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Denitrification in Presence of Benzene, Toluene, and *m*-Xylene

Kinetics, Mass Balance, and Yields

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Abstract

Denitrification of the electron donors toluene-C (15–100 mg/L), *m*-xylene-C (15–70 mg/L), benzene-C (5–25 mg/L), and acetate-C as experimental reference (50–140 mg/L) was carried out in batch culture. An initial concentration of 1.1 ± 0.15 g of volatile suspended solids/L of denitrifying sludge without previous exposure to aromatic compounds was used as inoculum. The results showed toluene and nitrate consumption efficiency (E_T and E_N, respectively) of 100%. Toluene was completely mineralized (oxidized) to CO_2. In all cases, the N_2 (Y_{N_2}) and HCO_3^- yields (Y_{HCO_3}) were 0.97 ± 0.01 and 0.8 ± 0.05, respectively. The consumption efficiency (E_X) of *m*-xylene (53 ± 5.7%) was partial. The Y_{N_2} and Y_{HCO_3} were 0.96 ± 0.01 and 0.86 ± 0.02, respectively. Benzene was not consumed under denitrifying conditions. The specific consumption rates of toluene (q_T) and *m*-xylene (q_X) were lower than that of acetate (q_A). The differences in specific consumption rates were probably owing to the negative effect of benzene, toluene, and isomers of xylene on the cell membrane.

Index Entries: Toluene; *m*-xylene; benzene; denitrification; kinetics.

Introduction

Benzene, toluene, and isomers of xylene, also known as BTX, are widely used as solvents and raw materials in the synthesis of organic compounds. They are byproducts of the petroleum industry and are found in

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gasoline to the extent of approx 18%, with toluene accounting for nearly 28% of the BTX mixture *(1)*. These aromatic hydrocarbons are polluting soils and groundwater owing to petroleum spills or to leaks from storage tanks. Significantly high concentrations of these compounds have been detected in groundwater, ranging from 9 to 14 mg/L of benzene, 23 to 81 mg/L of toluene, and 13 to 171 mg/L of xylene isomers *(2)*. BTX may produce diverse toxic effects in humans such as skin irritation, depression of the central nervous system, and eventually cancer *(3)* and, therefore, must be removed from water and wastewater.

Sulfate reduction *(4)* and methanogenesis *(5)* using microbial consortium or axenic cultures previously exposed to BTX were able to mineralize these aromatic compounds. Microbial heterotrophic denitrification is of particular interest because of the simultaneous elimination of both the organic matter and nitrate. It has been reported that under denitrifying conditions toluene and xylene isomers can be mineralized *(6)*, but benzene seemed to be recalcitrant *(4,7)*. However, in most cases, the complete mineralization of BTX to CO_2 still remains to be clearly demonstrated; only their disappearance has been noted. A better understanding of the denitrification process in the presence of BTX will help researchers to improve the efficiency of the process, thereby eliminating these toxic products.

The purpose of the present work was to study in batch culture the physiologic behavior of a stabilized denitrifying sludge produced in steady state during the mineralization of different concentrations of toluene, *m*-xylene, and benzene.

Materials and Methods

Culture Medium

The composition of the mineral medium was as follows: 100 mg/L of $FeCl_3$, 20 mg/L of $CuSO_4$, 400 mg/L of $MgSO_4$, 120 mg/L of $Na_2MoO_4 \cdot 2H_2O$, 60 mg/L of $CaCl_2 \cdot 2H_2O$, 1000 mg/L of KH_2PO_4. Different concentrations of toluene, *m*-xylene, and benzene were used as electron donors and carbon source to reduce nitrate to molecular nitrogen (N_2). The nitrate was used by the culture as electron acceptor and nitrogen source. Nitrate concentration (NO_3^--N) was adjusted to obtain a C/N ratio close to stoichiometric value. The C/N stoichiometric ratio for acetate is illustrated in the following equation, which does not include consumption of substrates for cell synthesis and is close to 1. The C/N ratios for toluene, *m*-xylene, and benzene were calculated in a similar way. The initial pH was adjusted to 7.0 with 1 *N* NaOH.

$$5\ CH_3COOH + 8\ NO_3^- \rightarrow 4\ N_2 + 8\ HCO_3^- + 2\ CO_2 + 6\ H_2O$$

Inoculum Source

The inoculum was obtained from an upflow anaerobic sludge blanket (UASB) reactor operated at a hydraulic retention time of 1 d at 30°C. The reactor was fed with 400 mg of acetate-C/(L·d) and 250 mg of nitrate-

N/(L·d). The sludge concentration was maintained at 4 g of volatile suspended solids (VSS)/L. The sludge was withdrawn from the UASB reactor previous to each batch assay and washed with a saline solution (0.9% NaCl).

Culture Conditions

The kinetic assays were carried out in serologic bottles of 60-mL capacity. The bottles were prepared in duplicate. Each bottle was an independent experimental unit, and after sampling, the bottles were sacrificed at designated times. Forty-eight milliliters of culture medium supplemented with the electron source was placed in each bottle: (50, 95, and 140 mg/L of acetate-C; 15, 25, 40, 55, 70, 85, and 100 mg/L of toluene-C; 15, 25, 35, 50, and 70 mg/L of *m*-xylene-C; and 5, 15, and 25 mg/L of benzene-C). The required nitrate concentration was calculated to maintain a C/N ratio of 1. The bottles were seeded, obtaining a final concentration of 1.1 ± 0.15 g of VSS/L and a headspace volume of 5 mL. The bottles were sealed with a rubber plug and an aluminum hoop. The molecular oxygen was displaced by using a current of helium for 5 min. Two controls were used for each assay. One contained the culture medium, denitrifying sludge, nitrogen source, and no electron source. The second control contained the culture medium, denitrifying sludge, and electron donor but without nitrogen source. The bottles were placed on a shaker at 150 rpm and incubated at 30°C. The total experimental time ranged from 1 to 15 d, depending on the electron source. Carbon mineralization from acetate, benzene, toluene, and *m*-xylene to CO_2 was measured as HCO_3^- generation. The total HCO_3^- was determined as the difference between HCO_3^- produced by the culture and that measured in the controls without electron source.

Abiotic Assays

To determine volatilization of BTX and adsorption in the sludge, two types of abiotic assays were conducted. The assays were carried out in 60-mL serologic bottles in the same way as described in the kinetic assays for 15 d. Two concentrations of BTX were evaluated in each case (25 and 50 mg of C/L). To assess volatilization of BTX, only mineral medium was placed in the bottles. BTX concentration was measured in the headspace and liquid phase. Adsorption was evaluated with mineral medium and sterile sludge. BTX concentration was also measured in the headspace and liquid phase. Adsorption of BTX was determined as the difference in concentration between the initial and final BTX value.

Evaluation of Culture Behavior

Sludge behavior was evaluated by consumption efficiency (E, mg of substrate consumed/mg of substrate fed), yield (Y, mg of product/mg of substrate consumed, considered as the efficiency of the metabolic denitrifying pathway), specific substrate consumption rate (q_S, mg of substrate consumed/[mg of VSS·d]), and specific production rate (q_P, mg of prod-

uct/[mg of VSS·d]). Specific substrate consumption rate was calculated using the equation $q_S = dS/dt \times 1/X$, in which S is the substrate concentration (mg/L), t is time (d), and X is the biomass concentration in the culture (mg of VSS/L). The slope values obtained during the lineal phase consumption represent the q_S values as reported by Elmén et al. *(6)*.

Analytical Methods

The concentration of toluene, *m*-xylene, and benzene was determined in the liquid and gas phase by gas chromatography (GC) (Varian model Star 3400) with a flame ionization detector and a capillary column (30 m long and 0.53-mm internal diameter) with a stationary-phase carbowax/BTR (Quadrex, Woodbridge, CT). The temperatures of the column, detector, and injector were 60, 235, and 250°C, respectively. Nitrogen was used as carrier gas (4 mL/min). N_2, N_2O, and CO_2 were quantified by GC (Varian model 3350) with a thermal conductivity detector. The temperatures of the column, detector, and injector were 40, 100, and 100°C, respectively. The carrier gas was helium (18 mL/min). Nitrite and nitrate content was measured by capillary electrophoresis (Waters capillary ion analyzer, model 4000; Millipore, Bedford, MA) as described by Gomez et al. *(8)*. Organic and inorganic carbon (HCO_3^-) was analyzed in a total organic carbon analyzer (TOC-5000A; Shimadzu) as described by Cuervo-López et al. *(9)*. Total suspended solids and VSS contents were determined at the end of each assay following standard methods *(10)*.

Results and Discussion

Abiotic Assays

In all cases, it was found that 65 ± 2% of the BTX compound was soluble in the liquid phase while the rest remained in the headspace. Thus, volatilization was 35 ± 2%. The adsorption assays indicated that close to 1.2% of BTX remained in the sludge. The adsorption was therefore negligible.

Denitrifying Sludge Source: UASB Reactor

N_2 production rate was 248 ± 12 mg of N_2-N/(L·d) after 5 wk of seeding the UASB reactor. Acetate consumption efficiency (E_A) was 96 ± 2%, and nitrate consumption efficiency (E_N) was 92 ± 4%. Y_{N_2} and Y_{HCO_3} values were 0.98 ± 2 and 0.96 ± 3, respectively. No intermediaries of denitrification such as NO_2^- and N_2O were detected. Since the N_2 production rate remained constant in the continuous reactor for about 12 mo, it was presumed that the sludge was in steady-state denitrification. As a consequence, the sludge acquired a higher physiologic stability. The sludge mineralized the acetate carbon by denitrification, as indicated by very high Y_{N_2} and Y_{HCO_3} values from the process. The process had a dissimilative pattern mainly owing to the low C/N ratio used (1.3).

Table 1
Efficiencies in Substrate Consumption (E) and Yields in Product Formation ($Y_{P/S}$) of Denitrifying Process Using Acetate, Toluene, Benzene, and *m*-Xylene as Electron Donors[a]

Compound (mg/L)	E (%)[b]		$Y_{P/S}$[c]	
	N	C	N_2	HCO_3^-
Acetate-C				
50	98 ± 0.5	97 ± 0.8	0.99 ± 0.01	0.97 ± 0.01
95	99 ± 0.6	98 ± 0.5	0.98 ± 0.02	0.98 ± 0.01
150	97 ± 0.7	98 ± 1.2	0.98 ± 0.01	0.97 ± 0.02
Toluene-C				
15	100	100	0.96 ± 0.02	0.85 ± 0.05
25	100	100	0.98 ± 0.01	0.71 ± 0.04
40	100	100	0.96 ± 0.01	0.80 ± 0.02
55	100	100	0.98 ± 0.02	0.75 ± 0.02
70	100	100	0.97 ± 0.02	0.80 ± 0.03
85	100	100	0.98 ± 0.01	0.85 ± 0.02
100	100	100	0.96 ± 0.02	0.83 ± 0.02
m-Xylene-C				
15	65 ± 1.1	60 ± 0.9	0.95 ± 0.03	0.83 ± 0.02
25	65 ± 0.6	55 ± 0.6	0.96 ± 0.02	0.85 ± 0.01
35	50 ± 0.5	55 ± 0.4	0.98 ± 0.02	0.88 ± 0.02
50	45 ± 0.6	50 ± 0.5	0.95 ± 0.02	0.88 ± 0.02
70	45 ± 0.8	45 ± 0.6	0.96 ± 0.03	0.86 ± 0.01
Benzene-C				
15	0	0	0	0
25	0	0	0	0
35	0	0	0	0

[a]Values represent mean values and the ± range from two independent samples.
[b]E = (mg substrate$_{consumed}$/mg substrate$_{fed}$) × 100.
[c]$Y_{P/S}$ = (mg product/mg substrate$_{consumed}$).

Batch Assays: Consumption of Acetate, Toluene, m*-Xylene, and Benzene*

The levels of volatilization and adsorption of BTX in batch cultures were similar to those observed in abiotic assays. When the concentration in the liquid phase decreased owing to microbial consumption, the aromatic compounds present in the headspace (35% of BTX) were dissolved into the liquid phase, resulting in consumption of the BTX found in the bottle.

Y_{N_2} and Y_{HCO_3} values as well as consumption efficiency of acetate (E_A), toluene (E_T), *m*-xylene (E_X), benzene (E_B), and nitrate (E_N) are given in Table 1. In the range of acetate concentrations, as electron donors the E_A and the E_N in all cases were higher than 97%. Similarly, Y_{N_2} and Y_{HCO_3} values were also higher than 0.98 ± 0.006 and 0.97 ± 0.006, respectively. There was no noticeable increase in the VSS content; the respiratory process was dissimilative. The denitrifying behavior of the sludge in batch culture with

acetate was similar to the behavior of the sludge observed from the UASB. Therefore, this respiratory pattern was used as a reference to compare the effect of toluene, *m*-xylene, and benzene as electron donors on the same sludge.

In the presence of toluene an E_T of 100% was achieved in all cases, even when 100 mg/L of toluene-C was used. The average Y_{HCO_3} value was 0.80 ± 0.05, while the average Y_{N_2} remained constant at 0.97 ± 0.01. In the same range of toluene concentration no increase in VSS was observed. These results show that the toluene was mineralized to CO_2 during denitrification, regardless of the initial toluene concentration. The high denitrification yields suggest that neither the denitrification pathway nor the enzymatic toluene oxidation were significantly altered. However, Evans et al. *(11)* reported an E_T of 87% when a sludge with no previous contact to BTX was used. They did not, however, mention whether the toluene was oxidized and nitrate was reduced. Schocher et al. *(12)* reported an E_T of 80% and a Y_{CO_2} of 0.5 when an axenic culture acclimatized to hydrocarbon consumption was used. Fifty percent of the toluene consumed in this case was presumably assimilated for the formation of microbial biomass, which however, is undesirable in wastewater treatment.

In our study, a significant decrease in E_X (53 ± 5.7%) and E_N (54 ± 10.2%) values was observed when different initial concentrations of *m*-xylene were used as electron donors. However, Y_{N_2} and Y_{HCO_3} values were similar to those obtained with acetate and toluene, 0.96 ± 0.01 and 0.86 ± 0.02, respectively, suggesting that the *m*-xylene consumed was used for the reduction of nitrate to N_2. Thus, the *m*-xylene did not have any effect on the denitrifying pathway even at an initial concentration of 70 mg/L. Although only 53% of the *m*-xylene was consumed, the quantity of *m*-xylene that was eliminated was two- to threefold higher than the values reported *(7,13,14)*. Our results also show that the initial concentrations of toluene and *m*-xylene employed (100 and 70 mg/L, respectively) did not influence the efficiency of the denitrifying pathway, because the Y_{N_2} and Y_{HCO_3} values were similar to those for the same acetate concentration.

In the assays with benzene, no consumption of substrates was observed; the nitrate and benzene concentrations remained constant throughout the experiment. This was similar to the behavior when only nitrate or benzene was added. This finding is in accord with the published information about denitrifying conditions in which benzene was recalcitrant *(4,7,13,15–17)*.

Kinetics of Batch Assays

The kinetics of the denitrifying process with 50 mg of acetate-C/L is illustrated in Fig. 1. There was no formation of intermediaries such as NO_2^- or N_2O during the reduction of NO_3^- to N_2. Similar behavior was observed with 90 and 140 mg of acetate-C/L. For each initial acetate concentration, the specific consumption rate of acetate (q_A, mg of acetate-C/[mg of VSS·d]) and specific consumption rate of nitrate (q_N, mg of NO_3^--N/[mg of VSS·d])

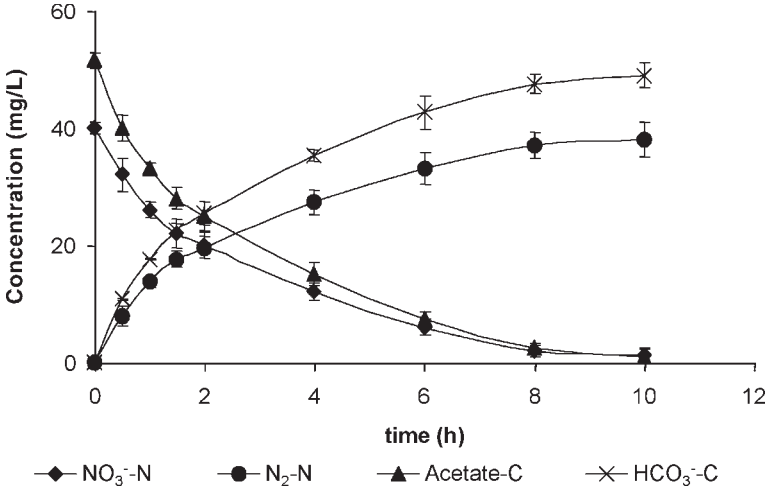

Fig. 1. Kinetic profiles of acetate-C and NO_3^--N consumption with denitrifying sludge. Values represent mean values and the ± range from two independent samples.

Table 2
Specific Rate of Acetate and Nitrate Consumption[a]

Acetate-C (mg/L)	NO_3^--N (mg/L)	q_S (mg substrate/[mg VSS·d])	
		Acetate	Nitrate
50	40	0.36 ± 0.01	0.29 ± 0.014
95	75	0.41 ± 0.02	0.34 ± 0.018
140	110	0.46 ± 0.01	0.39 ± 0.011

[a]Values represent mean values and the ± range from two independent samples.

increased proportionately with the increase in initial substrate concentration (Table 2). Similar q_N values of 0.66 and 0.33 mg of NO_3^--N/(mg of VSS·d) were also obtained *(18,19)* at nitrate concentrations of 200 and 75 mg of NO_3^--N/L, respectively. The kinetic pattern of the denitrifying sludge with 50–140 mg of acetate-C/L was used as a reference to compare its behavior in the presence of toluene and *m*-xylene.

Toluene oxidation (15 mg of toluene-C/L) and the formation of HCO_3^- are presented in Fig. 2A. Toluene was consumed within 2.5 d of batch culture, but there was no consumption of toluene in the control (toluene without nitrate). As a result of toluene oxidation, small quantities of acetate, propionate, butyrate, and benzoate were detected. Nitrate consumption and its reduction to N_2 are illustrated in Fig. 2B. Nitrate elimination was not observed in the control (nitrate without toluene). In the rest of the assays, only a transient formation of nitrite was observed, which was finally converted into N_2. A similar respiratory pattern was observed with toluene concentration ranging from 25 to 85 mg/L. A higher initial toluene concen-

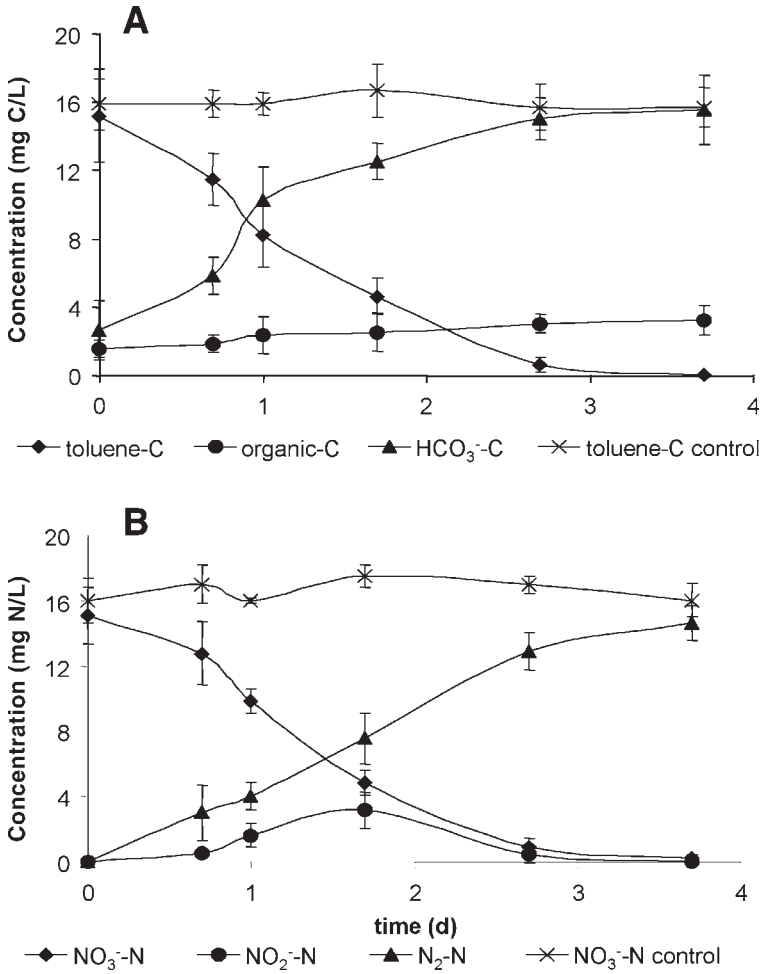

Fig. 2. Denitrification at 15 mg of NO_3^--N/L of toluene in batch culture: **(A)** profiles of toluene consumption; **(B)** profiles of nitrate reduction. Values represent mean values and the ± range from two independent samples.

tration resulted in an increase in the lag phase. Therefore, at 100 mg of toluene-C/L the time required to consume the toluene was two- to three-fold higher (7 d) with nitrite accumulation. This might be owing to the inhibitory effect of toluene on the nitrite oxide reductase (*Nir*) enzyme of the denitrification pathway.

Specific consumption rates of toluene (q_T, mg of toluene-C/[mg of VSS·d]) are shown in Fig. 3. It can be seen that the q_T increased to 4.5 times as the initial toluene concentration increased to 15–70 mg/L. Higher toluene concentrations induced a decrease in the q_T. Elmén et al. *(6)* found that 102 mg/L of toluene caused a decrease in the q_T value close to 25%. Our results indicate that toluene at a concentration of 85 mg/L produced a decrease in q_T only by 2.5%, but at 100 mg of toluene-C/L q_T was reduced

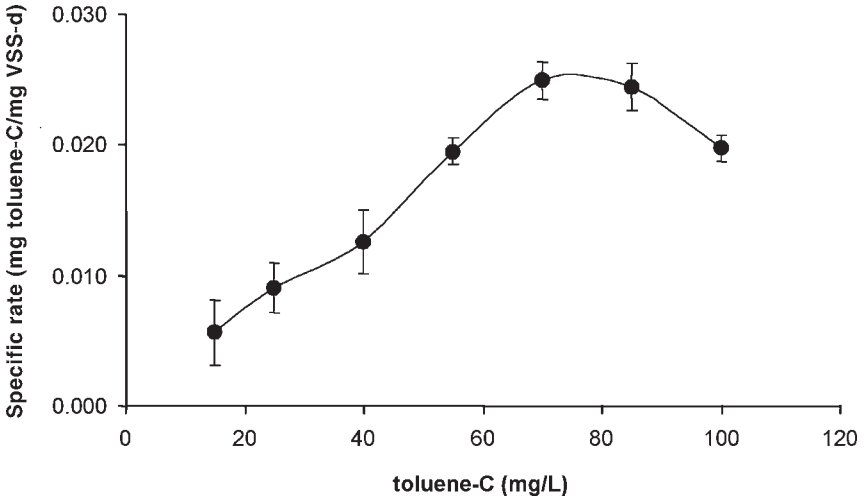

Fig. 3. The q_T in denitrifying process in batch culture. Values represent mean values and the ± range from two independent samples.

to 21%. The substrate inhibition profile in Fig. 3 shows a maximum experimental q_T value at 70 mg of toluene-C/L.

The results indicate that no substrate inhibition was detected at carbon concentrations >70 mg/L. The decrease in the specific rate for toluene was probably owing to the denaturing effect of toluene on the cytoplasmic membrane *(20)*. Regarding this, there are studies in *Pseudomonas putida (21,22)* indicating that when toluene was present the content of lipids in the cell membrane changed. In those studies, the aromatic hydrocarbon concentration was higher than in our work. The decrease in the specific rate at the toluene concentrations assayed (100 mg of C/L) might be owing to a higher sensibility of the denitrifying sludge to the BTX. As a consequence, the metabolic mineralization of toluene diminished resulting in a lower specific production rate of N_2. In fact, the specific toluene consumption rate was 17 times lower than the acetate consumption rate.

The behavior of the denitrifying process with 25 mg of *m*-xylene-C/L is presented in Fig. 4A, where three specific stages can be seen. The first stage corresponded to a lag phase of 4 d, followed by the consumption of *m*-xylene and nitrate in the second stage, and, finally, the third phase, which was stationary. The formation of acetate and propionate was detected during *m*-xylene oxidation. A significant increase in the dissolved organic carbon (DOC) concentration was observed at the end of this stage. The presence of this DOC could be owing to the effect of *m*-xylene on the cytoplasmic membrane structure and its permeability *(20–22)*. Nitrate consumption and its reduction to N_2 are shown in Fig. 4B. A transient formation of NO_2^- and N_2O was observed, which were both converted into N_2. A similar respiratory pattern was observed when *m*-xylene concentrations varied from 25 to 70 mg/L. Thus, *m*-xylene did not affect the efficacy of the denitrifying pathway because the Y_{N_2} was high (0.95–0.98); however,

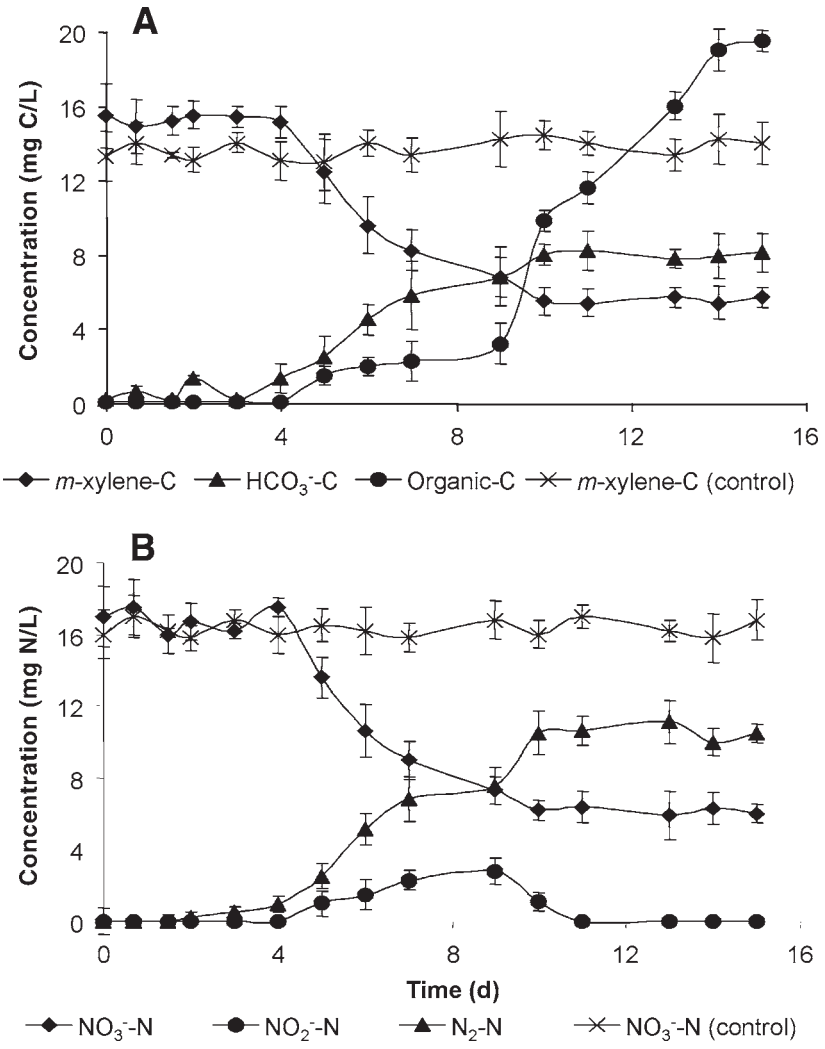

Fig. 4. Denitrification at 17 mg of NO_3^--N/L of *m*-xylene in batch culture: **(A)** profiles of *m*-xylene consumption; **(B)** profiles of nitrate reduction. Values represent mean values and the ± range from two independent samples.

because there was accumulation of intermediates, the rate of denitrifying enzymes *Nir* and nitrose oxide reductase enzyme (*Nos*) was decreased.

The specific consumption rate of *m*-xylene (q_X, mg of *m*-xylene-C/[mg of VSS·d]) showed an increase proportional to the increase in *m*-xylene concentration ranging from 15 to 50 mg of *m*-xylene-C/L, reaching a maximum value at 70 mg of *m*-xylene-C/L (Fig. 5). The q_X was 100 times lower than the q_A and 6 times lower than q_T.

The toxicologic effect of toluene and *m*-xylene on the denitrifying sludge was evaluated after every batch assay by adding nitrate and acetate and then measuring the rate of N_2 formation. The sludge control (toluene

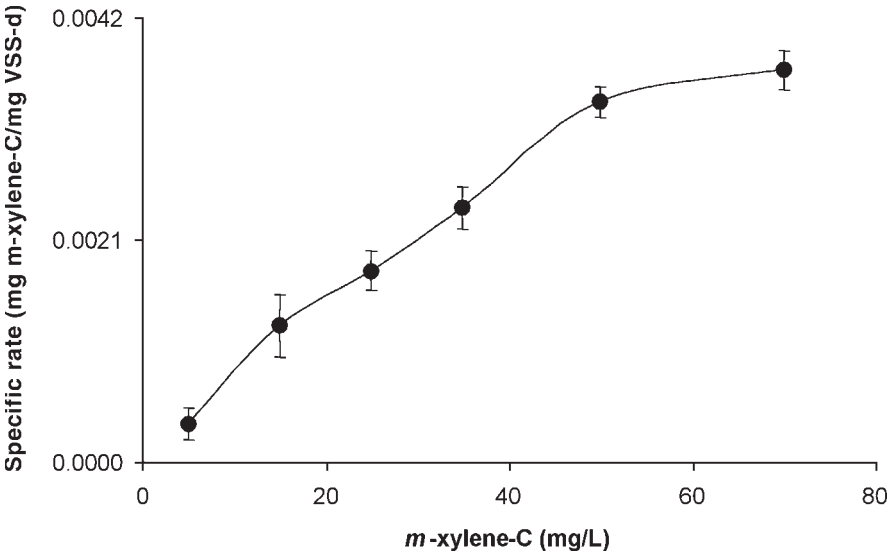

Fig. 5. The q_X in denitrifying process in batch culture. Values represent mean values and the ± range from two independent samples.

without nitrate) and the sludge exposed to toluene and nitrate totally consumed acetate and nitrate within 12 h irrespective of the initial toluene concentration, whereas 96% of these substrates was consumed within 24 h by the sludge exposed to *m*-xylene and nitrate. By contrast, the sludge control (*m*-xylene without nitrate) only recovered 45% of its initial consumption capacity. These results are evidence of the possible effect of toluene on the cytoplasmic membrane, which is different from the effect of *m*-xylene on the denitrifying sludge.

Material Balance During Denitrification Batch Process

The material balance of denitrification with acetate is shown in Table 3. It can be observed that the denitrification process was completely dissimilative as the acetate was mineralized to CO_2 and concomitantly nitrate was reduced to N_2.

When 15 mg of toluene-C/L and 15 mg of NO_3^--N/L were assayed, 12.7 mg of HCO_3^--C/L was produced and only 2.5 mg of C/L remained in a mixture of acetate, propionate, butyrate, and benzoate (Table 3). An intermediary compound was also detected that did not correspond to either benzyalcohol or benzaldehyde, which are some generally reported intermediaries *(12)*. There was no increase in the VSS content because toluene was not used as a carbon source, only as an energy source. Along with the nitrate reduction, the nitrite appeared transitorily, and finally was converted to 15 mg of N_2-N/L. This denitrifying performance was also observed in the assays in which toluene ranged from 25 to 100 mg/L.

Table 3
Balance of Materials of Denitrifying Process Using Different Electron Donors[a]

Acetate-C (mg/L)	Entry-C = Exit-C (mg/L)					Entry-N = Exit-N (mg/L)				
	Acetate-C	=	Inorganic-C	+	Organic-C	NO_3^--N	=	NO_3^--N	+	N_2-N
50	50 ± 2.5		48.5 ± 2.1		1.1 ± 0.15	40 ± 1.8		1.7 ± 0.04		38.8 ± 1.3
95	95 ± 3.9		91.2 ± 4.5		1.9 ± 0.2	76 ± 3		1.7 ± 0.08		73.1 ± 3
140	142 ± 5.3		136 ± 5.5		4.2 ± 2.2	112 ± 4.2		3.4 ± 0.15		105.4 ± 4.6
Toluene-C (mg/L)	**Entry-C = Exit-C (mg/L)**					**Entry-N = Exit-N (mg/L)**				
	Toluene-C	=	Inorganic-C	+	Organic-C	NO_3^--N	=	NO_2^--N	+	N_2-N
15	15 ± 1.1		12.7 ± 0.5		2.5 ± 0.3	15.1 ± 0.5		0		14.6 ± 0.8
25	24.6 ± 1.8		17.5 ± 0.5		5.8 ± 0.2	25.2 ± 0.8		0		24.5 ± 1.1
40	38.8 ± 2.5		31 ± 1.1		7.4 ± 0.5	40.5 ± 1		0		38.4 ± 1.3
55	55.4 ± 2.8		41.6 ± 2.1		10.9 ± 0.55	54.3 ± 1.4		0		53 ± 1.9
70	68.4 ± 4.1		54.7 ± 2.5		11.7 ± 0.6	70.1 ± 2.3		0		68.5 ± 2.8
85	83.5 ± 5.7		71 ± 2.9		10.8 ± 0.55	85.6 ± 3.6		0		83.3 ± 2.5
100	97.7 ± 6.5		81.1 ± 3.5		12.6 ± 0.62	98.2 ± 3.2		2.5 ± 0.4		95 ± 3.8
m-Xylene-C (mg/L)	Entry-C = Exit-C (mg/L)					Entry = Exit-N (mg/L)				
	m-Xylene-C	=	*m*-Xylene-C	+	Inorganic-C	NO_3^--N	=	NO_3^--N	+	N_2-N
15	14.5 ± 1		5.8 ± 0.5		7.2 ± 0.4	17 ± 0.35		6.1 ± 0.3		10.5 ± 0.41
25	25 ± 1.5		11.2 ± 0.8		11.7 ± 0.6	27 ± 1.1		9.5 ± 0.37		16.8 ± 0.54
35	36.3 ± 2.5		16.2 ± 1.2		17.4 ± 0.9	38.3 ± 1.3		13.3 ± 0.6		23.7 ± 0.8
50	52.6 ± 3		26 ± 1.5		22.9 ± 1.1	54 .4 ± 2		29.7 ± 1.1		23.3 ± 1.2
70	68.5 ± 4.2		37.5 ± 2.4		26.4 ± 1.3	75.2 ± 2.6		41.2 ± 1.6		32.4 ± 1.6

[a]Values represent mean values and the ± range from two independent samples.

Therefore, the capacity of toluene to reduce nitrate was similar to that of acetate.

It has been reported that both an excess of nitrate and the use of sludge previously exposed to hydrocarbon consumption increased the effectiveness of denitrification *(7,23)*; however, the excess nitrate resulted in NO_2^- and N_2O accumulation. Our results indicate that in spite of using a toluene concentration 10 times higher than those reported in the literature, excess nitrate was not required. However, the physiologic stability of the sludge was important.

At 15 mg of *m*-xylene-C/L, only 8.7 mg of *m*-xylene-C/L was consumed and converted into 7.2 mg of HCO_3^--C/L. Acetate and propionate were detected as intermediaries of the *m*-xylene oxidation but were not quantified owing to an interference of soluble organic matter in the culture medium. However, at the end of the culture, 10.5 mg of N_2-N/L and 6 mg of NO_3^--N/L were measured without any difficulty. There was no variation in the concentration of VSS during the culture time (1.1 ± 0.15 g of VSS/L of denitrifying sludge). Similar profiles were also observed in the assays in which *m*-xylene varied from 25 to 100 mg/L. Thus, the *m*-xylene and nitrate consumed corresponded to the stoichiometric ratio required for N_2 production from *m*-xylene oxidation (C/N stoichiometric ratio of 0.85). According to some studies, *m*-xylene concentrations between 7 and 11 mg/L were consumed under denitrifying conditions *(11,13,14,24)*, but there was no mention of whether the process was assimilative or dissimilative. Some researchers suggest the importance of using sludge acclimatized to BTX degradation *(7)*, whereas, according to our results, sludge acclimatization could be substituted by physiologic sludge stability.

Conclusion

Toluene and *m*-xylene concentrations ranging from 15 to 100 mg of toluene-C/L and 15 to 70 mg of *m*-xylene-C/L were used as electron sources for a denitrifying dissimilative respiratory process without previous acclimatization to BTX. Benzene was recalcitrant. Nitrate consumed were completely converted into N_2, while acetate, toluene, and *m*-xylene were completely converted into HCO_3^-. The efficiency of the denitrification pathway was not influenced by toluene or *m*-xylene; the denitrifying yield values were similar to the acetate reference.

The effect of the same range of concentrations of toluene and *m*-xylene on the microbial membrane was not the same. The lower specific consumption rate obtained for toluene and *m*-xylene (when compared with the specific consumption rate for acetate) was probably owing to injury of the microbial membrane. The q_T increased as the initial concentration of toluene increased (15–70 mg-C/L). The *qT* decreased by 21% at 100 mg of toluene-C/L. Kinetic studies showed that toluene and *m*-xylene inhibited the denitrifying pathway (NO_3^- to N_2) at different enzymatic levels owing to differences in the accumulated intermediaries.

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