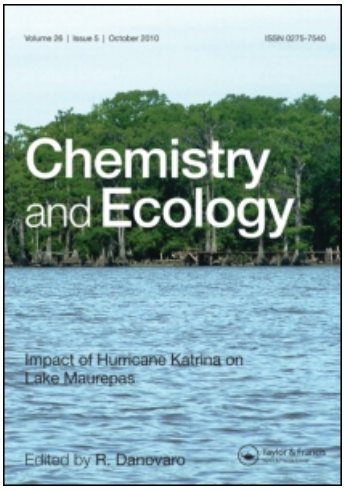

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Microbial Potential for the Anaerobic Transformation of Simple Homocyclic and Heterocyclic Compounds in Sediments of the Tsengwen River

Shiu-Mei Liu[a]; Cheng-Lung Kuo[a]
[a] Institute of Marine Biology, National Taiwan Ocean University, Taiwan, Republic of China

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MICROBIAL POTENTIAL FOR THE ANAEROBIC TRANSFORMATION OF SIMPLE HOMOCYCLIC AND HETEROCYCLIC COMPOUNDS IN SEDIMENTS OF THE TSENGWEN RIVER

SHIU-MEI LIU and CHENG-LUNG KUO

Institute of Marine Biology, National Taiwan Ocean University, Keelung 202, Taiwan, Republic of China

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The ability of microbial populations to mediate the anaerobic transformation of four aromatic compounds (aniline, benzoic acid, pyridine, and quinoline) in sediments of the Tsengwen River was examined. Along the river, from a freshwater (0.0‰ salinity) to an oceanic (37.0‰ salinity) environment, five sampling stations were chosen to collect the sediment. Sediment slurries were incubated in an anaerobic mineral salts medium that was amended with multiple electron acceptors, including manganese (IV) and ferric (III) oxides, and the concentrations of the aromatic substrates were followed over a 3- to 4-month period. Most sediment samples showed a complete loss of benzoic acid and quinoline (0.12–0.21 mM) within approximately three months. Pyridine was transformed after a lag period of 53 days in the sediment slurries from the freshwater environment which had been amended with both metal oxide (either Fe $(OH)_3$ or MnO_2) and inhibitor (either BESA or molybdate). Pyridine was not transformed in other sediment slurries. No significant metabolism of aniline was apparent in any of the sediments.

KEY WORDS: Anaerobic, transformation, sediment slurries, aromatic compounds.

INTRODUCTION

Tsengwen River is located in the southern part of Taiwan. Because of constantly receiving effluent from domestic sewage and industrial waste treatment, the river is seriously polluted. Bacteria in the sediments mediate a wide variety of diagenetic processes and the bottom sediments are important sites for organic matter degradation in the freshwater and marine environment. The possibility of using bioremediation to restore environments contaminated with organics is well known (Bouwer, 1992). Bioremediation may either be "intrinsic bioremediation" which naturally takes place in contaminated environments or engineered (Committee on *in situ* Bioremediation, 1993). Several studies have indicated that geochemical site conditions (e.g. oxidation-reduction potential, the presence of sulphate, the presence of iron-containing or Mn-containing minerals) will influence the potential for *in situ* anaerobic biological restoration of sediment contaminated with toxic chemicals. In particular, Fe (III) is the most plentiful potential anaerobic electron acceptor in most

soils and freshwater sediments (Ponnamperuma, 1972; Van Breemen, 1988) and is second only to sulphate as the most abundant electron acceptor in many marine sediments (Reeburgh, 1983). Manganese oxides are also abundant potential anaerobic electron acceptors in sedimentary environments (Dean *et al.*, 1981; Myers and Nealson, 1988; Edgington and Callender, 1970; Rossmann and Callender, 1968). Microorganisms, whether facultative or strictly anaerobic, can completely oxidize organic compounds with Fe(III) or Mn(IV) as the sole electron acceptors and that the energy yielded from oxidation of organic matter coupled to dissimilatory Fe(III) or Mn(IV) reduction can support microbial growth (Nealson, 1983; Myers and Nealson, 1988; Lovley and Phillips, 1986a, b; Lovley and Phillips, 1988), had been isolated. Thus, these metal oxide reduction-linked process may make substantial contributions to the overall transformation of xenobiotics in many anaerobic environments. However, until now only limited evidence links Fe(III) and Mn(IV) reduction to aromatic compound degradation in the sediment.

The potential for bioremediation of the Tsengwen River depends upon the microbial potential for the anaerobic degradation of organic compounds. In this study, the ability of microbial populations to mediate transformation of four (two homocyclic and two heterocyclic) aromatic compounds in sediments of the Tsengwen River under anaerobic conditions was examined. Multiple electron acceptors ($Fe(OH)_3$, MnO_2, sulphate, and CO_2) were included in the medium to allow for the activity of the major populations of anaerobic respirers (iron reducers, manganese reducers, sulphate reducers, and methanogens). Inhibitors of sulphate reduction and methanogenesis were included in some of the vials in an attempt to ascertain the potential contribution of these classes of bacteria.

MATERIALS AND METHODS

Chemicals and Reagents

Pyridine was obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan). Aniline, benzoic acid, quinoline, manganese dioxide (MnO_2), and ferric chloride-hexahydrate were purchased from Hayashi Pure Chemical Industries Ltd (Osaka, Japan). The 2-hydroxyquinoline and 2-bromoethanesulfonic acid-sodium salt (BESA) were obtained from Aldrich Chemical Co. (Milwaukee, Wis.). Sodium molybdate-2 hydrate was from Riedel-deHaen (Seelze, West Germany). All these chemicals were used without further purification. High-pressure liquid chromatography (HPLC) solvents were purchased from Fisons Scientific Equipment (Loughborough, England). All other chemicals are reagent or HPLC grade.

Amorphous $Fe(OH)_3$ was prepared as described by Beller *et al.* (1992). A 0.1 M ferric chloride solution was neutralized with sodium hydroxide. The precipitate was aged for 4 h after neutralization; during this period, the pH was adjusted periodically with sodium hydroxide to neutralize acidification resulting from ferric iron hydrolysis. The precipitate was then rinsed with Milli-Q water to reduce the residual chloride concentration to a level less than 1 mM. The amorphous $Fe(OH)_3$ was prepared with sterilized glassware and reagent prepared in sterilized Milli-Q water,

but the iron phase itself could not be autoclaved because the elevated heat and pressure would facilitate crystallization. The amorphous structure of the Fe(III) oxyhydroxides were confirmed by X-ray diffraction analysis.

Sediment Collection and Treatment

Sediment samples were obtained from 5 stations along the bank of the Tsengwen River (Fig. 1): station 1 (23°07′33″N, 120°14′″E); station 2 (23°06′17″N, 120°12′23″E); station 3 (23°05′23″N, 120°10′28″E); station 4 (23°05′08″N, 120°07′52″E); station 5 (23°02′42″N, 120°04′01″E). These stations included freshwater and oceanic environments. Salinity of the overlying site water of these sites were 0‰, 7.00‰, 10.0‰, 20.0‰, and 37.0‰ (Tab. I). Sand and silt percentage of the sediment collected from each station are different (Tab. II). To avoid contact with atmospheric oxygen, the top sediment (0 to 5 cm) was collected by completely filling jars with sediment and sealing. All samples were kept in the dark and sent back to the laboratory within 10 hours. Upon arrival in the lab, samples were flushed with a gas mixture of N_2: H_2 (19:1) and were then taken into the anaerobic glove box (Coy Labs, Grass Lake, MI) which was filled with a gas mixture of N_2:H_2 (19:1). To eliminate all residual traces of O_2, all sediments were equilibrated in the anaerobic glove box for at least 1 day before use.

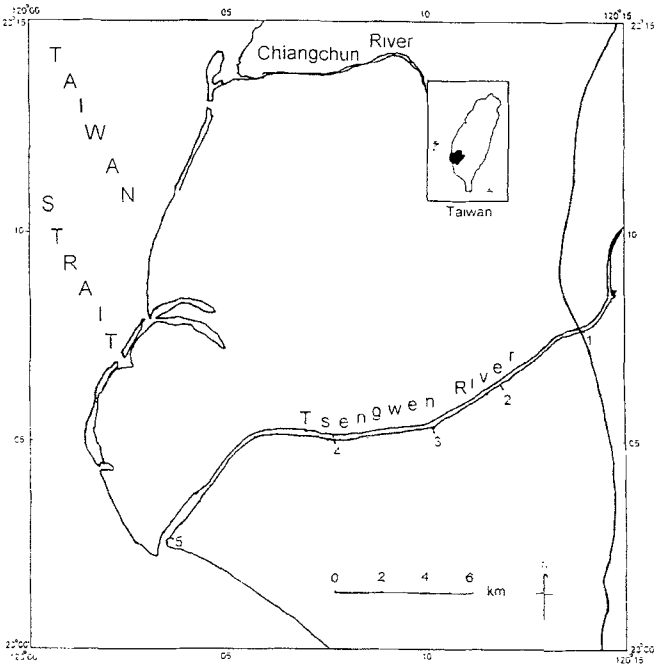

Figure 1 Location of the Tsengwen River and the 5 sampling stations in the river.

Table I Sampling stations along the Tsengwen River.

Station	*Location (GPS)*	*Surface water temperature (°C)*	*Salinity (ppt)*	*pH value*
1	N 23° 07'33" E 120° 14'10"	26.0	0	7.32
2	N 23° 06'17" E 120° 12'23"	23.0	7	7.53
3	N 23° 05'23" E 120° 10'28"	21.5	10	7.87
4	N 23° 05'08" E 120° 07'52"	21.5	20	7.90
5	N 23° 02'42" E 120° 04'01"	24.0	37	7.90

Table II Particle size analysis of the sediment collected from 5 sampling stations of the Tsengwen River.

Sample	*Sand (%, w/v)*	*Silt (%, w/v)*
Station 1	54.8	45.2
Station 2	85.8	14.2
Station 3	24.6	75.4
Station 4	27.9	72.1
Station 5	97.1	2.9

Media Preparation and Transformation Studies

Anoxic medium stock solutions were prepared as described previously (O'Connor *et al.*, 1989; O' Connor and Young, 1989; Owen *et al.*, 1979). In brief, all media stock solutions were prepared by boiling distilled water in a flask for 20 to 30 min then cooling to room temperature while being simultaneously purged with N_2. After cooling, corresponding chemicals were added to the distilled water and the flask was capped with a black butyl rubber stopper. These medium stock solutions were then taken into the anaerobic glove box. All subsequent manipulations were conducted in the anaerobic glove box. The glove box was equipped with a Coy model 10 continuous readout gas analyzer to confirm the absence of O_2 during all experimental procedures.

Sediment slurries were prepared by diluting sediment to 10% solid (w/v) using anoxic sterile water and medium stock solutions. The final concentration (mgl^{-1}) of the medium was: KH_2PO_4, 200; KCl, 300; NH_4Cl, 300; $CaCl_2$-$2H_2O$, 150; $MgCl_2$-$6H_2O$, 300; Na_2SO_4, 3000; $FeCl_2$-$4H_2O$, 1.5; $CuCl_2$-$2H_2O$, 0.015; Na_2MoO_4-$2H_2O$, 0.025; $NiCl_2$-$6H_2O$, 0.025; H_3BO_3, 0.06; $ZnCl_2$, 0.07; $MnCl_2$-$4H_2O$, 0.1; $CoCl_2$-$6H_2O$, 0.12; Na_2SeO_3, 0.003; biotin, 0.01; *p*-aminobenzoic acid, 0.05; B_{12}, 0.05; and thiamin, 0.1. In addition, $NaHCO_3$ was added separately from a stock solution saturated with CO_2 and its final concentration was 1.2 gl^{-1}. The pH of the medium was about 7.2. The medium was supplemented with either 2 mM MnO_2 or 2mM $Fe(OH)_3$. A reducing environment was established in the sediment slurries by the addition of 0.05% (w/v) Na_2S. Sediment slurries were dispensed into sterile serum

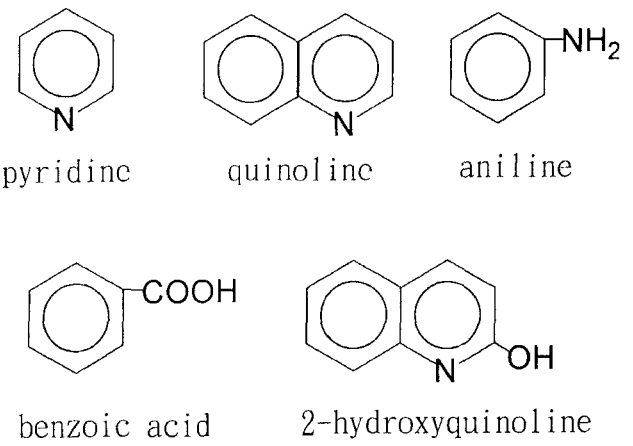

Figure 2 Chemical structure of pyridine, quinoline, aniline, benzoic acid and 2-hydroxyquinoline.

bottles (115 ml) which subsequently were capped with butyl rubber stoppers and crimp-sealed with aluminum caps. Thus, each bottle contained sterile defined medium, MnO_2 or $Fe(OH)_3$, and 10% (w/v) sediment slurry. Where indicated, filter-sterilized stock solution of sodium molybdate or 2-bromoethanesulphonic acid (BESA) were added to final concentrations of 10 mM and 2 mM, respectively.

Experiments were initiated by adding one of the aromatic compounds to the active and the control sediment slurries. Stock solutions of each aromatic compound (Fig. 2) were dissolved in acetonitrile to a final concentration of 20 mg ml^{-1} and amended to experimental and control sediment slurries to a final concentration of 0.12–0.21 mM. Controls consisted of autoclaved (121°C, 30 min for 3 consecutive days) sediment slurries and sediment slurries to which acetonitrile only was added. All experiments were replicated and the results are reported as the average of duplicates. The serum bottles were incubated at 23–25°C in the dark. At intervals, 1.0 ml subsamples were removed using sterile techniques from each active and control sediment slurry while swirling the slurry to ensure a uniform suspension. The subsamples were combined with 1.0 ml of acetonitrile and mixed with a vortex apparatus. The slurry-solvent mixtures were centrifuged at 1,800 g for 10 min, and the supernatant solution was filtered through a 0.22 μm pore-size filter (Lida Manufacturing Corp., Kenosha, WI) prior to HPLC analysis.

Analytical Methods

Filtered subsamples of sediment slurries from the different experiments were analyzed by HPLC for the appropriate substrate (aniline, benzoic acid, pyridine, or quinoline). The Hitachi HPLC apparatus consisted of a Lichrosorb RP-18 column (4.6 × 200 mm; 10 μm particle size; Hewlett Packard Corp., Palo Alto, Calif.), a model L-6200 intelligent pump, a model AS-2000 autosampler, a model L-4000 UV absorbance detector and a model D-2500 Chromato-integrator. The sample loop

was 100 µl. For the analysis of benzoic acid, the elution solvent, which consisted of a mixture of methanol, water and acetic acid (50:49.5:0.5, v/v/v) was introduced to the column at a flow rate of 1.0 ml min^{-1}. For the analysis of aniline, pyridine, quinoline, and 2-hydroxyquinoline, the elution solvent, which consisted of a mixture of methanol, water, and triethanolamine (600:399.32:0.68) adjusted to pH 6.8 by adding KH_2PO_4 4.4g l^{-1}, was introduced to the column at a flow rate of 1.0 ml min^{-1}. Quantification of aniline, benzoic acid, pyridine, quinoline and 2-hydroxyquinoline were carried out by the external standard method at a wavelength of 254 nm, 279 nm, 254 nm, and 310 nm, respectively.

RESULTS

Abiotic Reduction of Mn(IV) and Fe(III)

Over the incubation period of 4 months, there were no significant changes of the four test aromatic compounds in the autoclaved sediment slurries, which also means that there was no significant chemical interaction between the aromatic substrates and the metal oxides.

Aniline Biotransformation

Over the 4-month period, no significant loss of aniline was observed in any of the sediment slurries, which included $Fe(OH)_3^-$ or MnO_2^- amended sediment slurries from all stations along the Tsengwen River.

Benzoic Acid Biotransformation

Benzoic acid was transformed to unknown compound(s) in all sediment slurries amended with $Fe(OH)_3$ or MnO_2 after incubation for 21–80 days (Fig. 3a–3c and Table III). In most cases, the loss was complete within approximately 21 to 40 days. No lag periods were noted for benzoic acid transformation in sediment slurries collected from station 2, 3 and 5 amended with $Fe(OH)_3$. However, benzoic acid was transformed after a lag period of 12 days in the sediment slurries collected from the other stations. Benzoic acid transformation rates were slightly higher in sediment slurries amended with MnO_2 than in sediment slurries amended with $Fe(OH)_3$. As shown in Table III, sediment slurries collected from station 5 amended with MnO_2 had the highest benzoic acid transformation rate (18.86 µMd^{-1}) while station 3 amended with $Fe(OH)_3$ had the lowest (6.20 µMd^{-1}) benzoic acid transformation rate. There is no obvious difference in the rates of benzoic acid transformation in the freshwater (station 1) and oceanic environments (station 4 and 5). Benzoic acid was transformed after a lag period of 12 days in the sediment slurries amended with 2 mM BESA except in the sediment slurries collected from station 3 and 5 and amended with $Fe(OH)_3$. Addition of 2 mM BESA into the sediment slurries partially increased the removal rate of benzoic acid except in the sediment slurries collected from station 2 and 4 and amended with $Fe(OH)_3$. Benzoic acid was transformed

after a lag period of 12 days in most of the sediment slurries amended with 10 mM molybdate except in the sediment slurries collected from station 5 and amended with $Fe(OH)_3$. Addition of 10 mM molybdate partially or markedly inhibited the removal of benzoic acid. No significant losses of benzoic acid were observed in autoclaved-sediment slurries.

Pyridine Biotransformation

Pyridine was removed within 110 days after a lag period of 53 days in the sediment slurries collected from station 1 and amended with both metal oxide (either $Fe(OH)_3$

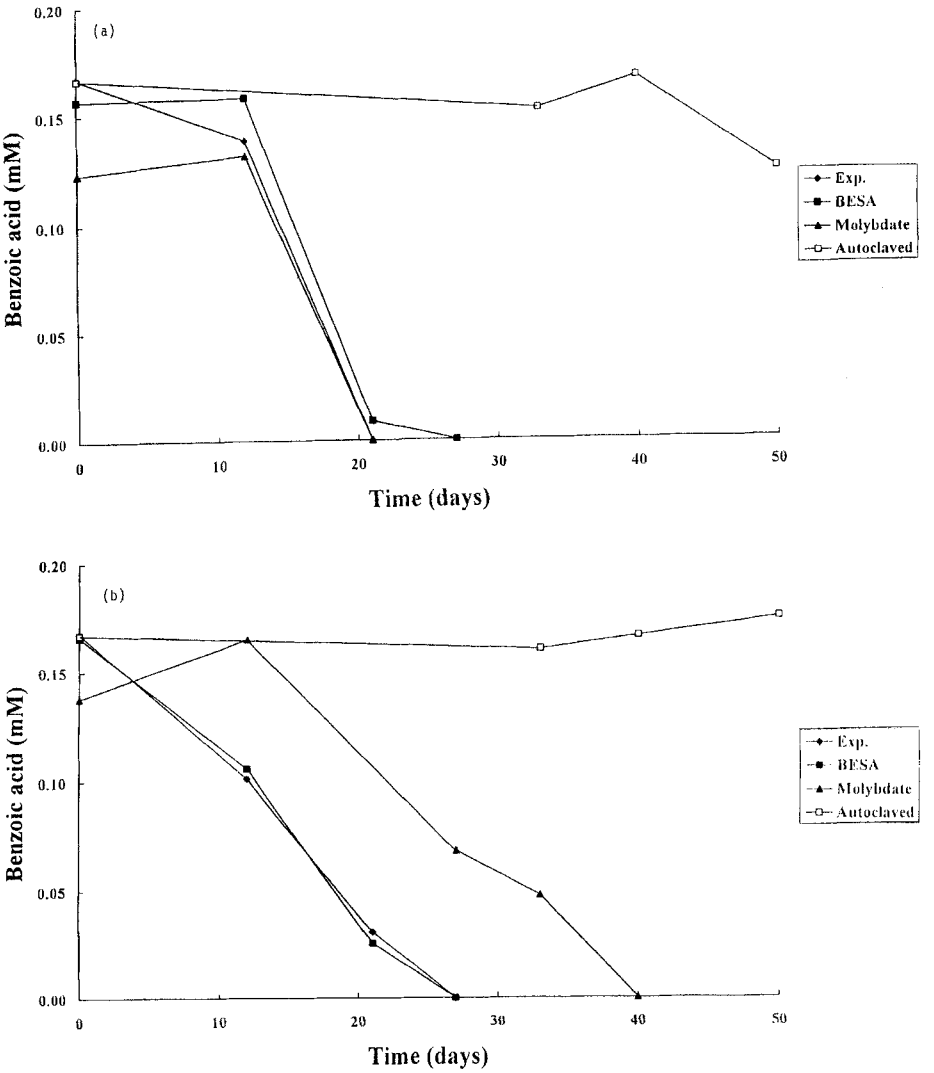

Figure 3 (See caption on next page)

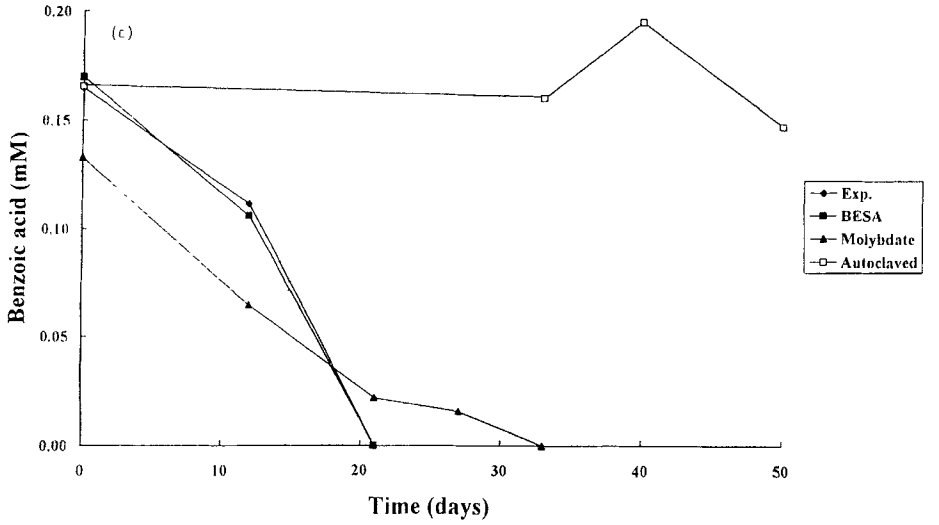

Figure 3 Examples of the anaerobic transformation of benzoic acid by sediment slurries from (a) station 1 (b) station 3 (c) station 5 amended with $Fe(OH)_3$.

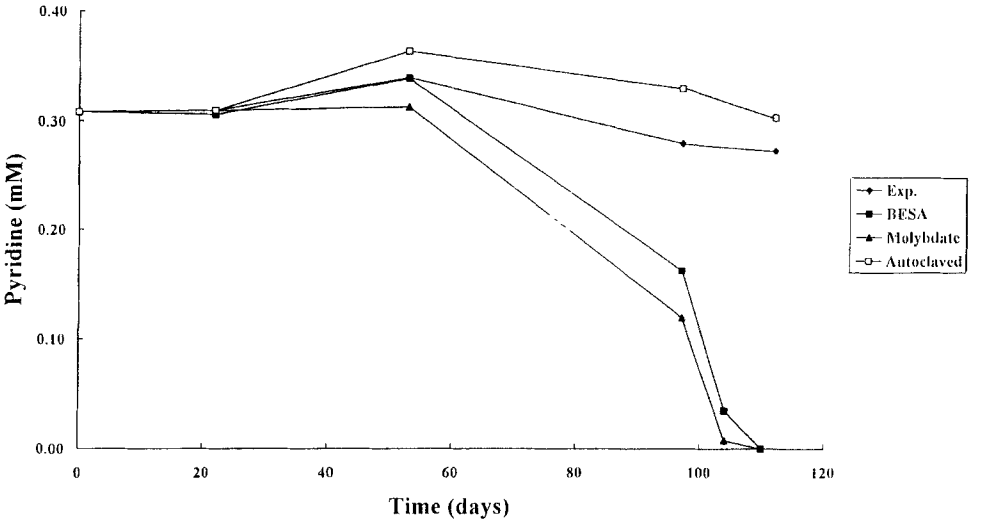

Figure 4 Examples of the anaerobic transformation of pyridine by sediment slurries from station 1 amended with $Fe(OH)_3$.

or MnO_2) and inhibitor (either BESA or molybdate) (Fig. 4). No transformation product was observed in these sediment slurries. Pyridine was persistent in all other sediment slurries up to 4 months. No significant losses of pyridine were observed in autoclaved-sediment slurries.

Table III Summary of benzoate removal by anaerobic slurries.

Station	*Added metal*	*Experimental*		*BESA*		*Molybdate*		*3 × autoclaved*
		rate[a] (μ*M*/*day*)	*lag period*[b] (*day*)	*ratc*[a] (μ*M*/*day*)	*lag period*[b] (*day*)	*rate*[a] (μ*M*/*day*)	*lag period*[b] (*day*)	*rate*[a] (μ*M*/*day*)
1	Mn(IV)	16.03	12	19.22	12	15.60	12	0.0
	Fe(III)	15.39	12	17.55	12	14.62	12	0.0
2	Mn(IV)	16.08	12	21.68	12	7.41	12	0.0
	Fe(III)	13.41	0	9.31	12	7.58	12	0.0
3	Mn(IV)	16.74	12	16.92	12	8.95	12	0.0
	Fe(III)	6.20	0	6.68	0	5.90	12	0.0
4	Mn(IV)	16.97	12	17.60	12	9.27	12	0.0
	Fe(III)	14.65	12	12.51	12	7.46	12	0.0
5	Mn(IV)	18.86	12	18.86	12	2.23	12	0.0
	Fe(III)	7.84	0	8.08	0	4.04	0	0.0

BESA, 2-bromoethanesulphonic acid.

[a]Removal rate was determined from the time course of substrate disappearance, using 2 to 4 points in the linear portion of graphs that relate substrate concentration to time.

[b]Lag was defined as the period in which no change in initial benzoate concentration (0.12–0.18 mM) was observed, and was terminated at the point that revealed a decrease in concentration of at least 10%.

Quinoline Biotransformation

Quinoline was transformed to an intermediate product in all sediment slurries amended with $Fe(OH)_3$ or MnO_2 after anaerobic incubation for 80 days (Fig. 5a–5c and Tab. IV). No lag periods were noted for quinoline transformation in sediment slurries collected from station 5. However, quinoline was transformed after a lag period of 26 days in the sediment slurries collected from the other 4 stations. With the exception of the sediment slurries collected from station 1, quinoline transformation rates were slightly higher in sediment slurries amended with MnO_2 than in sediment slurries amended with $Fe(OH)_3$. As shown in Table IV, sediment slurries

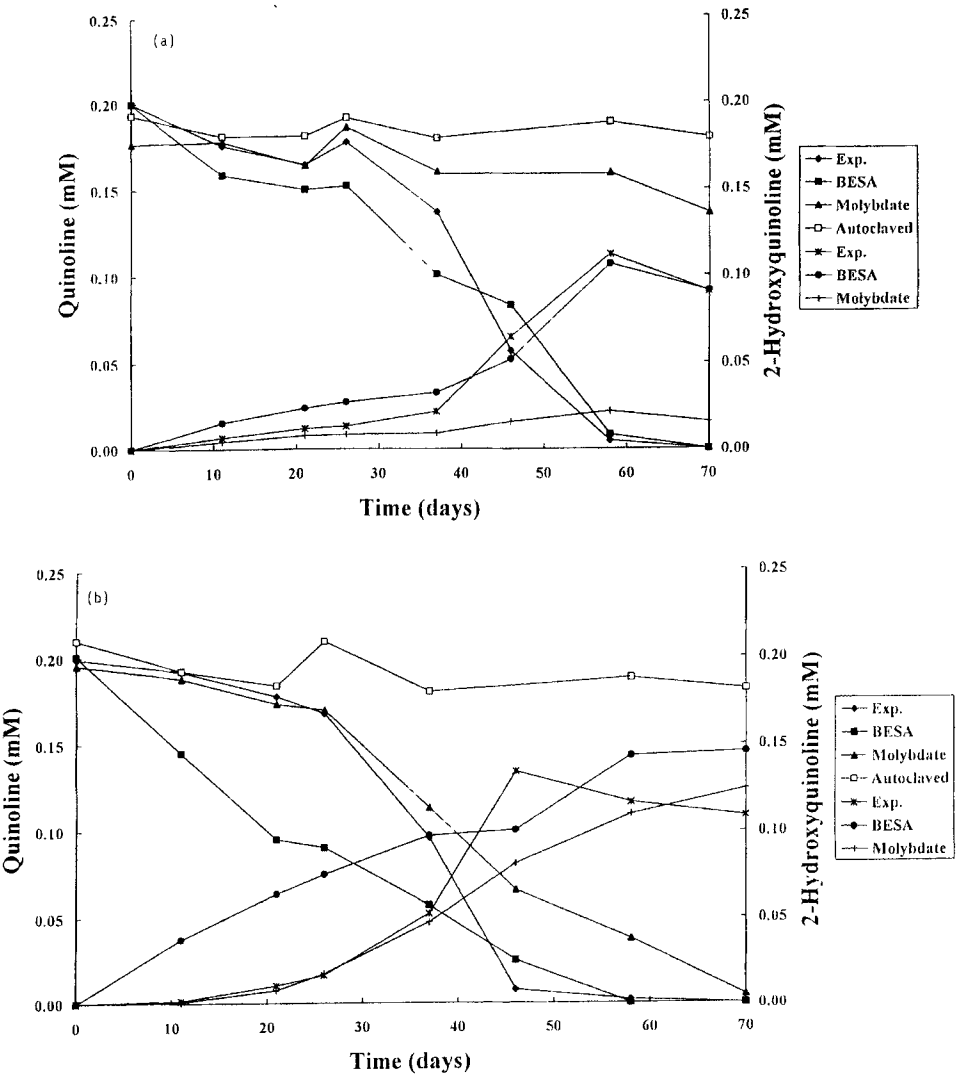

Figure 5 (See caption on next page)

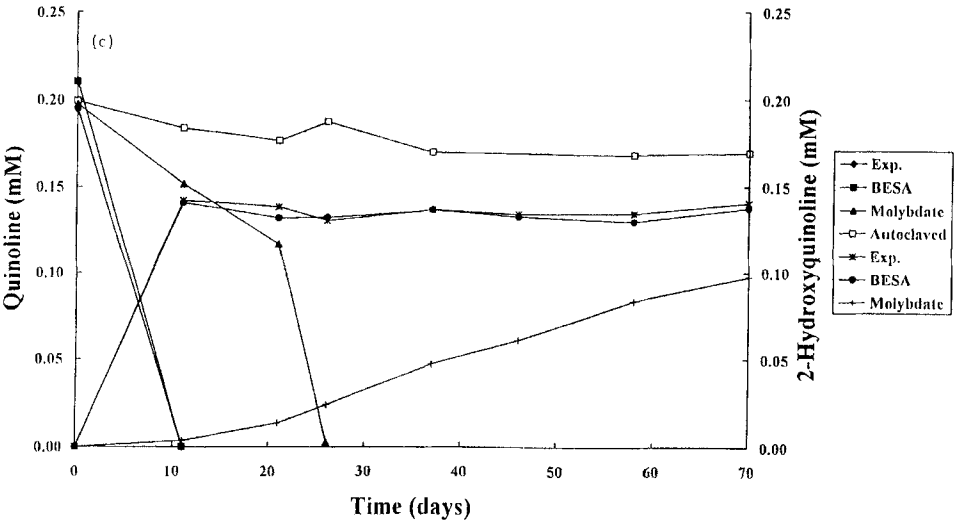

Figure 5 Examples of the anaerobic transformation of quinoline and the production of 2-hydroxyquinoline by sediment slurries from (a) station 1 (b) station 3 (c) station 5 amended with $Fe(OH)_3$.

collected from station 5 amended with MnO_2 (19.10 μMd^{-1}) or $Fe(OH)_3$ (17.67 μMd^{-1}) had the highest quinoline transformation rates. Quinoline transformation rates were higher in the oceanic environment than in the freshwater environment. Quinoline was transformed without a lag period in all the sediment slurries amended with BESA. However, with the exception of the sediment slurries collected from station 4 and 5 and amended with $Fe(OH)_3$, the addition of 10 mM BESA slightly decreased the removal rate of quinoline. In contrast, quinoline was transformed after a lag period of 26 days in most of the sediment slurries amended with 10 mM molybdate, except in the sediment slurries collected from station 5 amended with either $Fe(OH)_3$ or MnO_2, and station 4 amended with $Fe(OH)_3$. With the exception of the sediment slurries collected from station 4 and amended with MnO_2, the addition of 10 mM molybdate also partially or markedly inhibited the removal of quinoline. In each sediment slurry, transformation product was produced concomitant with the disappearance of the test compound (Fig. 5a–5c). HPLC retention time of the transformation product matches that of 2-hydroxyquinoline. In the sediment slurries collected from station 5, a stoichiometric amount of 2-hydroxyquinoline was accumulated rather than being further transformed to unknown compounds. In most other cases, the fact that no quantitative stoichiometric amount of 2-hydroxyquinoline was accumulated suggests the simultaneous production and consumption of 2-hydroxyquinoline. No significant losses of quinoline were observed in autoclaved-sediment slurries.

DISCUSSION

Although abundant literature exists on the biodegradability of chlorinated aniline, there are few data on the extent of unsubstituted aniline mineralization, toxicity, or

Table IV Summary of quinoline removal by anaerobic slurries.

Station	*Added metal*	*Experimental*		*BESA*		*Molybdate*		*3 × autoclaved*
		rate[a] (μ*M*/*day*)	*lag period*[b] (*day*)	*ratc*[a] (μ*M*/*day*)	*lag period*[b] (*day*)	*rate*[a] (μ*M*/*day*)	*lag period*[b] (*day*)	*rate*[a] (μ*M*/*day*)
1	Mn(IV)	4.09	26	3.23	0	0.79	26	0.0
	Fe(III)	5.41	26	3.30	0	0.58	26	0.0
2	Mn(IV)	5.49	26	4.86	0	3.06	26	0.0
	Fe(III)	5.08	26	3.80	0	4.26	26	0.0
3	Mn(IV)	8.54	26	6.00	0	3.79	26	0.0
	Fe(III)	8.00	26	3.46	0	3.75	26	0.0
4	Mn(IV)	4.18	26	3.58	0	4.25	26	0.0
	Fe(III)	3.34	26	3.51	0	2.52	0	0.0
5	Mn(IV)	19.10	0	17.77	0	11.71	0	0.0
	Fe(III)	17.67	0	19.10	0	7.48	0	0.0

BESA, 2-bromoethanesulphonic acid.

[a] Removal rate was determined from the time course of substrate disappearance, using 2 to 4 points in the linear portion of graphs that relate substrate concentration to time.

[b] Lag was defined as the period in which no change in initial quinoline concentration (0.16–0.21 mM) was observed, and was terminated at the point that revealed a decrease in concentration of at least 10%.

likely mechanisms of aniline metabolism. Nonetheless, the anaerobic degradation of aniline has been observed under nitrate reduction (Bollag and Russel, 1976), sulphate reduction (Schnell *et al.*, 1989), and methanogenic conditions (Myers *et al.*, 1994). Alexandra *et al.* (1994) reported that aniline was completely mineralized to CO_2 and N_2 in estuarine sediment under anaerobic denitrifying conditions amended with bicarbonate. Under anaerobic denitrifying conditions with no bicarbonate, aniline depletion was observed, however, stoichiometric quantities of N_2 were not produced from mineralization. Under methanogenic conditions, aniline concentration remained unchanged for > 31 weeks. However, they did not investigate aniline degradation under methanogenic conditions amended with bicarbonate. Schnell and Schink (1991) also demonstrated that aniline metabolism by the sulphate-reducing bacteria was enhanced in the presence of bicarbonate. However, in the present study, although bicarbonate (1.2 $g l^{-1}$) was amended to all sediment slurries, aniline remained persistent in all sediment slurries up to 4 months. Longer incubation periods might be needed for aniline biotransformation.

Myers *et al.* (1994) examined the potential for the anaerobic degradation of benzoic acid in sediments from Milwaukee Harbor, Green Bay and Lake Erie amended with $Fe(OH)_3$ or MnO_2, and found that while sulphate reducers might have an essential role in the degradation of benzoic acid, methanogens did not. In the present study, we found that either methanogens or sulphate reducers might have a role in benzoic acid removal in a freshwater environment, while sulphate reducers might have an essential role in benzoic acid removal in an oceanic environment. In any case, anaerobic benzoic acid degradation by sulphate (Cord-Ruwisch and Garcia, 1985) and iron reducing bacteria (Lovley *et al.*, 1989; Lovley and Lonergan, 1990) has been reported in other ecosystems.

Shanker *et al.* (1991) reported that pyridine degradation in deep subsurface sediments was observed only rarely under sulphate-reducing conditions and never under methanogenic conditions. Kaiser and Bollag (1991), using ^{14}C-pyridine, also reported that pyridine mineralization under sulphate-reducing conditions was only 10% of that under denitrifying conditions in anaerobic sewage sludge. Based on these and several other investigations (e.g. Liu *et al.*, 1994a; Sims and O'Loughlin, 1989), it seems that pyridine is more readily degraded in the sediment under denitrifying than either sulphate-reducing or methanogenic conditions. In the present study we observed that with the exception of sediment slurries from the freshwater environment which had been amended with both metal oxide (either $Fe(OH)_3$ or MnO_2) and inhibitor (either BESA or molybdate), pyridine was persistent in essentially all the sediment slurries up to 4 months. Since the addition of BESA and molybdate to the sediment slurries would inhibit methanogens and sulphate reducers respectively, these results suggest that iron or manganese reducers may play an essential role in pyridine removal in the freshwater environment.

In a previous study, we reported that quinoline (10 mg l^{-1}) was completely transformed without a lag phase under methanogenic and sulphate-reducing conditions after incubation for 23 and 45 days respectively in sediment collected from Athens, Georgia, U.S.A. (Liu *et al.*, 1994b). Quinoline transformation rate was higher under methanogenic conditions than under sulphate-reducing conditions. In

the present study, which used sediment slurries collected from the Tsengwen River, we observed that quinoline was more readily transformed in the sediment slurries collected from an oceanic environment than from a freshwater environment. Addition of BESA to the sediment slurries decreased or slightly increased the rate of quinoline transformation, while addition of molybdate markedly inhibited the rate of quinoline transformation in the sediment slurries either from freshwater or oceanic environments. These results suggest that sulphate reducers have an essential role in quinoline transformation. Hydroxylation is the intial step of biodegradation for some N-heterocyclic compounds under anaerobic conditions. Evidence for hydroxylation of quinoline at the number 2 carbon has been reported (Liu *et al.* 1994a, b; Pereira *et al.*, 1987 a, b). A similar pattern of hydroxylation of quinoline was observed in this study; 2-hydroxyquinoline was assumed to be the transformation product of quinoline. As shown in Fig. 5c, a stoichiometric amount of 2-hydroxyquinoline was produced in 11 days in some sediment slurries collected from station 5 and amended with $Fe(OH)_3$. However, it was not further transformed during another 60 days incubation. In contrast, 2-hydroxyquinoline was further transformed before stoichiometric amounts were accumulated in some sediment slurries collected from station 1 and amended with $Fe(OH)_3$. It seems that microbial communities responsible for quinoline transformation are different from the microbial communities responsible for 2-hydroxyquinoline transformation.

In our studies, the inhibitory effects of molybdate on benzoic acid and quinoline transformation suggest a partial to marked role for sulphate reducers in the transformation of these compounds. Sulphate reducers are sometimes dismissed as trivial components in freshwater sediments because the levels of sulphate are much lower than those in marine systems. However, sulphate reducers evidently have the potential to play a role in the transformation of aromatic compounds in freshwater sediments.

In the present study, we investigated the role of methanogens, sulphate reducers, and metal reducers in the degradation of aromatic compounds by including BESA or molybdate in some sediment slurries amended with $Fe(OH)_3$ or MnO_2. Although Myers and Nealson (1988) observed that addition of 9 mM molybdate does not affect the Mn(IV)-reducing activity of *Shewanella putrefaciens* MR-1, it is possible that the effects of molybdate were not directly solely at sulphate reducers.

In most cases, transformation rates of benzoic acid and quinoline were higher in the sediment slurries amended with Mn(IV) than those amended with Fe(III). Also neither the methanogen nor the sulphate reducer had an essential role in pyridine removal in freshwater environment. These data suggest, but do not prove, a potential role for metal reducers. At present it is still premature to affirm or disaffirm a role for metal-reducing bacteria in the transformation of aromatic compounds in these sediments.

Sand and silt composition of the sediment collected from the 5 stations were different. Sediment collected from station 1, 2 and 5 contained more sand, while sediment collected from station 3 and 4 contained more silt. In the present study, no correlation was found between sand and silt composition and the microbial activities. In spite of the microbiological and geochemical complexities of these systems, the findings in the present study suggest the possibility that toxic aromatics in sediment

collected from Tsengwen River may be transformed by *in situ* populations. Additional studies are necessary to gain further insight into the degradative potential of these sediments.

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