Journal of Hazardous Materials 173 (2010) 151–158

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Nitrate-dependent biodegradation of quinoline, isoquinoline, and 2-methylquinoline by acclimated activated sludge

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ARTICLE INFO

Article history:
Received 14 May 2009
Received in revised form 16 July 2009
Accepted 14 August 2009
Available online 22 August 2009

Keywords:
Quinolines
Denitrifying
COD/NO_3–N ratio
Activated sludge
Metabolites

ABSTRACT

The anaerobic degradation of quinoline, isoquinoline and 2-methylquinoline was investigated under nitrate-reducing conditions with acclimated activated sludge. Quinoline was completely transformed during degradation with an optimum COD/NO_3–N ratio of 7. Isoquinoline and 2-methylquinoline were also completely transformed; however, nitrate consumption was much lower with the optimum COD/NO_3–N ratios being in the ranges of 83–92 and 21–26, respectively. GC-MS analyses showed that during degradation, quinoline and isoquinoline were transformed by hydroxylation into 2(1H)-quinolinone and 1(2H)-isoquinolinone, respectively. While quinoline was completely mineralized, only 92% of isoquinoline was mineralized, and 1(2H)-isoquinolinone remained in the effluent. 2-Methylquinoline was transformed by hydrogenation to 1,2,3,4-tetrahydro-2-methyl-quinoline, and further degradation resulted in cleavage of the heterocyclic ring leaving 4-ethyl-benzenamine. Both the metabolites remained in the effluent, resulting in the low mineralization of 2-methylquinoline (58%). This is the first time that 2-methylquinoline is observed degradable under denitrifying conditions, and its metabolites are identified.

1. Introduction

Quinolinic compounds, including quinoline, isoquinoline, and methylquinoline, occur in many products such as coal tar, oil, creosote, pharmaceuticals, pesticides and dyes [1–3]. As many of these compounds are considered toxic, carcinogenic and mutagenic [4,5], it is essential to understand their biodegradability to assess their fate in the environment and in the wastewater treatment plants (WWTP). Most quinolinic compounds were considered recalcitrant under anaerobic conditions [6,7]. In recent years however, this view is changing as the anaerobic biodegradation of quinolines has been observed under nitrate-reducing, sulfate-reducing, and methanogenic conditions [8–13].

Most research on the biodegradation of quinolines has used pure cultures [6,7,12–17], but a few studies used soil microcosms to degrade quinolines [9,10,18]. Under aerobic conditions, biodegradation pathways for the microbial transformation of quinoline and isoquinoline involve hydroxylation, ring cleavage of the homocyclic and heterocyclic rings, and carboxylation [7,19]. Under anaerobic conditions, the degradation of quinolines involves the initial hydroxylation at position 2 [9–12,20,21]. Johansen et al. [12] proposed that the sulfate-reducing bacterium *Desulfobacterium indolicum*, after this initial hydroxylation, transforms quinoline to 3,4-dihydro-2(1H)-quinolinone, which is further transformed into unidentified products. 6- and 8-methylquinoline were converted to 6- and 8-methyl-3,4-dihydro-2(1H)-quinolinone by this microorganism, whereas 3- and 4-methyl-2(1H)-quinolinone were not degraded. Isoquinoline and 2-methylquinoline were not degraded by *D. indolicum* in their study. Reineke et al. [21] investigated the degradation of methylquinolines under nitrate-, sulfate- and iron-reducing conditions in microcosms with aquifer material of a former coke manufacturing site. The degradation activities were only revealed under sulfate-reducing conditions, and the hydroxylated methylquinolines, with the exception of 4-methyl-2(1H)-quinolinone, were recalcitrant. Little data is available on the metabolism of quinolinic compounds under denitrifying conditions. Johansen et al. [11] found that quinoline and 3-, 4-, 6-, 8-methylquinoline were transformed by hydroxylation into their 2-hydroxyquinoline analogues, and isoquinoline was transformed to 1-hydroxyisoquinoline. The hydroxylated metabolites of isoquinoline and quinolines methylated at the heterocylic ring were not transformed further, whereas metabolites of quinoline and quinolines methylated at the homocyclic ring were hydrogenated at position 3 and 4, and the resulting 3,4-dihydro-2-quinolinone analogues accumulated. They also found that 2-methylquinoline was not degraded at all.

Wastewaters from coal-coking and pharmaceutical industries usually contain relatively high concentrations of ammonia and

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doi:10.1016/j.jhazmat.2009.08.061

N-heterocyclic compounds [22,23]. In WWTPs, ammonia is usually removed by nitrification under aerobic conditions followed by denitrification. Therefore, during the biological treatment of these wastewaters, N-heterocyclic compounds will also be exposed to nitrate-reducing conditions. Previous studies by Li et al. [8,23] indicated that a nitrate containing anaerobic reactor is effective in eliminating most N-heterocyclic compounds, including quinolines. However, detailed information about the degradation pathways, rates, optimal conditions, etc. is limited.

As denitrification is a process for both organic compound degradation and nitrate reduction, and neither organics nor nitrate is desired to be released into the environment during wastewater treatment or in situ remediation, it is also of interest to investigate the optimum C/N ratio for both the degradation of quinolines and denitrification. The optimum C/N ratio in this paper is defined as the COD/NO_3–N ratio at which COD is completely degraded and nitrate or nitrite is completely reduced.

In the present study, batch experiments were conducted to investigate the degradation potential of quinoline, isoquinoline and 2-methylquinoline under nitrate-reducing conditions with the three quinolines as sole carbon sources, respectively. The optimum COD/NO_3–N ratios for quinoline degradation and denitrification were determined, and the degradation metabolites of the three quinolines were also analyzed. These studies have important engineering implications for the treatment of wastewater containing quinolinic compounds.

2. Materials and methods

2.1. Synthetic wastewaters

Quinoline, isoquinoline and 2-methylquinoline were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Synthetic wastewaters were prepared using tap water supplemented with minimal media containing KH_2PO_4 (0.6 g/L), $CaCl_2$ (0.1 g/L), and $MgSO_4$ (0.1 g/L). One of the three quinolines was spiked and sodium nitrate was supplemented at desired COD/NO_3–N ratio. The COD for quinoline, isoquinoline and 2-methylquinoline was theoretically estimated based on the following reactions:

$$C_9H_7N + 10O_2 \rightarrow 9CO_2 + 2H_2O + NH_3 \quad (1)$$

$$C_{10}H_9N + \tfrac{23}{2}O_2 \rightarrow 10CO_2 + 3H_2O + NH_3 \quad (2)$$

where 1.0 g of quinoline or isoquinoline is equivalent to 2.48 g of COD; 1.0 g of 2-methylquinoline is equivalent to 2.57 g of COD.

2.2. Acclimation of inocula

The seed sludge was obtained from a coal-coking wastewater treatment facility in Baoshan Steel Factory (Shanghai, China). This facility has been in operation for more than 10 years, and consists of an anoxic and aerobic treatment system. The seed sludge was obtained from the anoxic tank and to avoid impurities such as ammonia, nitrate and N-heterocyclic compounds, the seed sludge was washed using tap water and separated by static sedimentation three times in the laboratory. After decanting the supernatant, the mixed liquor suspended solid (MLSS) was 12 g/L. This was evenly divided into three cylindrical plexiglass sequencing batch reactors (SBR) and filled the top with synthetic wastewaters containing quinoline, isoquinoline and 2-methylquinoline, respectively. The working volume of each SBR was 8 L. The reactors were sealed with plexiglass lids. In each reactor, mixing was achieved by a motor-driven mixer with two four-blade propellers (at 1/3 and 2/3 of the reactor height). It was set to a speed of 60–70 rpm that was sufficient to completely mix the wastewater and activated sludge, but not to shear the flocs.

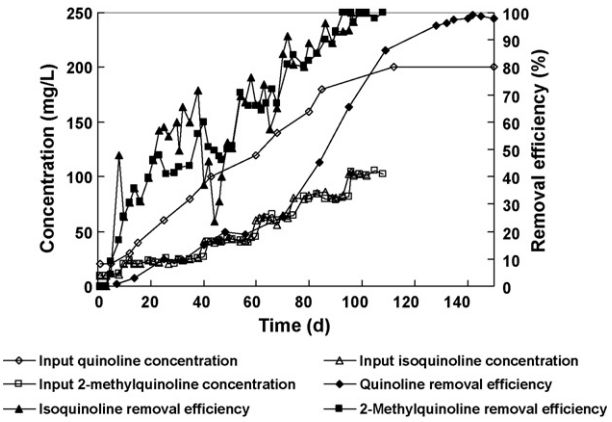

Fig. 1. Input concentrations and removal efficiencies of the three quinolines during activated sludge acclimation.

The reactors were incubated at a temperature-controlled room at around 25 °C.

When quinoline was used as the sole carbon source, the SBR was operated in a cycle time of 24 h, and each cycle consisted of 5 phases: fill (0.25 h), react (21 h), settle (2.0 h), draw (0.25 h) and idle (0.5 h). When isoquinoline and 2-methylquinoline were used as the sole carbon source, respectively, the SBR cycle time was 30 h with the fill, react, settle, draw and idle time of 0.25 h, 27 h, 2.0 h, 0.25 h and 0.5 h, respectively. Initially, the MLSS in each reactor was about 7000 mg/L, and concentrations of quinoline, isoquinoline and 2-methylquinoline were 20 mg/L, 10 mg/L, and 10 mg/L, respectively. When quinoline removals and denitrification rate were stable, quinoline, isoquinoline and 2-methylquinoline concentrations were gradually increased to 200 mg/L, 100 mg/L, and 100 mg/L, respectively (steps of 10–20 mg/L). Nitrate was amended to a COD/NO_3–N ratio of 5. Fig. 1 shows the concentrations and removal efficiencies for the three quinolines during acclimation. After 3–4 months, the removal efficiencies of all the three quinolines were greater than 95% and MLSS concentrations in the three reactors had reached steady state. The final MLSS concentrations in the reactors were 3250 mg/L, 3080 mg/L and 2940 mg/L when quinoline, isoquinoline and 2-methylquinoline were used as the sole carbon source, respectively.

2.3. Determination of the optimum COD/NO_3–N ratio

Optimum COD/NO_3–N ratios for the degradation of quinolines were investigated using 1-L Erlenmeyer flasks. Acclimated sludge was removed from the SBRs, and was washed using tap water to eliminate residual quinolines. After gravity separation and decanting the supernatant, the sludge was re-suspended in the minimal media. About 600 mL of the mixed liquor containing 1.8 g of MLSS was transferred to each flask. The number of the flasks depended on the COD/NO_3–N ratios tested. To determine the optimum COD/NO_3–N ratio for both quinoline degradation and denitrification, the flasks were spiked with quinoline concentration in the range of 40–150 mg/L, and nitrate was added for COD/NO_3–N ratios of 3, 5, 7, 8, and 10, respectively. When isoquinoline or 2-methylquinoline was used as the sole carbon source, as only small amounts of nitrate were consumed, a wider range of COD/NO_3–N ratio was tested. The flasks were capped with butyl rubber stoppers after spiking, and their headspace was purged with nitrogen for 20 min. Mixing was accomplished using a magnetic stir bar (~90 rpm).

2.4. Degradation of quinolines at optimum COD/NO_3–N ratios

The degradation of quinolines at the optimum COD/NO_3–N ratios was also investigated using 1-L Erlenmeyer flasks. The preparation of the sludge and the addition of the mixed liquor were similar to those in determining the optimum COD/NO_3–N ratios. Quinolines were spiked into the flasks at the initial concentration of ~100 mg/L. Nitrate was added to make the COD/NO_3–N ratios at the optimum values. Three replicates of each quinoline were performed.

2.5. Analyses

Quinoline concentrations were analyzed using a gas chromatograph (Aglient 6890N) coupled to a flame ionization detector. The column was a HP-5 (30 m × 0.32mm × 0.25 μm). Nitrogen was the carrier gas. The system was operated under constant flow conditions (63 mL/min). Injector and detector temperatures were kept at 250 °C and 280 °C, respectively. The column temperature control program was as follows: 100 °C for 1 min, ramp to 160 °C at 10 °C/min. The final temperature was held for 2 min. The samples were extracted with dichloromethane prior to injection. Ammonia and nitrite measurements were performed in accordance with the Standard Methods [24]. In the Standard Methods, measurement of UV absorption at 220 nm and 275 nm was made for the determination of nitrate [24]. However, because quinolines also have strong absorbance at 220 nm and 275 nm, Standard Method was abandoned. Instead, nitrate was measured by rapid spectrophotometric determination using phenol [25]. This method measures the absorbance at 388 nm and 302 nm, thus the interference of quinolines was avoided. Total organic carbon (TOC) was determined using a Shimadzu TOC-Vcpn analyzer.

Quinoline metabolites were analyzed by GC-MS. The samples were prepared as per Li et al. [23]. Samples were extracted with dichloromethane and divided into acidic, basic, and neutral fractions; then qualitatively analyzed by an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer. The gas chromatograph was equipped with a capillary column (HP-5MS, 30 m × 0.25 mm × 0.25 μm), and nitrogen was the carrier gas. The system was operated under constant flow conditions (63 mL/min) and the injector was operated in splitless mode at a temperature of 280 °C. The temperature control program was as follows: 80 °C for 3 min, ramp to 300 °C at 10 °C/min. The final temperature was held for 2 min. The electron impact (EI) conditions were: ionization energy, 70 eV; electron multiplier voltage, 1823.5 V; ion source temperature, 200 °C; mass range *m*/*z*, 35–500. The metabolites of 2-methylquinoline were also analyzed by a couple of Thermo Focus DSQ gas chromatograph and mass spectrometer. This gas chromatograph was equipped with a HP-5MS capillary column, and helium was the carrier gas. The system was operated under constant flow conditions (1 mL/min) and the injector temperature was 250 °C. The temperature control program was as follows: 60 °C for 2 min, ramp to 300 °C at 20 °C/min. The final temperature was held for 10 min. The electron impact conditions were: ionization energy, 70 eV; electron multiplier voltage, 1823.5 V; ion source temperature, 250 °C; mass range *m*/*z*, 41–450.

Table 1
Effect of COD/NO_3–N ratio on quinoline degradation efficiency and denitrification.

(a) Quinoline as the sole carbon source

Quinoline input concentration = 50 mg/L				Quinoline input concentration = 100 mg/L				Quinoline input concentration = 150 mg/L			
C/N[a]	Quinoline removal efficiency (%)	NO_3–N reduction efficiency (%)	NO_2–N[b] (mg/L)	C/N	Quinoline removal efficiency (%)	NO_3–N reduction efficiency (%)	NO_2–N (mg/L)	C/N	Quinoline removal efficiency (%)	NO_3–N reduction efficiency (%)	NO_2–N (mg/L)
3	92.80	96.70	19.98	3	95.85	96.56	35.34	3	97.17	99.08	56.32
5	91.52	96.94	9.62	5	95.81	98.63	12.01	5	97.45	99.73	22.65
7	91.88	97.32	0.21	7	95.67	99.30	0.84	7	97.29	99.79	0.52
8	78.40	100	0.11	8	80.28	100	0.56	8	86.85	100	0.26
10	60.56	100	n.d.[c]	10	69.66	100	0.09	10	75.89	99.44	n.d.

(b) Isoquinoline as the sole carbon source

Iso-Q[d] input concentration = 50 mg/L				Iso-Q input concentration = 80 mg/L				Iso-Q input concentration = 100 mg/L			
C/N	Iso-Q removal efficiency (%)	NO_3–N reduction efficiency (%)	NO_2–N (mg/L)	C/N	Iso-Q removal efficiency (%)	NO_3–N reduction efficiency (%)	NO_2–N (mg/L)	C/N	Iso-Q removal efficiency (%)	NO_3–N reduction efficiency (%)	NO_2–N (mg/L)
23	91.47	26.98	0.72	27	93.65	19.13	0.38	50	92.33	31.90	0.76
46	92.25	29.82	0.56	35	89.09	28.16	0.30	77	91.20	72.73	0.24
90	93.39	93.28	0.33	54	96.98	51.91	0.15	92	93.73	96.11	0.08
141	59.72	100	n.d.[c]	83	93.07	80.62	0.55	216	56.64	100	0.09

(c) 2-Methylquinoline as the sole carbon source

2-MQ[e] input concentration = 40 mg/L				2-MQ input concentration = 70 mg/L				2-MQ input concentration = 100 mg/L			
C/N	2-MQ removal efficiency (%)	NO_3–N reduction efficiency (%)	NO_2–N (mg/L)	C/N	2-MQ removal efficiency (%)	NO_3–N reduction efficiency (%)	NO_2–N (mg/L)	C/N	2-MQ removal efficiency (%)	NO_3–N reduction efficiency (%)	NO_2–N (mg/L)
14	89.47	80.38	0.39	14	92.75	69.42	1.12	14	98	49.87	1.25
21	92.47	97.42	1.24	25	95.78	96.88	0.89	25	95.81	94.23	0.48
26	92.41	96.09	0.31	34	75.82	100	n.d.[c]	48	56.44	100	n.d.

[a] C/N, COD/NO_3–N ratio.
[b] NO_2–N, remaining nitrite in the effluent.
[c] n.d., not detected.
[d] Iso-Q, isoquinoline.
[e] 2-MQ, 2-methylquinoline.

3. Results and discussion

3.1. The optimum COD/NO_3–N ratio for quinoline degradation and denitrification

Table 1(a) shows that the optimum COD/NO_3–N ratio is 7 for quinoline concentrations ranging from 50 mg/L to 150 mg/L. At this COD/NO_3–N ratio, quinoline removals were greater than 90%, and nitrate and nitrite were almost completely reduced. For COD/NO_3–N ratios greater than 7, there was not enough nitrate to completely degrade quinoline; for COD/NO_3–N ratio less than 7, nitrite remained in the effluent.

Unexpectedly, only small amounts of nitrate were consumed during isoquinoline degradation. For isoquinoline and COD/NO_3–N ratios between 10 and 50, there was nearly no change for isoquinoline removal efficiency (>90%); but as isoquinoline was almost completely transformed, only 8–30% of the nitrate was removed (data not shown). Therefore, a wider range of COD/NO_3–N ratios from 23 to 216 was tested (Table 1(b)). The optimum COD/NO_3–N ratio for the anoxic degradation of isoquinoline is between 83 and 92 for isoquinoline concentrations between 50 mg/L and 100 mg/L. In this range, isoquinoline was significantly degraded, while over 80% nitrate was reduced. However, when COD/NO_3–N ratios were much higher than 92 (nitrate concentrations were less than 1 mg/L), the degradation of isoquinoline was incomplete. This indicates that the presence of a certain amount of nitrate is necessary for the degradation of isoquinoline. Compared to isoquinoline, the nitrate consumption was slightly higher for the degradation of 2-methylquinoline. The optimum COD/NO_3–N ratio for both 2-methylquinoline degradation and denitrification was between 21 and 26 (Table 1(c)). The relatively high COD/NO_3–N ratios during denitrification with isoquinoline or 2-methylquinoline as the sole carbon source remain to be evaluated.

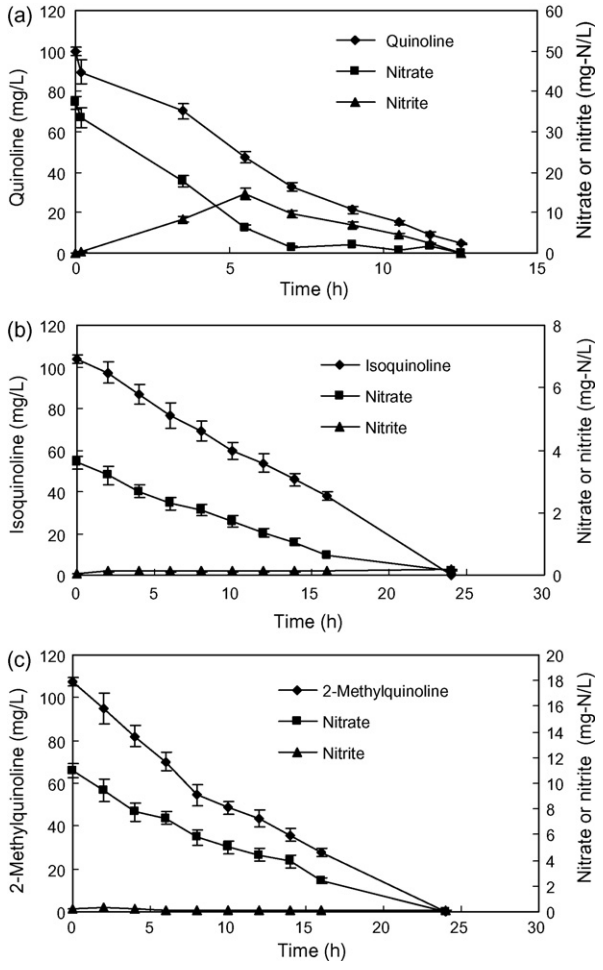

Fig. 2. Concentration profiles of quinolines, nitrate and nitrite at their optimum COD/NO_3–N ratios with the initial quinoline concentration of around 100 mg/L when (a) quinoline, (b) isoquinoline and (c) 2-methylquinoline was used as the sole carbon source, respectively.

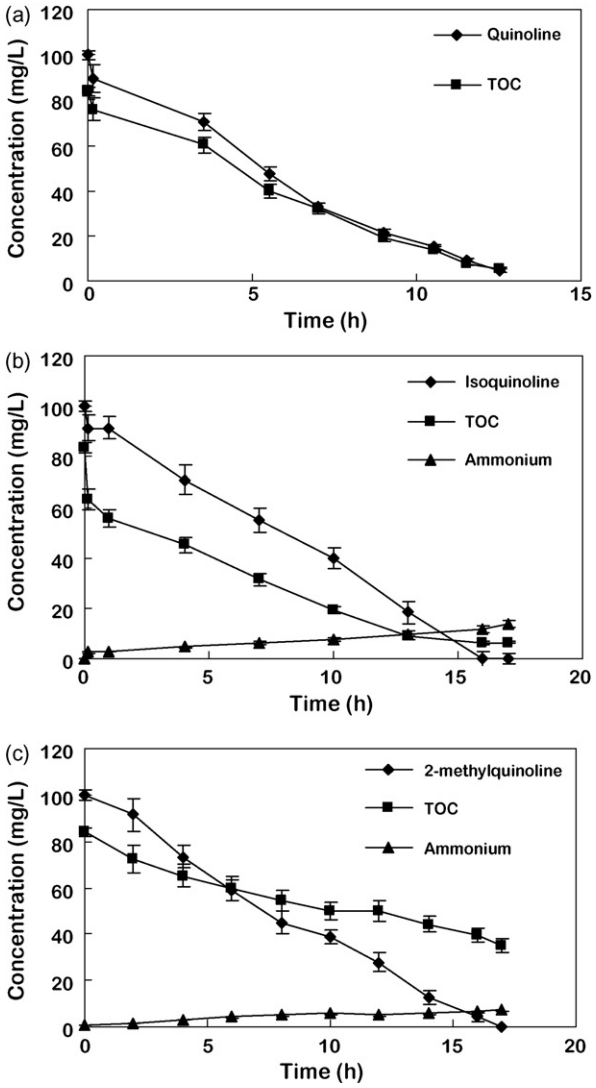

Fig. 3. Concentration profiles of quinolines, TOC and ammonia during the degradation experiments of quinoline (a), isoquinoline (b) and 2-methylquinoline (c). Ammonia was not measured during the degradation of quinoline.

3.2. Degradation of quinolines at optimum COD/NO_3–N ratios

Concentration profiles for quinoline, isoquinoline and 2-methylquinoline at their optimum COD/NO_3–N ratios are illustrated in Fig. 2, and nitrate and nitrite are also plotted. At quinoline concentrations of 100 mg/L, quinoline was almost completely degraded in 13 h (Fig. 2(a)). During degradation, nitrate is reduced, and when nitrate reduction almost ceased, nitrite reached its maximum concentration and subsequently decreased. However, the maximum nitrite concentrations were 52% of the input nitrate concentration. This indicates that despite competition between nitrate and nitrite reductases for electron donors

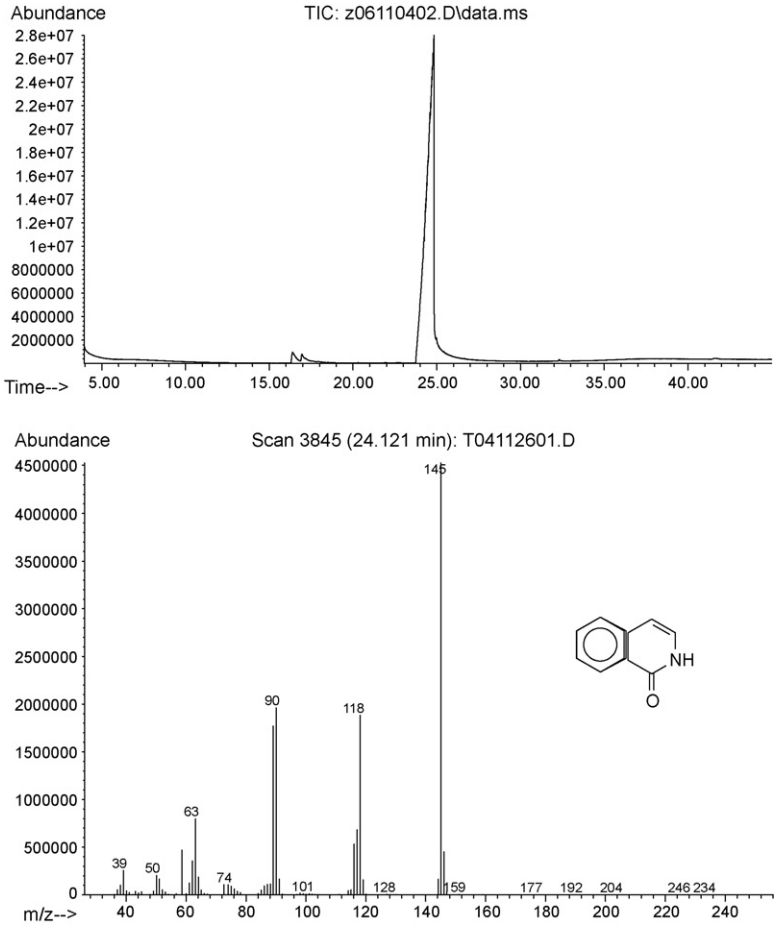

Fig. 4. Total ion chromatogram (TIC) of the sample taken after isoquinoline was almost completely attenuated (top) and the mass spectrum of the peak at retention time of 24.121 min (bottom).

[26,27], nitrite could still be partly reduced. Nitrate was depleted earlier than quinoline, and as quinoline degradation proceeded, nitrite was used as an electron acceptor until it was completely consumed.

At initial concentration of 104 mg/L, isoquinoline was transformed in 24 h (Fig. 2(b)); 2-methylquinoline was also transformed in 24 h for initial concentration of 107 mg/L (Fig. 2(c)). Nitrate was completely consumed at their optimum COD/NO_3–N ratios, while nitrite concentration remained at low (<0.5 mg/L) during denitrification. This is likely a result of the low initial nitrate concentrations (<4 mg/L and 11 mg/L for isoquinoline and 2-methylquinoline degradation, respectively) in the flasks. At the optimum COD/NO_3–N ratios, the specific degradation rates for quinoline, isoquinoline and 2-methylquinoline were 0.0023 g/g MLSS/h, 0.0014 g/g MLSS/h and 0.0015 g/g MLSS/h, respectively. This indicated that the degradation rates of isoquinoline and 2-methylquinoline were similar, and they were degraded much slower than quinoline.

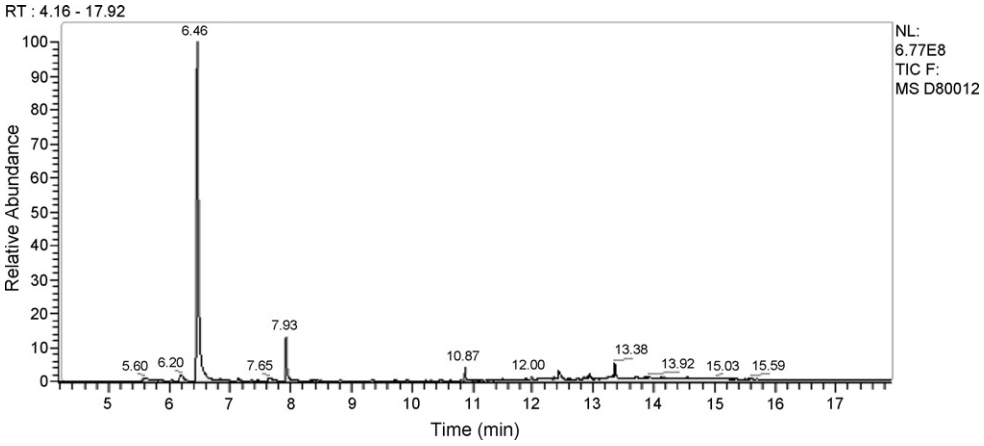

Fig. 5. Total ion chromatogram (TIC) of the sample taken after 2-methylquinoline was completely attenuated.

3.3. Mineralization and degradation metabolites of quinolines

Change of total organic carbon and the production of ammonia were used as indicators for the occurrence and extent of mineralization of quinolines. Fig. 3 shows TOC and ammonia profiles during the degradation of quinoline, isoquinoline, and 2-methylquinoline. Because COD/NO_3–N ratios have no effect on quinoline removal efficiencies at values less than the optimum ratios, the COD/NO_3–N ratios for quinoline, isoquinoline and 2-methylquinoline were set at 7, 8 and 8, respectively, to allow comparisons between each compound.

During the degradation of quinoline, TOC and quinoline profiles tracked each other closely until quinoline was almost completely transformed (Fig. 3(a)). This suggested that quinoline was completely degraded without the accumulation of metabolites. Based on this deduction, ammonia was not measured during the

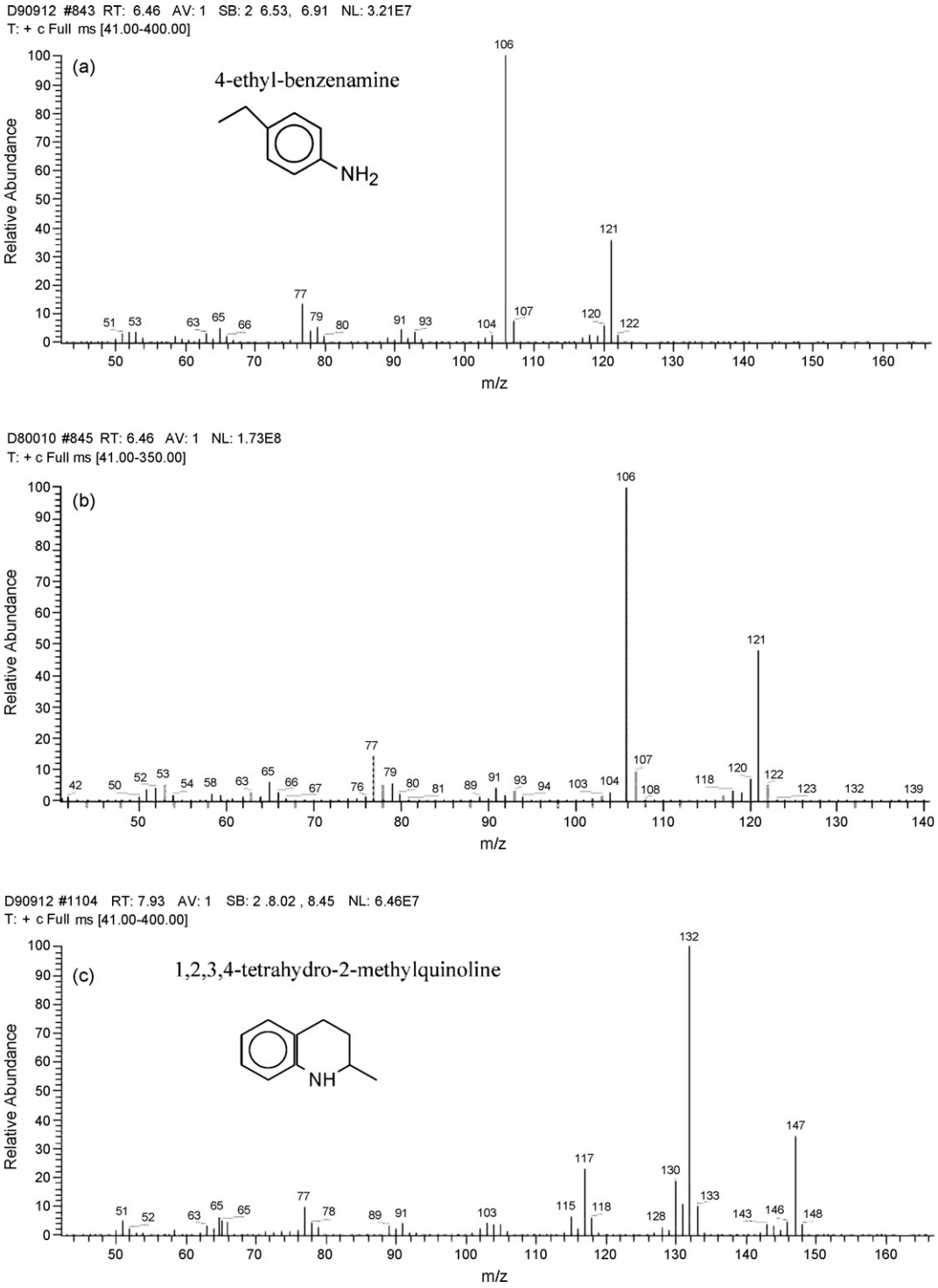

Fig. 6. Comparison between the mass spectrums of the authentic standard chemicals, 4-ethyl-benzenamine (a) and 1,2,3,4-tetrahydro-2-methylquinoline (c), and the mass spectrums of the metabolites produced during the transformation of 2-methylquinoline (b) and (d) (the peaks at retention time of 6.46 min and 7.93 min in the TIC of Fig. 5).

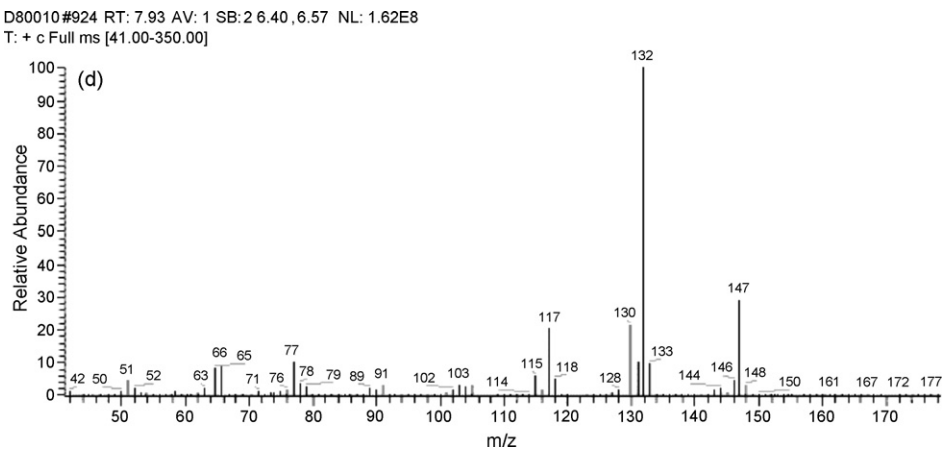

Fig. 6. (*Continued*)

degradation of quinoline. Johansen et al. [11] found that two quinoline metabolites, 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone, were more rapidly metabolized than quinoline in a biofilm system under denitrifying conditions. Further degradation products were not identified in their research. In our experiments, we also identified 2(1H)-quinolinone in the samples taken 2 h after spiking, but no degradation products were observed in samples taken near the end of the experiment (data not shown). This indicates that 2(1H)-quinolinone was being further transformed.

For isoquinoline microcosms, TOC values tracked isoquinoline values to low concentration, and reached a relatively high TOC removal efficiency (92%) (Fig. 3(b)). This indicated the significant mineralization of isoquinoline. The results were confirmed by ammonia production. Since 1 mol of isoquinoline mineralization is expected to produce 1 mol of ammonia, and no ammonia was added to the synthetic wastewaters, the amount of ammonia in the flasks can be used to indicate the degree of isoquinoline mineralization. When isoquinoline was almost completely attenuated, a total of 11.5 mg NH_4^+-N/L (0.82 mmol NH_4^+-N/L) was produced (Fig. 3(b)). This value is in accordance with the amount of ammonia released if 92% of the spiked isoquinoline is degraded (0.72 mmol NH_4^+-N/L). The slightly higher value of the measured ammonia concentration may result from the microorganism decay.

In Fig. 3(b) from 13 h onwards, while isoquinoline concentration continuously decreased, TOC values became stable at 6.3 mg/L, which suggested some recalcitrant metabolites were produced during isoquinoline degradation. GC-MS analyses of samples taken in the end of the experiment showed the presence of a metabolite which is at the retention time of 24.121 min in the total ion chromatogram (TIC) (Fig. 4). The mass spectrum of the metabolite was characterized by a M^+ peak at *m*/*z* 145, and other main peaks at *m*/*z* 118 (M-HCN), 90 (M-HCN, -CO) and 89 ($C_7H_5^+$). It was identified as 1(2H)-isoquinolinone by referring to the library of mass spectrometry fragment patterns provided by the National Institute of Standards and Technologies (NIST) and literature [11]. The residual 1(2H)-isoquinolinone could explain the residual measured TOC in Fig. 3 (b). Several researchers have reported that the first step in the microbial catabolism of isoquinoline under aerobic conditions is the hydroxylation at C-1 to 1(2H)-isoquinolinone [2,28]. Under anaerobic conditions, isoquinoline degradation was not as well studied, but Johansen et al. [11] identified that 1-hydroxyisoquinoline was formed and further transformed to 1(2H)-isoquinolinone under denitrifying conditions. This indicates that the initial step for the degradation of isoquinoline is the same under both aerobic and denitrifying conditions. The further transformation of 1(2H)-isoquinolinone was not observed by Johansen et al. [11]. In the present study, the remaining of 1(2H)-isoquinolinone in the effluent also suggested its recalcitrant potential.

TOC profiles in Fig. 3(c) show a different trend during the degradation of 2-methylquinoline. When 2-methylquinoline was almost completely transformed in 17 h, TOC concentrations were still high (35 mg/L) and the TOC removal efficiency was only 58%. This suggested the production of some recalcitrant metabolites during 2-methylquinoline degradation. Ammonia measurements confirm the incomplete mineralization of 2-methylquinoline. After 2-methylquinoline was completely attenuated, only 6.4 mg NH_4^+-N/L (0.46 mmol NH_4^+-N/L) was produced. This corresponds well with the ammonia production (0.41 mmol/L) calculated theoretically based on 58% 2-methylquinoline degradation. Like isoquinoline, the measured ammonia was a slightly higher than the theoretical mineralizing ammonia. Several researchers [9,11,12,21] have observed the complete inhibition of 2-methylquinoline degradation under anaerobic conditions, and postulate that it is a result of blockage at position 2 by the methyl group. The degradation of 2-methylquinoline in the present study might indicate a novel degradation pathway under denitrifying conditions.

TIC for the sample taken after 2-methylquinoline was completely attenuated is shown in Fig. 5. The peaks at retention time of 6.46 min and 7.93 min in TIC were identified as 4-ethyl-benzenamine and 1,2,3,4-tetrahydro-2-methyl-quinoline, respectively, by comparison against the NIST library; then authentic standard chemicals were analyzed under the same conditions to confirm the identification (Fig. 6). Johansen et al. [11] investigated the degradation pathway of methylquinolines using mixed culture under denitrifying conditions, and using *D. indolicum* (DSM 3383) under sulfate-reducing conditions [12], and found that after the initial hydroxylation at position 2, 6- and 8-methyl-2(1H)quinolinones were further hydrogenated at position 3 and position 4, and resulting in the accumulation of methylated 3,4-dihydro-2(1H)quinolinones, while the 3- and 4-methylquinoline were not further transformed. They also found that 2-methylquinoline was recalcitrant to hydroxylation at position 2 because of steric hindrance from the methyl group. In the present study, it appears that 2-methylquinoline can be hydrogenated at positions 1, 2, 3 and 4, and results in the production of 1,2,3,4-tetrahydro-2-methylquinoline. Cleavage of the heterocyclic ring should result in 4-ethyl-benzenamine. The presence of both products in the samples taken after 2-methylquinoline was completely transformed indicated their resistance to the biodegradation under denitrifying conditions.

4. Conclusions

Quinoline, isoquinoline and 2-methylquinoline were transformed under nitrate-reducing conditions. The optimum COD/NO_3–N ratio for both quinoline degradation and denitrification was 7, while for isoquinoline and 2-methylquinoline, the optimum COD/NO_3–N ratios were in the range of 83–92 and 21–26, respectively. Using quinoline (50–100 mg/L) as the sole carbon source, nitrite accumulation was transiently observed; no nitrite accumulated when isoquinoline or 2-methylquinoline were used as the sole carbon source. Quinoline and isoquinoline were transformed by hydroxylation into 2(1H)-quinolinone and 1(2H)-isoquinolinone, respectively. 2(1H)-quinolinone was further completely degraded, whereas 1(2H)-isoquinolinone remained in the effluent despite 92% of isoquinoline mineralization. When 2-methylquinoline was used as the sole carbon source, TOC removal efficiency of 58% suggests that 2-methylquinoline was incompletely mineralized, and GC-MS analyses of the effluent identified the remaining metabolites of 2-methylquinoline as 1,2,3,4-tetrahydro-2-methyl-quinoline and 4-ethyl-benzenamine. This is the first time that 2-methylquinoline is observed degradable under anaerobic conditions, and its metabolites are identified.

Acknowledgments

The authors wish to express all their gratitude to Dr. John Montgomery-Brown (Department of Civil and Environmental Engineering, Stanford University) for help with the English language. This work was funded by the Natural Science Foundation of China (Grant no. 50108009 and 50878165) and Program for New Century Excellent Talents in University (NCET-08-0403). This work was also partly supported by the State Key Laboratory of Pollution Control and Resource Reuse, China (Grant no. PCRRY08009 and PCRRCF07001).

References

[1] K.V. Padoley, S.N. Mudliar, R.A. Pandey, Heterocyclic nitrogenous pollutants in the environment and their treatment options—an overview, Bioresour. Technol. 99 (2008) 4029–4043.
[2] S. Fetzner, Bacterial degradation of pyridine, indole, quinoline, and their derivatives under different redox conditions, Appl. Microbiol. Biotechnol. 49 (1998) 237–250.
[3] M. Mundt, J. Hollender, Simultaneous determination of NSO-heterocycles, homocycles and their metabolites in groundwater of tar oil contaminated sites using LC with diode array UV and fluorescence detection, J. Chromatogr. A 1065 (2005) 211–218.
[4] J. Neuwoehner, A.-K. Reineke, J. Hollender, A. Eisentraeger, Ecotoxicity of quinoline and hydroxylated derivatives and their occurrence in groundwater of a tar-contaminated field site, Ecotoxicol. Environ. Safety 72 (2009) 819–827.
[5] A.S. Sideropoulos, S.M. Secht, Evaluation of microbial testing methods for the mutagenicity of quinoiline and its derivatives, Mutat. Res. 11 (1984) 59–66.
[6] J. Aislabie, A.K. Bej, H. Hurst, S. Rothenburger, R.M. Atlas, Microbial degradation of quinoline and methyquinolines, Appl. Environ. Microbiol. 56 (1990) 345–351.
[7] R. Miethling, V. Hecht, W.D. Deckwer, Microbial degradation of quinoline: kinetic studies with Comamonas scidovorans DSM 6426, Biotechnol. Bioeng. 42 (1993) 589–595.
[8] Y. Li, G. Gu, J. Zhao, H. Yu, Anoxic degradation of nitrogenous heterocyclic compounds by acclimated activated sludge, Process Biochem. 37 (2001) 81–86.
[9] S.M. Liu, W.J. Jones, J.E. Rogers, Biotransformation of quinoline and methylquinolines in anoxic freshwater sediment, Biodegradation 5 (1994) 113–120.
[10] D. Licht, B.K. Ahring, E. Arvin, Effects of electron acceptors, reducing agents, and toxic metabolites on anaerobic degradation of heterocyclic compounds, Biodegradation 7 (1996) 83–90.
[11] S.S. Johansen, E. Arvin, H. Mosbaek, A.B. Hansen, Degradation pathway of quinolines in a biofilm system under denitrifying conditions, Environ. Toxicol. Chem. 16 (1997) 1821–1828.
[12] S.S. Johansen, D. Licht, E. Arvin, H. Mosbaek, A.B. Hansen, Metabolic pathways of quinoline, indole and their methylated analogs by *Desulfobacterium indolicum* (DSM 3383), Appl. Microbiol. Biotechnol. 47 (1997) 292–300.
[13] B. Liu, F. Zhang, X. Feng, Y. Liu, X. Yan, X. Zhang, L. Wang, L. Zhao, *Thauera* and *Azoarcus* as functionally important genera in a denitrifying quinoline-removal bioreactors revealed by microbial community structure comparison, FEMS Microbiol. Ecol. 55 (2006) 274–286.
[14] W. Jianlong, Q. Xiangchun, H. Liping, Q. Yi, W. Hegemann, Microbial degradation of quinoline by immobilized cells of *Burkholderia pickettii*, Water Res. 36 (2002) 2288–2296.
[15] P. Röger, G. Bar, F. Lingens, Two novel metabolites in the degradation pathway of isoquinoline by *Pseudomonas diminuta* 7, FEMS Microbiol. Lett. 129 (1995) 281–286.
[16] S.-N. Zhu, D.-Q. Liu, L. Fan, J.-R. Ni, Degradation of quinoline by *Rhodococcus* sp. QL2 isolated from activated sludge, J. Hazard. Mater. 160 (2008) 289–294.
[17] J.J. Griese, R.P. Jakob, S. Schwarzinger, H. Dobbek, Xenobiotic reductase A in the degradation of quinoline by *Pseudomonas putida* 86: physiological function, structure and mechanism of 8-hydroxycoumarin reduction, J. Mol. Biol. 361 (2006) 140–152.
[18] A.B. Thomsen, K. Henriksen, C. GrØn, A.P. MØldrup, Sorption, transport, and degradation of quinoline in unsaturated soil, Environ. Sci. Technol. 33 (1999) 2891–2898.
[19] G. Schwarz, R. Bauer, M. Speer, T.O. Rommel, F. Lingens, Microbial metabolism of quinoline and related compounds. II. Degradation of quinoline by *Pseudomonas putida* 86 and *Rhodococcus* spec. B1, Biol. Chem. Hoppe-Seyler 370 (1989) 1183–1189.
[20] A.-K. Reineke, T. Göen, A. Preiß, J. Hollender, Quinoline and derivatives at a tar oil contaminated site: hydroxylated products as indicator for natural attenuation? Environ. Sci. Technol. 41 (2007) 5314–5322.
[21] A.-K. Reineke, A. Preiß, M. Elend, J. Hollender, Detection of methylquinoline transformation products in microcosm experiments and in tar oil contaminated groundwater using LC-NMR, Chemosphere 70 (2008) 2118–2126.
[22] S. Brond, C. Sund, Biological removal of nitrogen in toxic industrial effluents high in ammonia, Water Sci. Technol. 29 (1994) 231–240.
[23] Y.M. Li, G.W. Gu, J.F. Zhao, H.Q. Yu, Y.Z. Peng, Treatment of coke-plant wastewater by biofilm systems for removal of organic compounds, Chemosphere 52 (2003) 997–1005.
[24] American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, AWWA and WEF, Washington, D.C., 2005.
[25] N. Velghe, A. Claeys, Rapid spectrophotometric determination of nitrate with phenol, Analyst 108 (1983) 1018–1022.
[26] J.S. Almeida, M.A.M. Reis, M.J.T. Carrondo, Competition between nitrate and nitrite reduction in denitrification by *Pseudomonas fluorescens*, Biotechnol. Bioeng. 46 (1995) 476–484.
[27] Y.-M. Li, J. Li, G.-H. Zheng, J.-F. Luan, Q.S. Fu, G.-W. Gu, Effects of the COD/NO_3^--N ratio and pH on the accumulation of denitrification intermediates with available pyridine as a sole electron donor and carbon source, Environ. Technol. 29 (2008) 1297–1306.
[28] P. Röger, A. Erben, F. Lingens, Microbial metabolism of quinoline and related compounds. IV. Degradation of isoquinoline by *Alcaligenes faecalis* Pa and *Pseudomonas diminuta* 7, Biol. Chem. Hoppe-seyler 371 (1990) 511–513.