Acidogenic pretreatment of wastewaters containing 2-nitrophenol

K.B.S.N. Jinadasa, C.H. Mun, M.A.Aziz and W.J. Ng

Centre for Water Research, Department of Civil Engineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260 (E-mail: *engngwj@nus.edu.sg*)

Abstract Anaerobic Toxicity Assay (ATA) tests were conducted on acidogenic cultures to assess the feasibility of using acidogenic processes to treat wastewaters containing 2-nitrophenol. Results indicated 2-nitrophenol could be removed with a removal efficiency of more than 98%. 2-aminophenol was identified as the major metabolite of the biotransformation of 2-nitrophenol. Reduction in inhibition potential of acidogenic pretreated effluent was observed in the aerobic process. EC_{50} values of 2-nitrophenol and 2-aminophenol were found to be 0.065 mM and 1.83 mM respectively.

Keywords Acidogenesis; 2-aminophenol; biotransformation; 2-nitrophenol

Introduction

Industrial development has led to increased usage of chemicals, a portion of which are xenobiotic. As such, many industrial wastewaters may contain persistent organics that are potentially inhibitory to microorganisms. When these wastewaters are discharged into water bodies, they may cause long-term adverse effects on water quality and human health.

Biological treatment is often the most economical means for removing the bulk of organic pollutants in wastewaters. The biodegradability of persistent organics such as nitroaromatic compounds had been reported under aerobic and anaerobic conditions (Donlon *et al*., 1996; Uberoi and Bhattacharya, 1997; Razo-Flores *et al*., 1997). However, the removal of potentially inhibitory organic compounds by aerobic processes alone may be insufficient to meet increasingly stringent effluent discharge standards (Ng *et al*., 1999).

Anaerobic processes are widely used to treat industrial wastewaters with high organic strength. Recent studies have revealed their ability to treat wastewaters containing aromatic compounds (Karim and Gupta, 2001; Razo-Flores *et al*., 1997). However, anaerobic processes have been shown to be sensitive to potentially inhibitory compounds and long acclimation periods are often needed before their successful degradation can take place. The need to accommodate such sensitivities has meant anaerobic biotreatment has often proved to be not cost competitive.

Nitrophenols are nitroaromatic derivatives present in some industrial wastewaters as they are widely used as intermediates in industries such as pesticides, herbicides, dyes and manufacturing solvents. They are considered to be potentially carcinogenic and mutagenic. As such, 2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol have been classified as the priority pollutants by US EPA (1976). In addition, US EPA has restricted their concentration in natural water to be less than 10 μg/L.

Nitrophenols are reported to be biodegradable in the anaerobic process, but a long degradation period is required. Acidogens, on the other hand, have been reported to be dissipating excess reducing capability on nitroaromatic compounds (Spain *et al*., 2000). Hence, the acidogenic phase could be feasible as a pretreatment stage to improve the biodegradability of nitrophenols. This study was conducted to assess the feasibility of using the acidogenic process as pretreatment for wastewater containing 2-nitrophenol.

Water Science and Technology **Vol 50 No 8 pp 119–124** © IWA Publishing 2004

Materials and methods

Stock culture

Seed sludge was obtained from an anaerobic digester at a local sewage treatment plant. The collected sludge was filtered using a laboratory sieve with an aperture of 600 µm prior to seeding. A 5.0 L conical flask was used as the Acidogenic Sequencing Batch Reactor (AcSBR). Temperature and pH were maintained at 35 ± 1°C and 4.8–5.2 respectively. MLVSS, HRT and SRT were maintained at 8,000 mg/L, 24 hours and 10 days respectively. The experimental setup is shown in Figure 1. Synthetic wastewater with sucrose as the primary carbon source was used to cultivate the biomass. The composition of the feed, inorganic nutrients and trace elements are shown in Tables 1 to 3. The reactor had been operated continuously for 2 years.

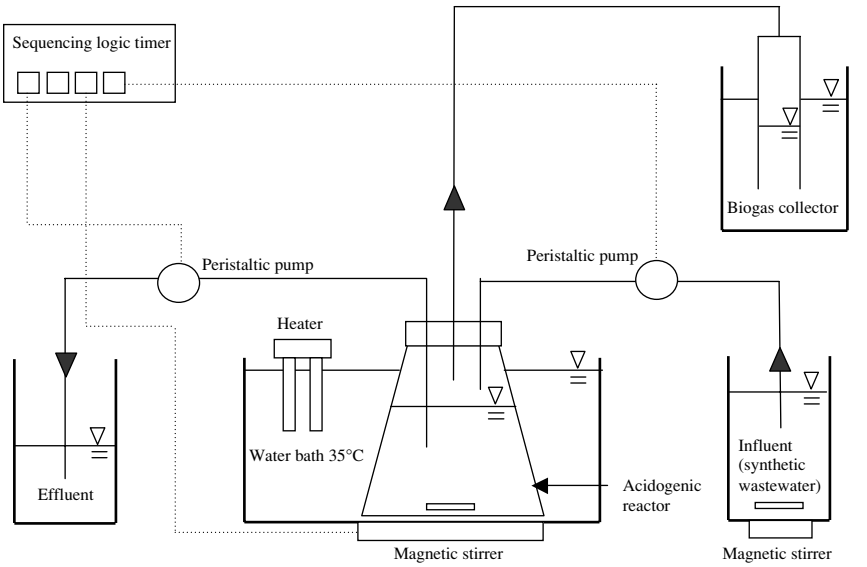

Figure 1 Experimental setup of the acidogenic sequencing batch reactor

Table 1 Feed composition for the acidogenic sequencing batch reactor

Component	Functional role	Concentration
Sucrose	Carbon source	–8.82 g/L
Inorganic nutrients	Nutritional supplement	–30 mL/L
Trace elements	Nutritional supplement	–1 mL/L
Sodium bicarbonate	pH buffer	–3.40 g/L

Table 2 Composition of stock solution of inorganic macronutrients supplement

Chemical name	Chemical formula	Concentration (g/L)
Calcium chloride dihydrate	$CaCl_2.2H_2O$	3.67
Magnesium chloride hexahydrate	$MgCl_2.6H_2O$	4.17
Iron (III) chloride hexahydrate	$FeCl_3.6H_2O$	0.50
Ammonium chloride	NH_4Cl	14.33
Dipotassium hydrogen phosphate anhydrous	K_2HPO_4	3.00
Potassium dihydrogen phosphate	KH_2PO_4	1.00
Sodium sulphate anhydrous	Na_2SO_4	2.22

Table 3 Composition of stock solution of trace elements supplement

Chemical name	Chemical formula	Concentration (g/L)
Manganese (II) chloride dehydrate	$MnCl_2.2H_2O$	0.500
Zinc chloride	$ZnCl_2$	0.250
Sodium molybdate	$NaMoO_4.2H_20$	0.020
Cobalt (II) chloride hexahydrate	$CoCl_2.6H_2O$	0.025
Nickel (II) chloride hexahydrate	$NiCl_2.6H_2O$	0.250
Boric acid	H_3BO_4	0.250
Thiamine chloride hydrochloride	$Cl_2H_{18}C_{12}N_2OS$	2.000

Anaerobic toxicity assay (ATA) test

The ATA test was performed according to the method reported by Owen *et al.* (1979). Serum bottles (150 mL capacity) were cleaned with 1:1 HCl acid solution and rinsed with tap water and distilled water. The following were added to each serum bottle: 75 ml of acidogenic sludge from the reactor, 3 ml of inorganic nutrients, 0.1 ml of trace metal solution, 0.882 g of sucrose, 0.17 g of sodium bicarbonate and varying concentrations of 2-nitrophenol. Distilled water was then added to obtain a final volume of 100 mL. Serum bottles were sealed with rubber septa and aluminium caps. The bottles were then flushed with nitrogen gas to ensure anaerobic conditions.

Analytical methods

2-nitrophenol and its metabolite, 2-aminophenol, were determined using a Shimadzu HPLC with a UV-VIS spectrophotometric detector. A reverse phase 4.6 mm × 100 mm and packing size of 3.5 μm Xterra C18 column was used. The eluent composition was 30/70 HPLC grade methanol/deionised water for 2-nitrophenol and 50/50 0.5 M sodium acetate/deionised water for 2-aminophenol. Eluent flow rate was 1.0 mL/min while the detection wavelength used was 254 nm with a sample volume of 20 μL.

EC_{50} inhibition test was performed according to the experimental procedures as described in the International Standards Organisation's (ISO) "Water quality – Test for Inhibition of Oxygen Consumption by Activated Sludge" (ISO 8192-1986-E Method B). A modified version of this test was used to determine the inhibition potential of the influent and the effluent. In this test, 50 mL samples were used as test material. Measurements of pH, suspended solids and volatile suspended solids were made in accordance with *Standard Methods* (1998). The experimental results reported herein represent the average of at least three measurements.

Results and discussions

Removal efficiency of 2-nitrophenol

Removal of 2-nitrophenol is shown in Figure 2. It can be seen that even at a concentration of 100 mg/L, the removal efficiency was around 99% in the batch ATA test. This suggested that the acidogenic phase could remove 2-nitrophenol effectively. This result is in contrast to the long degradation required in conventional anaerobic processes (Donlon *et al.*, 1996; Uberoi and Bhattacharya, 1997).

Biotransformation of 2-nitrophenol to 2-aminophenol

It was noted from Figure 3 that the effluent contained high concentrations of 2-aminophenol. Figures 2 and 3 suggest 2-aminophenol is the major metabolite of 2-nitrophenol. On a molar basis, approximately 70% of 2-nitrophenol was recovered through this metabolite. The remaining percentage could be due to biosorption of 2-nitrophenol, formation of other metabolites or biotransformation of 2-aminophenol.

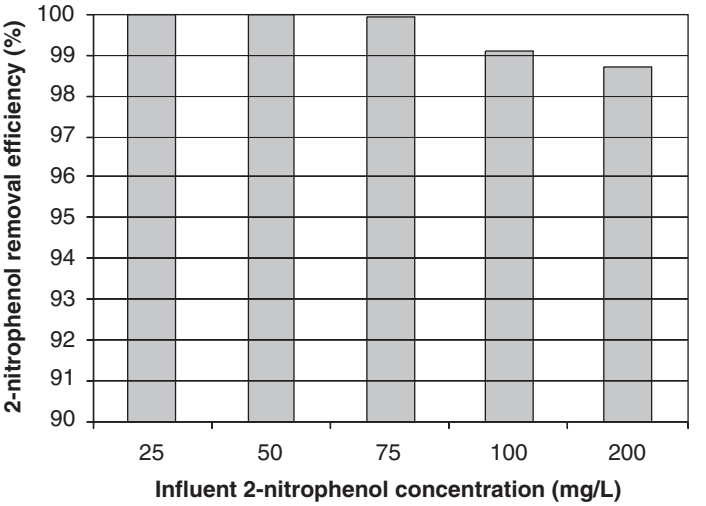

Figure 2 Removal efficiency of 2-nitrophenol at different concentrations

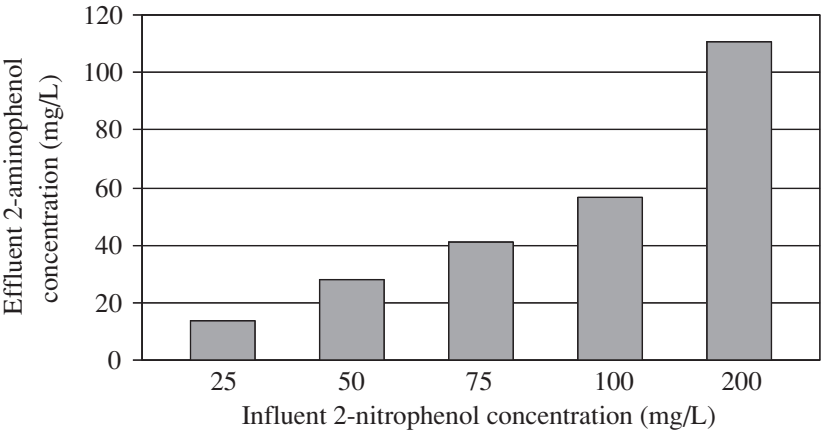

Figure 3 Metabolite production of 2-aminophenol at different concentrations of 2-nitrophenol

Karim and Gupta (2002) had demonstrated the sorptive capacity of nitrophenols on anaerobic granular sludge and autoclaved acidogenic biomass. Although biotransformation of 2-aminophenol under acidogenic conditions is possible, preliminary findings from the acidogenic batch ATA test indicated that 2-aminophenol could not be further biotransformed in the short time period.

70% recovery of 2-nitrophenol was obtained in the present study compared to the higher recoveries reported by Razo-Flores *et al*. (1997) and Donlon *et al*. (1996) who reported biodegradation of 2-nitrophenol to 2-aminophenol in stoichiometric quantities under methanogenic conditions.

Influent and effluent inhibition test

As 2-nitrophenol was transformed to 2-aminophenol and other metabolites during acidogenesis, it is necessary to investigate whether these metabolites will inhibit the subsequent treatment process i.e. aerobic biotreatment.

The results of the inhibition test conducted on influent and effluent are presented in Table 4. Activated sludge dosed with effluent had higher respiration rates compared to activated sludge dosed with the influent. The higher respiration rate is indicated by the

increase in oxygen uptake rate. Negative inhibition (%) indicates possible stimulatory effects while positive inhibition (%) represents potential inhibitory effects on the aerobic process by the test compounds. It was observed that there was a reduction in the inhibition potential of the effluent compared to the influent. The decrease in inhibition could be due to the conversion of the potentially inhibitory organics present in the wastewater into less inhibitory or non-inhibitory forms. Other studies have also reported similar results and a study has reported 2-aminophenol was completely mineralized under aerobic conditions (Melgoza *et al.*, 2000).

Table 4 Inhibition test results of influent and effluent with sucrose as co-substrate

		Respiration rate (mg/L DO.min)	Inhibition (%)	Decrease in inhibition (%)
Control		0.8985	–	–
25 mg/L	Influent	0.894	–0.50	51.98
	Effluent	1.370	–52.48	
50 mg/L	Influent	0.925	–2.95	17.58
	Effluent	1.083	–20.53	
75 mg/L	Influent	0.932	–3.73	13.07
	Effluent	1.050	–16.80	
100 mg/L	Influent	0.851	5.29	13.92
	Effluent	0.976	–8.63	
200 mg/L	Influent	0.638	29.00	23.50
	Effluent	0.849	5.50	

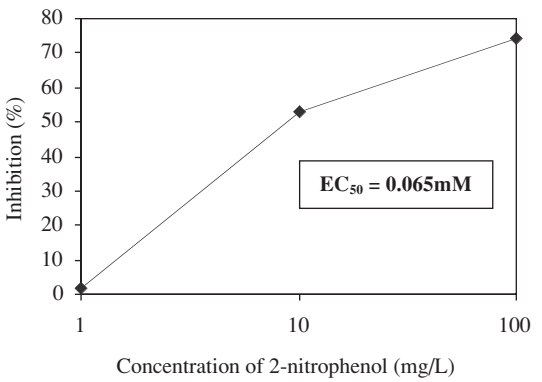

Figure 4 EC_{50} of 2-nitrophenol

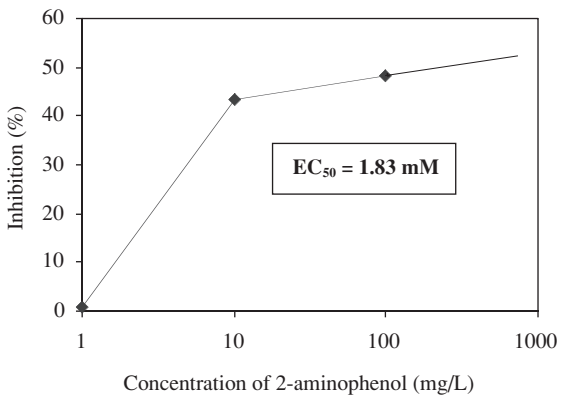

Figure 5 EC_{50} of 2-aminophenol

EC_{50} of 2-nitrophenol and its metabolite

The EC_{50} values of 2-nitrophenol and 2-aminophenol were found to be 0.065 mM and 1.83 mM as shown in Figures 4 and 5. On a molar basis, 2-aminophenol is approximately 28 times less inhibitory to the aerobic process compared to 2-nitrophenol. Similar findings (3.21 mM and 0.089 mM respectively) were reported by Donlon *et al*. (1995) where 2-aminophenol and 2-nitrophenol inhibited acetoclastic methanogenic bacteria.

Conclusions

From the experimental results presented and discussed herein, the following conclusions are drawn.

- The acidogenic phase of the anaerobic process is effective in removing 2-nitrophenol from wastewaters.
- 2-aminophenol was identified as a possible metabolite. Around 70% of 2-nitrophenol was recovered through this metabolite.
- Acidogenic biotreatment has a potential as a pretreatment process for industrial wastewaters containing persistent organic compounds.

References

Donlon, B.A., Razo-Flores, E., Field, J.A. and Lettinga, G. (1995). Toxicity of N-substituted aromatics to acetoclastic methanogenic activity in granular sludge. *Appl. Environ. Microb.*, **61**(11), 3889–3893.

Donlon, B.A., Razo-Flores, E., Lettinga, G., and Field, J.A. (1996). Continuous detoxification, transformation, and degradation of nitrophenols in upflow anaerobic sludge blanket (USAB) reactors. *Biotechnol. Bioeng.*, **51**(4), 439–449.

International Standards Organisation (ISO). *Water quality – Test for inhibition of oxygen consumption by activated sludge*. ISO 8192. 1986 edition.

Karim, K. and Gupta, S.K. (2001). Biotransformation of nitrophenols in upflow anaerobic sludge blanket reactors. *Bioresource Technol.*, **80**(3), 179–186.

Karim, K. and Gupta, S.K. (2002). Biosorption of nitrophenols on anaerobic granular sludge. *Environ. Technol.*, **23**(12), 1379–1384.

Melgoza, R.M., Chew, M. and Buitron, G. (2000). Start-up of a sequential anaerobic/aerobic batch reactor for the mineralization of p-nitrophenol. *Wat. Sci. Tech.*, **42**(5), 289–292.

Ng, W.J., Hu, J.Y., Ong, S.L. and Aziz, M.A. (1999). Effect of acidogenic stage on aerobic toxic organic removal. *J. Environ. Eng.*, **125**, 495–500.

Owen, W.F., Stuckey, D.C., Healy, J.B., Jr., Young, L.Y. and McCarty, P.L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Wat. Res.*, **13**(6), 485–492.

Razo-Flores, E., Donlon, B., Lettinga, G. and Field, J.A. (1997). Biotransformation and biodegradation on N-substituted aromatics in methanogenic granular sludge. *FEMS Microbiol. Rev.*, **20**(3–4), 525–538.

Spain, J., Hughes, J.B. and Knackmuss, H. (2000). *Biodegradation of Nitroaromatic Compounds and Explosives*. Lewis Publishers. New York, NY, USA.

Standard Methods for the Examination of Water and Wastewater (1998). 20th ed, American Public Health Association, Washington DC, USA.

Uberoi, V. and Bhattacharya, S.K. (1997). Toxicity and biodegradability of nitrophenols in anaerobic systems. *Wat. Environ. Res.*, **69**(2), 146–156.

US EPA (1976). *Water Quality Criteria*. US Environmental Protection Agency. Washington DC, USA.