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INHIBITION OF METHANOGENIC ACTIVITY OF STARCH-DEGRADING GRANULES BY AROMATIC POLLUTANTS

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ABSTRACT

The effects of nine common aromatic pollutants from chemical industry on the bioactivity of anaerobic granules were examined. The granules were obtained from an upflow anaerobic sludge blanket (UASB) reactor treating wastewater containing colloidal starch. The specific methanogenic activities (SMA) of granules were measured at 37°C in serum vials using 3000 mg/l of colloidal starch as substrate, plus individual pollutants at various concentrations. The toxicity was expressed by the IR_{50} and IC_{50} values, i.e. the toxicant/biomass ratio and concentration at which levels the granules exhibited only 50% of their original bioactivities. Results showed that in general the granules exhibited mild resistance to toxicity of aromatic pollutants, probably due to the granules' layered microstructure. The toxicities, which were dependent on the nature of chemical functional group, of the aromatic pollutants were in the following descending order: cresols > phenol > hydroxyphenols/phthalate > benzoate. There was only marginal difference between the toxicity of the steric isomers. For the seven phenolic pollutants, the more hydrophobic the functional group the higher the toxicity. The granules' resistance to toxicity suggested the plausibility of anaerobic treatment of wastewater from the chemical industry. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Anaerobic; aromatic; granule; inhibition; microstructure; phenol; SMA; starch; toxicity; UASB.

INTRODUCTION

Wastewater of chemical industries commonly contains aromatic pollutants, such as benzoate, phthalate, phenol and its derivatives, etc. Benzoate is a pharmaceutical raw material, while phthalate is used in the plastic industry as plasticizer as well as the raw material for making resins. Phenol and its derivatives, on the other hand, are used for making a variety of specialty chemicals, such as synthetic resins for construction materials and adhesives, antioxidants, herbicides, photo-developing chemicals, etc. (Kirk-Othmer, 1978). In addition, phenolic pollutants are often found in wastewater from coal conversion facilities, coke-oven batteries, oil refinery and petrochemical plants (Patterson, 1975). It is often believed that aromatic chemicals are refractory to biodegradation and are likely toxic to microorganisms in biological wastewater systems.

Anaerobic technology has become viable for the treatment of industrial wastewater in the last two decades, mainly because of the development of high-rate reactors (Speece, 1983). Among them, the upflow anaerobic sludge blanket (UASB) process has been widely accepted in recent years particularly for the treatment of wastewaters from agricultural and food/beverage industries (Lettinga and Hulshoff Pol, 1991). In a UASB reactor, biomass aggregate to form granules. These granular biomass exhibit increased settleability, which prevents them from being washed out, as well as high bioactivity, which enhances the reactor efficiency (Lettinga *et al.*, 1980). Fang and Chui (1994) compared the startup performance of four types of high-rate anaerobic reactors for the treatment of wastewater containing concentrated carbohydrate pollutants. UASB was found superior to anaerobic filter, fluidized-bed and expanded-bed reactors. Another study (Fang and Chui, 1993) found that UASB reactors were capable of removing over 90% of COD for loading rates as high as 160 g-COD/l.d in treating wastewater containing soluble carbohydrates such as sucrose.

In addition, a recent study also demonstrated that UASB could be effective for the treatment of wastewater containing high levels of biodegradable suspended solids, such as colloidal starch (Kwong and Fang, to be published). Furthermore, a more recent study showed that UASB granules exhibited higher resistance to the toxicity of heavy metals than the flocculent digester sludge (Fang and Hui, 1994). The capability of resisting toxicity of the granules could be attributed to their layered microstructure, as observed under electron microscopes (Fang *et al.*, 1994).

This study was conducted to investigate the toxic effect of nine individual aromatic pollutants on the methanogenic activity of starch-degrading UASB granules.

MATERIALS AND METHODS

A 2.8 litre UASB reactor, as illustrated in Figure 1, was used for this study. About 1 litre of starch-degrading UASB granules obtained from a previous study (Kwong and Fang, to be published) were used to seed the reactor along with 1.5 litres of additional sludge from the digester of a local municipal sewage treatment plant. The reactor was then continuously fed with synthetic wastewater containing starch as the sole organic carbon source, plus nutrients, alkalinity and trace elements based on the formulation used in a previous study (Fang and Chui, 1993). The COD loading gradually increased from 3 g/l.d, initially, to 15 g/l.g over a two-month period, during which a granular sludge bed was developed and the reactor steadily removed over 95% of COD in wastewater. The reactor was then kept at the latter loading throughout the rest of the study.

After the reactor was steady at 15 g-COD/l.d, samples of sludge granules were periodically taken from the UASB reactor for bioactivity analysis. The specific methanogenic activity (SMA) of the sampled granules was measured by the rate of converting starch into methane in the 157 ml serum vials (Owen *et al.*, 1979; Dolfing and Mulder, 1985). Each vial was filled with 100 ml of synthetic wastewater which contained 3000 mg/l of colloidal starch as substrate along with nutrients, vitamins, trace elements, etc., plus individual aromatic pollutants as controlled dosage. After granules were transferred, each vial was capped with butyl rubber and submerged in 37°C water in a shaking bath. About 100 mg of biomass were added to each vial; the exact amount was measured at the end of each test by the content of volatile suspended solids (VSS) following the standard methods (APHA, 1985). Transfers of wastewater and granules to the serum vials were conducted in an anaerobic environment.

Biogas production volume in each vial was monitored every few hours by a syringe. The methane content in the biogas was measured using a gas chromatograph (Hewlett Packard, model 5890A) equipped with a thermal conductivity detector and a 2m x 2mm (inside diameter) stainless-steel column packed with Porapak N (80-100 mesh). Injector and detector temperatures were respectively kept at 130°C and 200°C, while column temperature was increased from 90°C to 110°C. Based on these measurements, the accumulated methane productions were calculated. Each SMA measuring program lasted about one week by which time the methane production had normally levelled off. The quantity of biomass in each vial was then measured based on the VSS content. Previous study (Fang and Kwong, 1994) has shown that colloidal starch was readily hydrolysed and contributed little to the VSS content in the serum vial after one week.

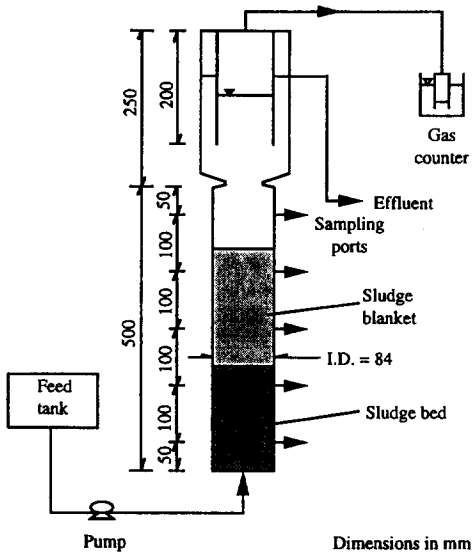

Figure 1. UASB reactor setup.

The initial slope of specific methane production against time represented the specific methanogenic activity (SMA) of the granules using colloidal starch as substrate. The SMA in each serum vial was dependent on the dosage of individual aromatic pollutants. All SMA measurements were conducted in duplicates. Up to 30 vials were monitored in each batch; at least two of the 30 vials without aromatic pollutants served as control. The toxicities of individual aromatic pollutants at various concentrations were indicated by the decrease in SMA compared with the controls.

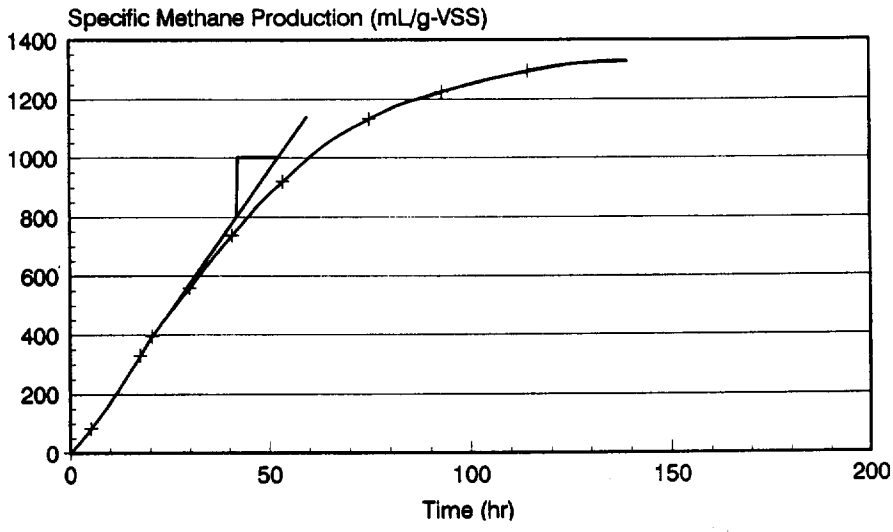

Figure 2. Specific methane production of starch-degrading granules.

RESULTS AND DISCUSSION

Figure 2 illustrates a typical plot of the methane production of control granules. The initial starch concentration in each vial was 3000 mg/l, at which level the supply of starch to the microorganisms was not a limiting factor. However, as starch became depleted, the methane production rate gradually levelled off. The initial slope, as illustrated in Figure 2, represents the SMA of the granules on the degradation of starch without the influence of any aromatic pollutants. Each gram of granules on average had the maximum hourly capacity of producing 19.6 ml of methane at 37°C, corresponding to an average SMA of 1.19 g-methane-COD/g-VSS.d.

For each aromatic pollutant, a plot similar to Figure 2 was drawn for each dosage level and the corresponding SMA was estimated. Results showed that the SMA decreased as the dosage of the individual aromatic pollutant increased, indicating an inhibition effect by the pollutant. The toxicities of individual aromatic pollutants are illustrated in Figures 3-5, in which the relative SMA (compared with the control) was plotted against the toxicant/biomass ratio for each pollutant. Figure 3 illustrates the toxicities of benzoate, phenol and phthalate over a wide range of toxicant/biomass ratio. In general, phenol was more toxic than phthalate, which was in turn more toxic than benzoate.

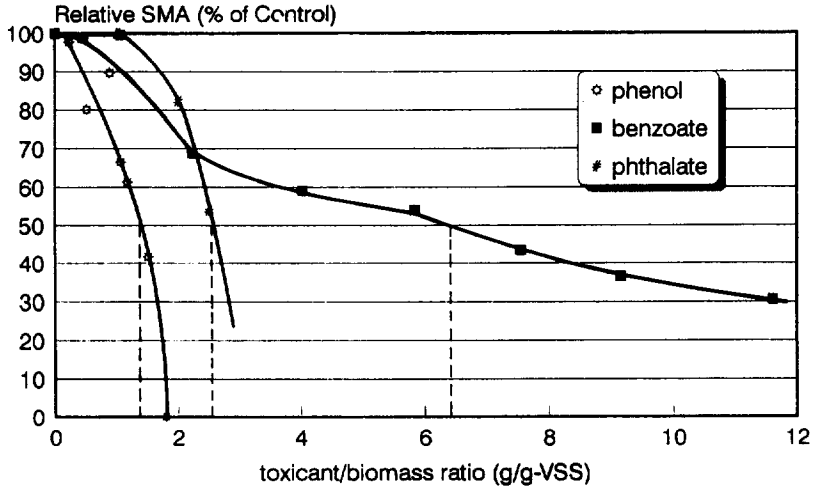

Figure 3. Inhibition of bioactivity of granules by benzoate, phenol and phthalate.

The toxicity of each aromatic pollutant to starch-degrading granules can be expressed by the IR_{50} value, which represents the ratio of toxicant to biomass at which level the methanogenic activity was reduced to 50% of the control. From Figure 3, the IR_{50} for benzoate, phthalate and benzoate were 6200, 2300 and 1500 mg-toxicants/g-VSS, respectively. The corresponding IC_{50}, i.e. the concentration of toxicant at which level the SMA of the granules was reduced by 50%, were 6600, 2500 and 1370 mg/l, respectively.

Figures 4 and 5 illustrate the toxicities of hydroxyphenol isomers, and cresol isomers. Figure 4 illustrates that, for the hydroxyphenols, catechol was more toxic than resorcinol, which in turn was more toxic than hydroquinone. Judging from the respective IR_{50} values of 1900, 2000 and 2600 mg-hydroxyphenol/g-VSS, which correspond to IC_{50} of 1920, 2100 and 2420 mg/l, the toxicities of catechol, resorcinol and hydroquinone were mild, and the difference between the isomers were small. Figure 5 illustrates that, the difference in the toxicities of the three cresol isomers were also marginal, with respective IR_{50} values of 670, 740 and 830 mg-cresol/g-VSS for ortho-, meta- and para-cresols, which correspond to IC_{50} of 675, 710 and 800 mg/l. Cresols exhibited higher inhibition to the methanogenic activity to the granules than other pollutants examined in this study.

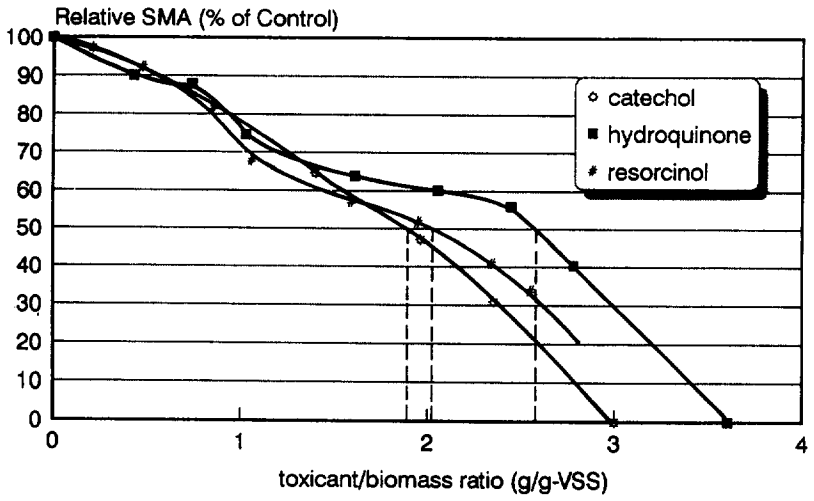

Figure 4. Inhibition of bioactivity of granules by hydroxyphenols.

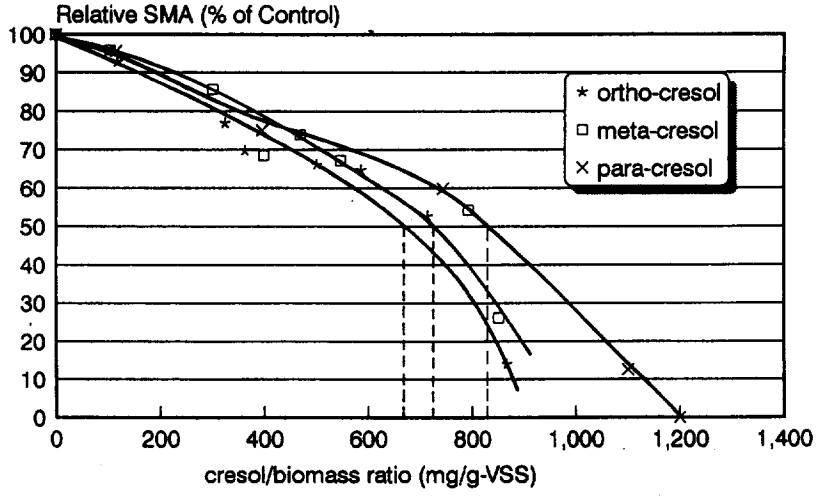

Figure 5. Inhibition of bioactivity of granules by cresol.

Table 1 summarizes the IR_{50} and IC_{50} data observed in this study for starch-degrading granules. Sierra-Alvarez and Lettinga (1991) investigated the toxic effect of organic pollutants on UASB granules treating distillery wastewater by measuring the reduction of the granules' bioactivity on converting acetate to methane. More recently, Fang $_{et\ al.}$ (1994) examined the toxic effect of phenolic pollutants on the bioactivity of converting benzoate to methane for UASB granules which had been fed with benzoate as substrate. the corresponding IC data from these two types of granules are also listed in Table 1 for comparison.

Table 1. IC50 and IR50 of aromatic pollutants for UASB granules

	Starch-Degrading		Benzoate-Degrading		Distillery
pollutants	IR_{50} (mg/g-VSS)	IC_{50} (mg/L)	IR_{50} (mg/g-VSS)	IC_{50} (mg/L)	IC_{50} (mg/L)
benzoate	6200	6600	>6000	>6000	>4880
phthalate	2300	2500	N/A	N/A	N/A
phenol	1500	1370	1840	1850	1100
catechol	1900	1920	1890	2250	1813
resorcinol	2000	2100	2340	2400	N/A
hydroquinone	2600	2420	2270	2500	N/A
ortho-cresol	670	675	870	850	N/A
meta-cresol	740	710	950	925	N/A
para-cresol	830	800	925	975	568

N/A: data not available

The three types of UASB granule in Table 1 were acclimated, prior to the SMA tests, in wastewaters containing different organic substrates, i.e. colloidal starch, benzoate and acetate. results show that there was not much difference between the three types of UASB granule in response to the toxicity of individual aromatic pollutants. Benzoate-degrading granules exhibited, in general, slightly better resistance to toxicity, probably because they had been acclimated to benzoate, which is an aromatic chemical, while the starch-degrading granules and the distillery granules had not had prior exposure to aromatic chemicals.

Results in Table 1 also show that there were only marginal differences among steric isomers on the response to the toxicity of aromatic pollutants. The toxicity was mainly dependent on the chemical nature of the pollutant's functional group. Phenols with a hydrophobic methyl functional group (i.e. cresols) were more toxic than phenol, which in turn was more toxic than those with an extra hydrophillic hydroxy functional group (i.e. hydroxyphenols). This finding was in agreement with that observed by Sierra-Alvarez and Lettinga (1991), namely, the more hydrophobic the functional group of an aromatic chemical the higher the inhibition effect to the methanogenic activity.

Although phenol and its derivatives were perceived as inhibitory to biodegradation, results of this study demonstrated that their inhibition to methanogenic activity was not severs for the UASB granules. The mild resistance towards toxicity exhibited by the UASB granules could probably be attributed to their microstructure. Observations using electron microscope have illustrated that UASB granules often exhibit a layered microstructure (MacLeod *et al.*, 1990; Fang *et al.*, 1994; Kwong and Fang, to be published). The outer layer of the granules was mostly composed of fermentative and acidogenic bacteria which were capable of converting colloidal starch and other complex pollutants into fatty acids. These bacteria shielded the toxicity-sensitive methanogens, which were populated in the interior of granule, from being exposed to the toxic pollutants.

CONCLUSION

Aromatic pollutants exhibited toxic effects on the bioactivity of anaerobic starch-degrading granules. There was only marginal difference between the toxicity of steric isomers. The toxicity was more dependent on the nature of the chemical functional group of the pollutant. For the phenolic pollutants, the more hydrophobic the functional group the more toxic to the granules. The starch-degrading granules exhibited mild resistance to the toxicity of pollutants, probably due to their layered microstructure. The toxicity resistance exhibited

by the anaerobic granules suggested the plausibility of using UASB process for the treatment of wastewater from chemical industries containing aromatic pollutants.

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