994. Removal of formate from wastewater by anaerobic process. J. Environ. Eng., ASCE 120 (5), 1308–1320.

Donlon, B.A., Razo-Flares, E., Lettinga, G., Field, J.A., 1996. Continuous detoxification, transformation, and degradation of nitrophenols in upflow anaerobic sludge blanket (UASB) reactors. Biotechnol. Bioeng. 51, 439–449.

Duff, S.J.B., Kennedy, K.J., Brady, A.J., 1995. Treatment of dilute phenol/PCP wastewaters using the upflow anaerobic sludge blanket (UASB) reactor. Water Res. 29 (2), 645–651.

Environmental Protection Agency, 1980. Ambient water quality for nitrophenols. EPA 440/5 80-063.

Fang, H.H.P., Chui, H.K., 1993. Maximum COD loading capacity in UASB reactor at 37°C. J. Environ. Eng., ASCE 119 (1), 103–119.

Fang, H.H.P., Zhou, G., 1999. Interactions of methanogens and denitrifiers in degradation of phenols. J. Environ. Eng., ASCE 125 (1), 57–63.

Ghangrekar, M.M., 1997. Studies on granulation, start-up and performance of upflow anaerobic sludge blanket reactor. Ph.D. Thesis, CESE, Indian Institute of Technology, Bombay, Mumbai, India.

Gorontzy, T., Kuver, J., Boterogel, K.H., 1993. Microbial Transformations of nitroaromatic compounds under anaerobic conditions. J. General Microbiol. 139, 131.

Haghighi-Podeh, M.R., Bhattacharya, S.K., Mingbo, Q., 1995. Effects of nitrophenols on acetate utilizing methanogenic systems. Water Res. 29 (2), 391.

Hendriksen, H.V., Ahring, B.K., 1996. Integrated removal of nitrate and carbon in an upflow anaerobic sludge blanket (UASB) reactor: operation performance. Water Res. 30 (6), 1451–1458.

Krumme, M.L., Boyd, S.A., 1988. Reductive degradation of chlorinated phenols in anaerobic upflow bioreactors. Water Res. 22, 171–177.

Liehr, S.K., 1995. Effect of pH on metals precipitation in denitrifying biofilms. Water Sci. Technol. 32 (8), 179–183.

Michael, A.H., Valerie, C., Thomas, J.R., William, J.A., 1990. Biodegradation of P-nitrophenol in an aqueous waste stream by immobilized bacteria. Appl. Environ. Microbiol. 56 (10), 2967.

Prakash, S.M., Gupta, S.K., 2000. Biodegradation of tetrachloroethylene in upflow anaerobic sludge blanket reactor. Bioresour. Technol. 72, 47–54.

Shelley, M.D., Autenrieth, R.L., Wild, J.R., Dale, B.E., 1996. Thermodynamics analysis of trinitrotoluene biodegradation and mineralization pathways. Biotechnol. Bioeng. 50, 198–205.

Tarre, S., Armon, R., Shelef, G., Green, M., 1994. Effects of water characteristics on granular sludge formation in a USB reactor for denitrification of drinking water. Water Sci. Technol. 30 (9), 141–147.

Thomas, F.H., Silverstein, J.A., Schonidt, S.K., 1993. Effect of secondary substrate on the degradation of nitrophenols. Water Environ. Res. 65 (1), 73.

Tseng, S.K., Lin, M.R., 1994. Treatment of organic wastewater by anaerobic biological fluidized bed reactor. Water Sci. Technol. 29 (12), 157.

Uberoi, V., Bhattacharya, S.K., 1997. Toxicity and degradability of nitrophenols in anaerobic systems. Water Environ. Res. 69 (2), 146.

Visser, A., Alphenaar, P.A., Gao, Y., Rossum, G. van, Lettinga, G., 1993. Granulation and immobilization of methanogenic and sulfate reducing bacteria in high rate anaerobic reactors. Appl. Microbiol. Biotechnol. 40, 575–581.

Zoh, K.D., Daniels, J.I., Knezovich, J.P., Stenstrom, M.K., 1999. Treatment of hydrolysats of the high explosives hexahydro-1,3,5-trinitro-triazin and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine using biological denitrification. Water Environ. Res. 71 (2), 148–155.