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Effects of alternative carbon sources on biological transformation of nitrophenols

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Abstract

The removal of nitrophenols under denitrifying conditions was studied in bench-scale upflow anaerobic sludge blanket (UASB) reactors (R1, R2, R3 and R4) using three different carbon sources. Initially acetate was used as carbon source (substrate) in all the four reactors followed by glucose and methanol. Reactor R1 was kept as control and R2, R3, R4 were fed with 30 mg/l concentration of 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), and 2,4-dinitrophenol (2,4-DNP), respectively. Throughout the study the hydraulic retention time (HRT) and COD/NO_3^-–N ratio were kept as 24 h and 10, respectively. 2-Aminophenol (2-AP), 4-aminophenol (4-AP) and 2-amino,4-nitrophenol (2-A,4-NP) were found as the major intermediate metabolites of 2-NP, 4-NP and 2,4-DNP degradation, respectively. Methanol was found to be a better carbon source for 4-NP and 2,4-DNP degradation as compared to acetate and glucose, while 2-NP degradation was not influenced much by the change of substrate. Nitrate nitrogen removal was always more than 99%. COD removal efficiency of the nitrophenol fed reactors varied from 85.7% to 97.7%. The oxidation-reduction potential (ORP) inside the reactors dropped, up to –300 mv, with glucose as carbon source. As the reactors were switched over to methanol, ORP increased to –190 mv. The granular sludge developed inside the reactors was light brown in colour when acetate and glucose were used as substrate, which turned dark brown to black at the end of methanol run. Biomass yield in terms of volatile suspended solids was observed as 0.15, 0.089 and 0.14 g per gram of COD removal for acetate, glucose and methanol, respectively.

Introduction

Nitrophenols are among the most widely used industrial organic compounds. They are frequently used as an intermediate in the production of explosives, pharmaceuticals, pesticides, pigments, dye, wood preservatives and rubber chemicals (Uberoi & Bhattacharya, 1997). They also arise from microbial degradation of pesticides and photochemical reaction in the atmosphere.

Among mononitrophenols, 4-NP is probably the most important in terms of the quantities used and potential environmental contamination. The annual production of 4-NP alone is 20 million kg (Donlon et al. 1996). Of the six possible isomers of dinitrophenols, 2,4-DNP is commercially the most important. Nitrophenols are highly toxic to man and mammals being easily reduced by enzymes to nitroso and hydroxylamine derivatives. These derivatives may lead to the formation of either methamoglobin, which is unable to bind oxygen, or of nitrosoamines, which are carcinogenic (Kriek 1979). The toxicity of nitrophenols has been attributed to the fact that they cause uncoupling of oxidative phosphorylation (Donlon et al. 1995). Terada (1981) reported uncoupling of oxidative phosphorylation by 2.4-DNP at a concentration of 9.2 mg/l (50 μM). 2-NP, 4-NP and 2,4-DNP are listed on the US Environmental Protection Agency's (USEPA's) 'Priority Pollutants List' and recommends restricting their concentrations in natural waters to below 10 ng/L (EPA 1980).

Although physical and chemical changes, volatilization, photo degradation etc., occur in nature and are the eventual fate of many organic pollutants, biodegradation is perhaps the ultimate degradation mechanism. Many workers have conducted studies to understand the biodegradation mechanism of nitrophenols (Hess et al. 1990, 1993; Gorontzy et al. 1993; Tseng & Lin 1994; Donlon et al. 1996). Nitroaromatic compounds are difficult for oxygenolytic attack (Shelley et al. 1996). However, under anaerobic conditions these compounds readily get transformed to aromatic amines. Aromatic amines are on the average 500-folds less toxic than their corresponding nitroaromatic analogous (Donlon et al. 1995). This suggests that anaerobic treatment processes will at least detoxify nitroaromatic compounds if not completely mineralize them. Boopathy et al. (1993) have also reported better removal of nitroaromatic compounds under denitrifying conditions.

Gupta and Karim (2000) reported the effect of denitrifying conditions on the degradation of nitrophenols in UASB reactor. Nitrophenols (2-NP, 4-NP and 2,4-DNP) degradation was assessed at different COD/NO_3^-–N ratio. Percentage degradation of the total input 2-NP, 4-NP and 2,4-DNP concentration increased from 57.2 to 72.7, 31.24 to 89.42, and 54 to 83.3, respectively, as the COD/NO_3^-–N ratio was decreased from 20.8 to 10.

The presence of supplemental carbon source has been found to enhance the degradation of nitrophenols. Schmidth et al. (1987) studied the effect of glucose on the kinetics of 4-NP degradation by a pure culture of *Pseudomonas* sp. Their results suggested that glucose enhanced the specific rate of 4-NP degradation. Hess et al. (1990, 1993) found that glucose enhanced the removal rate of 2,4-DNP, provided glucose was not added to levels exceeding 1000 mg/l. The enhancement was attributed to higher levels of 2,4-DNP degrading bacteria produced via growth on glucose and retained in the reactor. Donlon et al. (1996) studied the effect of different primary substrates on the specific nitrophenol reducing activity of the methanogenic granular sludge. They found that acetate and methanol did not stimulate nitrophenol reduction. Other researchers have also reported poor removal of nitrophenols by acetate using methanogens (Haghighi-Podeh et al. 1995; Uberoi & Bhattacharya 1997).

The present study was intended to reveal the effect of acetate, glucose and methanol on biological removal of nitrophenols under the nitrate reducing conditions.

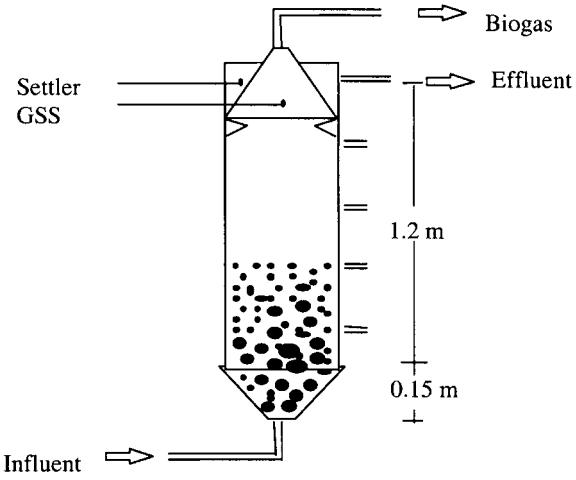

Figure 1. Experimental set-up.

Materials and methods

Experimental set-up

Continuous experiments were conducted in four identical bench-scale UASB reactors (R1, R2, R3 and R4) having 12.5 L volume (Figure 1). Inner dimensions of the reactors were 10 × 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 (GLSS). The reactors were made of transparent acrylic plastic sheet of 6.0 mm thickness and maintained at room temperature (25 ± 2 °C).

Experimental design

Reactors were inoculated with 5 L anaerobic granular sludge (VSS = 25 g/l, granule size = 0.25–4 mm), collected from bench-scale UASB reactors treating chlorinated aliphatic compounds, and acclimated with nitrophenols. During acclimation phase, sodium acetate was used as electron donor (substrate) and sodium nitrate was used as electron acceptor in all of the four reactors. COD/NO_3^-–N ratio was kept constant as 20. Reactor R1 was kept as control whereas reactors R2, R3 and R4 were started with 2 mg/l of 2-NP, 4-NP and 2,4-DNP, respectively, along with substrate and nutrients. Subsequently nitrophenol concentration was increased in steps to 5, 10, 20 and 30 mg/l. Initially influent COD was kept as 1000 mg/l and after 45 days

of acclimation influent COD was increased to 2000 mg/l. At each increment the reactors were acclimated for 30 to 53 days to achieve nitrophenols removal more than 75%. After 45 days of acclimation, the dry weight fraction of granular sludge inside the reactors having settling velocity more than 15 m/h were about 45% (W/W) of the total sludge, which increased to about 75% after 80 days of continuous operation. The weight fraction was determined by allowing granular sludge samples to settle through a 75 cm long and 7.5 cm diameter glass column (filled with tap water) for 3 min. The settled fractions were collected and dried at 105 °C to calculate dry weight. It took about 170 days to acclimate granular sludge with 30 mg/l of nitrophenols and a COD of 2000 mg/l at 24 h HRT. Detail about the reactors performance during acclimation phase has been given elsewhere (Karim & Gupta 2001).

In the present study three different carbon sources (sodium acetate, methanol and glucose) were used to study their effect on the biological removal of nitrophenols under denitrifying conditions. Sodium nitrate ($NaNO_3$) was used as the NO_3^-–N source. All the four reactors (R1, R2, R3 and R4) were fed with synthetic feed having 2000 mg/l COD, 200 mg/l NO_3^-–N, 120 mg/l NH_4Cl, 200 mg/l KH_2PO_4, 49.8 mg/l K_2HPO_4, 267 mg/l $CaCl_2$ $2H_2O$, 250 mg/l $MgCl_2$, and 300 mg/l KCl. The trace metals were added to the synthetic feed as reported by Prakash & Gupta (2000). Along with the above mentioned carbon, nitrogen and nutrient sources, reactor R2 was fed with 30 mg/l of 2-NP, reactor R3 was fed with 30 mg/l of 4-NP, reactor R4 was fed with 30 mg/l of 2,4-DNP, were as reactor R1 was kept as control. Throughout the study HRT were kept constant as 24 h and COD/NO_3^-–N ratio was kept as 10, as found suitable in another study to achieve complete denitrification (Gupta & Karim 2000). The study was initiated with sodium acetate as carbon source. After operating the reactors under steady-state conditions for about 20 days carbon source was changed to glucose. 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 reactors were operated with glucose under steady-state conditions for about 20 days before changing over to methanol as carbon source. After achieving steady-state conditions with methanol, reactors were operated under steady-state conditions for about 18–20 days.

Analytical methods

The influent and effluent samples were analyzed for pH, COD, respective nitrophenols and aminophenols, NO_3^-–N, NO_2^-–N and NH_4^+N. The analytical procedures for all tests were as outlined in the Standard Methods for the Examination of Water and Wastewater (APHA 1989), unless specified otherwise. Daily measurements were taken for the rate of gas production. Sludge samples from the reactors were analyzed for suspended solids (SS) and volatile suspended solids (VSS).

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 × 4.6 mm, 5 μ ODS, Hypersil, UK). The detection wavelength 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 50% de-ionized water and 50% HPLC grade methanol at a flow rate of 1 ml/min. Detection limit was 0.5 mg/l for each nitrophenol and aminophenol.

Volume of the biogas produced was measured using water displacement method (Jr. Safely & Westerman 1994). Methane and nitrogen percentage in the biogas was analyzed by injecting 1ml sample to gas chromatograph (Perkin Elmer, Sigma 2000) equipped with thermal conductivity detector. Proper care was taken to prevent contamination of air from outside while sampling and injecting into the gas chromatograph. Gas samples were collected using gas-tight syringe and septum containing sampling port in the gas collection bags. Septum was changed after every five injections. 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 × 1/8″ column (Netel Chromatograph, India). The carrier gas was helium applied at a flow rate of 30 ml/min. Thermal conductivity detector filament current was kept around 300 mv.

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. Temperature correction factor was applied for the measured temperature as per Standard Methods (APHA 1989).

Average settling velocity of the granular sludge was measured using a glass column of 7.5 cm diameter and 75 cm height. Diluted granular sludge sample (20

ml) was poured at the top of the glass column filled with tap water. Fractions of the sludge settled at the bottom of the glass column was collected at 0.5, 1.0, 1.5, 3.0, 7.5, 15, 30 and 60 min time intervals. Collected fraction of the granular sludge was filtered through GFC filter paper and dried at 105 °C. The average settling velocity was calculated using the approach adopted by Ghangrekar (1997).

For granules size distribution analysis, granular sludge samples were screened through a 0.18 mm sieve in order to eliminate small particles. Thereafter, 10 ml of the sieved sample was collected in a 7-cm diameter petri dish and scanned at 6X magnification. Photographs were developed and the size of the granules was physically measured (least count 1 mm). Then the actual size of the granules was calculated by dividing by the magnification factor.

Oxidation-reduction potential was monitored using an ORP meter (Model – 108, Orion, USA). At the end of the operation of the UASB reactor with acetate and methanol as carbon source, mineral contents of the granular sludge were analyzed. The granular sludge samples were dried in oven at 105 °C and digested in Microwave Labstation (mls 1200 mega, Milestone, Italy). Digested sludge samples were analyzed for different elements (including Ca, Mg, Na, etc.) using argon plasma in inductively coupled plasma atomic emission spectrophotometer (ICP-AES, 8440 M Plasma Lab, GBC, Australia). The wavelengths used in AES ranges from 160 nm to 800 nm. Plasma was generated from radio frequency of 27.12 MHz. The inductive heating maintained the plasma flame at a temperature of 6000 K and up to 10,000 K at its hottest point. The flow rate of the carrier gas (Argon) was kept as 0.8–1.2 l/min.

Results and discussion

Nitrophenols transformation

The average performance data of the four reactors (R1, R2, R3 and R4) with three different carbon sources (acetate, glucose and methanol) have been presented in Tables 1–4. Table 2 shows almost complete removal (>98%) of 2-NP with the three different carbon sources. 2-Aminophenol (2-AP) was found as the only intermediate metabolite by HPLC analysis. Average concentration of 2-AP in the effluent was observed as 6.5, 5.6 and 6.9 mg/l with acetate, glucose and methanol as the carbon source, respectively. The 2-AP concentration in the effluent accounted for about 30, 23.8 and 29.3% (on molar basis) of the total input 2-NP with acetate, glucose and methanol as the carbon source, respectively. Table 3 shows the average performance data of the reactor fed with 4-NP (R3). 4-Aminophenol (4-AP) was found as the only intermediate metabolite by HPLC analysis in this case. Concentration of 4-AP in the effluent was 8.9, 6.9 and 7.7 mg/l which accounted for 13.5, 18.9 and 5.8% (on molar basis) of the total input 4-NP with acetate, glucose and methanol as carbon source, respectively. Table 4 shows the average performance data of the reactor R4 (fed with 2,4-DNP). In this case 2-amino,4-nitrophenol (2-A,4-NP) was found as the only major intermediate metabolite. Almost completely removal (>98%) of 2,4-DNP was observed with all the three carbon sources. 2,4-DNP recovery in the form of 2-A,4-NP in the effluent was calculated as 16.3, 24 and 10.4% of the total input 2,4-DNP (on molar basis) with acetate, glucose and methanol, respectively.

To elucidate further, the data were subjected to analysis of variance (ANOVA). There was no significant difference for 2-NP degradation at the 1% level ($F = 1.70$, $df = 2, 12$) with the three carbon sources. 4-NP degradation was found to be about 86.5% with acetate, 81% with glucose and 94.2% with methanol. The results show that the degradation data of 4-NP varied significantly at the 1% level ($F = 19.7$, $df = 2, 12$). On molar basis about 16.3, 24 and 10.4% of input 2,4-DNP concentration was detected in the effluents as 2-A,4-NP with acetate, glucose and methanol, respectively. 2,4-DNP degradation data varied significantly at the 1% level ($F = 30$, $df = 2, 12$).

The results show almost complete removal (>98%) of the three nitrophenols with the three different carbon sources (acetate, glucose and methanol) and a part of the removed nitrophenols was observed in the effluents in the form of their respective aminophenols. The remaining part of the input nitrophenols was transformed and degraded further. It is important to note that in the present study methanol was observed as better substrate for degradation of 4-NP and 2,4-DNP as compared to acetate and glucose under denitrifying conditions. However, no nitrophenols reduction was observed (Tables 3–4) when acetate and methanol was used as primary substrates under methanogenic conditions (Donlon et al. 1996). This suggests that denitrifying conditions are more favourable for nitrophenols reduction than that of methanogenic conditions. A separate study was conducted by the authors to evaluate the role of biosorption in nitrophenols removal, reported else where (Karim & Gupta 2002;

Table 1. Performance of R1 (blank reactor) with three different carbon sources

Carbon source	Sludge loading rate (kgCOD/ kgVSS-d)	COD removal (%)	Effl. pH	Total alkalinity (as $CaCO_3$ mg/l)		Biogas (l/d)		
				Infl.	Effl.	Total	CH_4	N_2
Acetate	0.172	98.7	9.1	1200	2226	7.4	4.3	2.5
Glucose	0.187	97.7	6.8	400	1280	9.4	4.9	2.4
Methanol	0.179	97.8	7.3	390	1696	9.0	6.4	1.8

Infl. – influent; Effl. – effluent.

Table 2. Performance of R2 (2-NP fed reactor) with three different carbon sources

Carbon source	Sludge loading rate (kgCOD/ kgVSS-d)	COD removal (%)	2-NP (mg/l)		Effl. 2-AP mg/l)	Effl. pH	Total alkalinity (as $CaCO_3$ (mg/l)		Biogas (l/d)		
			Infl.	Effl.			Infl.	Effl.	Total	CH_4	N_2
Acetate	0.19	94.4	30.3	<0.5	6.5	9.0	1200	2283	4.8	2.6	1.9
Glucose	0.19	90.2	30.2	<0.5	5.6	6.8	400	1271	6.5	2.9	2.3
Methanol	0.20	96.1	30.7	<0.5	6.9	7.5	400	1640	5.8	3.5	1.7

Infl. – influent; Effl. – effluent.

Table 3. Performance of R3 (4-NP fed reactor) with three different carbon sources

Carbon source	Sludge loading rate (kgCOD/ kgVSS-d)	COD removal (%)	4-NP (mg/l)		Effl. 4-AP mg/l)	Effl. pH	Total alkalinity (as $CaCO_3$ (mg/l)		Biogas (l/d)		
			Infl.	Effl.			Infl.	Effl.	Total	CH_4	N_2
Acetate	0.19	95.3	30.2	<0.5	2.53	8.9	1200	2293	6.1	3.3	2.4
Glucose	0.19	95.9	30.4	<0.5	4.44	6.9	410	1311	8.3	4.2	2.2
Methanol	0.19	97.7	31.1	<0.5	1.34	7.7	388	1635	8.2	5.4	1.8

Infl. – influent; Effl. – effluent.

Table 4. Performance of R4 (2,4-DNP fed reactor) with three different carbon sources

Carbon source	Sludge loading rate (kgCOD/ kgVSS-d)	COD removal (%)	2,4-DNP (mg/l)		Effl. 2-A,4-NP mg/l)	Effl. pH	Total alkalinity (as $CaCO_3$ (mg/l)		Biogas (l/d)		
			Infl.	Effl.			Infl.	Effl.	Total	CH_4	N_2
Acetate	0.19	93	30	<0.5	4.3	8.9	1090	2326	5.4	2.6	2.4
Glucose	0.19	86	30	<0.5	6.2	6.7	395	1305	6.1	2.7	2.4
Methanol	0.18	89	31	<0.5	2.6	7.4	383	1605	5.9	3.5	1.9

Infl. – influent; Effl. – effluent.

Karim 2001). Sorption of 2-NP, 4-NP and 2,4-DNP on anaerobic granular sludge was found to be only 8.42 μg/g VSS, 10.20 μg/g VSS and 6.80 μg/g VSS, respectively. Since the UASB reactors were having a long sludge retention time (>40 days), the contribution of biosorption was found insignificant as compare to the total removal of nitrophenols.

Transformation pathway

Literature suggests that under methanogenic and nitrate reducing conditions nitroaromatic compounds initially get reduced to their respective amino derivatives (Donlon et al. 1996; Tseng & Lin 1994; Boopathy et al. 1993). Further metabolisation of aminophenols takes place via deamination to phenol (Tseng & Lin 1994), or by carboxylation dehydroxylation to 3-aminobenzoate (Bisaillon et al. 1993). In another study Spain et al. (1979) identified an enzyme 2-nitrophenol oxygenase from *Pseudomonas*, capable of removing nitrite group from 2-NP and 4-NP, and converting them to catechol.

In the present study HPLC analysis detected 2-AP, 4-AP and 2-A,4-NP as the only intermediate metabolite present in the effluents from 2-NP, 4-NP and 2,4-DNP fed reactors, respectively. Catechol and 3-aminobenzoate were never detected in the effluents. Therefore, further transformation and degradation of aminophenols probably took place via deamination as suggested by Tseng & Lin (1994). Stoichiometrically complete reduction of 30 mg/l of 2-NP, 4-NP and 2,4-DNP would result in 23.52, 23.52 and 25.10 mg/l of 2-AP, 4-AP and 2-A,4-NP respectively. However, maximum effluent concentration of 2-AP, 4-AP and 2-A,4-NP observed during this study were only 6.9, 4.4 and 6.2 mg/l, respectively (Tables 2–4). This shows further degradation of aminophenols under denitrifying conditions. Earlier reports in the literature suggest that 2-AP and 4-AP can be mineralized under methanogenic conditions (O'Connor & Young 1989; Donlon et al. 1996). However, accumulation of amino derivatives of 4-NP and 2,4-DNP was observed by Razo Flores et al. (1997) under methanogenic conditions.

In the present study relatively high levels (up to 30% of the input nitrophenol, on molar basis) of aminophenol was observed in the effluents. Increased retention time would have helped in further degradation of the remaining aminophenols.

COD and NO_3^-–N removal

COD removal and biogas generation data show that the COD removal and methane production in nitrophenol-fed reactors were less than that in the control reactor (Tables 1–4). This indicates inhibitory effect of nitrophenols on COD removal. Analysis of variance shows that there was a significant difference in COD removal at the 1% level ($F = 9.70$, $df = 2, 15$) with the three carbon sources in control reactor (R1). However the t-test result shows that least significant difference was 0.43, which depicts that there was no significant difference in the COD removal in case of glucose and methanol. COD removal efficiency varied significantly in the nitrophenol fed reactors, R2 ($F = 131.4$, $df = 2, 15$), R3 ($F = 34.95$, $df = 2,15$) and R4 ($F = 140.2$, $df = 2, 15$), at 1% level with the three carbon sources. Biogas generation varied significantly in the reactors, R1 ($F = 31.7$, $df = 2, 15$), R2 ($F = 20$, $df = 2, 15$) and R3 ($F = 67$, $df = 2,15$) except reactor R4 ($F = 4.07$, $df = 2, 15$), at 1% level with the three carbon sources.

Influent NO_3^-–N concentration in the present study was kept as 200 mg/l in order to keep the COD/NO_3^-–N ratio as 10, which was found suitable for complete denitrification (Gupta & Karim 2000). Simultaneous production of both methane and nitrogen gas was observed throughout the study. This indicates combined methanogenesis and denitrification inside the reactors. Effluent NO_3^-–N concentration was nil in all cases with non-detectable level of NO_2^-–N except with glucose as carbon source, when NO_2^-–N was detected occasionally. Oxidation-reduction potential (ORP) inside the reactors were –210 to –250 mv, –250 to –300 mv and –190 to –200 mv with acetate, glucose and methanol as carbon source. Decrease in the ORP inside the reactors with glucose as carbon source was believed to be the result of fermentative conditions. Fermentative conditions are known to favour facultative anaerobes, which reduce NO_3^-–N to NO_2^-–N but not further as they are not true denitrifiers (Manoharan et al. 1989; Carley & Mavinic 1991). This could be the reason why NO_2^-–N was detected occasionally with glucose as carbon source.

Influent pH was 7.0 – 7.2 and influent alkalinity was 1000, 400 and 380 mg/l (as $CaCO_3$), with acetate, glucose and methanol, respectively. Effluent pH and alkalinity have been shown in Tables 1–4. Denitrification resulted in high effluent pH and alkalinity. Effluent pH was observed as about 9 for all the four reactors, when acetate was used as carbon

source. However, effluent pH for reactors dropped considerably when glucose was used as carbon source. This was because of the development of fermentative conditions inside the reactors during glucose run as explained in the previous paragraph. Average alkalinity generated per g NO_3^-–N removal was calculated as 5.5, 4.24 and 5.7 during acetate, glucose and methanol run, respectively, which is on higher side of the theoretical value 3.57. Higher alkalinity generation could be attributed to methanogenesis (van Haandel & Lettinga 1994), simultaneously taking place in the reactors along with denitrification. The two pathways of methanogenesis have been shown as equations 1 and 2. About 72% of the methane formation comes from acetate cleavage (Equation 1) and remaining 28% results from reduction of carbon dioxide (Equation 2) (Fanning et al. 1983). The produced carbon dioxide (CO_2) gets dissolved in the bulk liquid inside the reactors and forms bicarbonate alkalinity.

$$CH_3COOH \rightarrow CH_4 + CO_2, \quad (1)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O. \quad (2)$$

Characteristics of the granular biomass

The granular sludge developed inside the reactors was light brown in colour when acetate and glucose were used as substrate, which turned dark brown to black at the end when methanol was used as substrate. Carley & Mavinic (1991) reported similar colour changes with the change of substrate. Biomass yield was calculated as net sludge production by considering biomass wasted, biomass lost in the effluent (as suspended solids) and increase in biomass inside reactor, for a particular time period per unit weight of COD removed. Biomass yield was observed to be 0.15 g VSS, 0.089 g VSS and 0.14 g VSS per gram of COD removed for acetate, glucose and methanol, respectively. The VSS fraction of the granular biomass was found to be 0.34–0.38, 0.35–0.46 and 0.48–0.60 when acetate, glucose and methanol were used as the carbon source, respectively. Low VSS fraction can be attributed to increase in alkalinity and pH allowing Ca^{2+} to precipitate in the sludge (Arvin & Kristensen 1982). In order to verify this, dried granular sludge samples were digested and analyzed for calcium content using argon plasma in ICP-AES. Calcium content in the granular sludge during acetate and methanol run was found to be 17.2–18% and 5.5–9.6% (w/w of SS), respectively. Fang & Chui (1993) have also reported low VSS/SS ratio (0.27) due to precipitation of calcium salt. Biomass had density of 1017–1049 kg/m^3, which is comparable with the observation (1064–1059 kg/m^3) reported by Hendriksen & Ahring (1996).

Conclusions

Almost complete removal (>98%) of nitrophenols (2-NP, 4-NP and 2,4-DNP) along with simultaneous denitrification and methanogenesis were achieved with three different carbon sources (acetate, glucose and methanol). 2-AP, 4-AP and 2-A,4-NP were found as major intermediate metabolite of 2-NP, 4-NP and 2,4-DNP, respectively. Methanol was found to be a better substrate for degradation of 4-NP and 2,4-DNP as compared to acetate and glucose, while 2-NP degradation was not much affected with the change of carbon source. COD removal efficiency of the nitrophenol fed reactors was found to be 85.7–97.7%. However, methane percentage in the biogas, generated with methanol substrate was much better (60.2–70.9%) compared to acetate and glucose substrate. With glucose as substrate, ORP inside the reactors dropped to –250 to –300 mv, which resulted in fermentative conditions. As the reactors were switched over to methanol ORP increased to –190 to –200 mv.

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