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# CO-DEGRADATION OF PHENOL AND M-CRESOL IN A UASB REACTOR

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**Abstract**

*An upflow anaerobic sludge blanket (UASB) reactor operated at 37°C, one-day hydraulic retention plus effluent recycle, was able to degrade up to 98% of phenol and 20% of m-cresol without a carbohydrate co-substrate, for wastewaters containing up to 900 mg/l of phenol and 320 mg/l of m-cresol. Further increases of phenol and m-cresol concentration in wastewater impaired the phenol-degrading of the biomass activity. However, biomass was able to regain activity once the phenolic concentrations were lowered. In treating a wastewater containing 600 mg/l of phenol, m-cresol had a threshold toxicity of 600–800 mg/l in a continuous reactor; but in a batch reactor the toxicity was progressive with a $IC_{50}$ value of 330 mg/l. The absence of intermediate acids in the reactor effluent and other results of batch experiments suggested that the initial acidogenesis was likely to be the rate-limiting step for phenol degradation, instead of the subsequent acetogenesis or methanogenesis. © 1997 Elsevier Science Ltd.*

*Key words:* Anaerobic, bioactivity, degradation, m-cresol, phenol, toxicity, UASB.

## INTRODUCTION

Phenol and its derivatives are used for making a variety of specific chemicals, such as antioxidants, herbicides, photo-developer, adhesives, synthetic resins for construction materials, etc. (Kirk-Othmer, 1978). Wastewaters from these industries naturally contain high levels of phenols. In addition, phenols are often found in wastewaters from coal gasification, coke-oven batteries, refinery and petrochemical plants (Patterson, 1975; Blum *et al.*, 1986). Phenolic pollutants are recalcitrant to biodegradation, and are toxic to microorganisms.

It was recently reported that individual simple aromatic chemicals, such as phenol and benzoate, could be effectively degraded in UASB (upflow anaerobic sludge blanket) reactors. Over 97% of phenol at concentrations up to 1260 mg/l was removed at COD (chemical oxygen demand) loading rates as high as 6 g/l/d (Fang *et al.*, 1996); and 97–99% of benzoate up to 12 500 mg/l was removed at loading rates as high as 30.6 g-COD/l/d (Li *et al.*, 1995). In a UASB reactor (Lettinga *et al.*, 1980), biomass is effectively retained in the sludge bed due to a built-in gas–liquid–solid separator on top of the reactor. The retained biomass gradually develops into granules with superb settleability. In addition, each biogranule has a micro-environment in which bacteria interact syntrophically, and thus considerably enhance the overall reactor efficiency (Fang *et al.*, 1995). It is estimated that about 1000 UASB treatment systems have been installed worldwide for the treatment of various types of high-strength industrial wastewaters.

Wastewaters from industries often contain more than one type of phenolic pollutant. Those with more complex structures are often more toxic than the simple phenol, and yet little is known about the efficiency of treating wastewater containing a mixture of phenolic pollutants. This study was thus conducted to investigate the effectiveness of treating a wastewater containing two phenolic pollutants, simple phenol and m-cresol, in a UASB reactor. Two major objectives were to determine (1) whether the two phenolic pollutants could be effectively degraded without a carbohydrate co-substrate, and, if so, (2) the maximum phenolic concentrations which could be effectively treated by the UASB process. Degradation characteristics of the biogranules were further investigated in a number of batch tests.

## METHODS

### Continuous treatment

The experiment was conducted in a 2.8-l UASB reactor (Fang *et al.*, 1996), at 37°C and at a constant

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retention time of 24 h, for 444 days. The wastewater contained phenol and m-cresol, balanced nutrient, trace elements and buffer chemical in a previously established formula (Fang *et al.*, 1996). Sucrose was added as a co-substrate only during the startup. The reactor was seeded with 1.5 l of methanogenic sludge, including 1.0 l of flocculent sludge from the anaerobic sludge digester of a local wastewater treatment plant, plus 0.5 l of partially granulated sludge treating sucrose-containing wastewater in a 65-l UASB reactor. Influent pH was at a constant 8.1.

The experiment was divided into three phases. Phase 1 (days 1–130) was the startup, which allowed the methanogenic sludge to gradually become acclimated to the increased phenolic concentrations. The concentrations of phenol and m-cresol reached 950 mg/l and 350 mg/l, respectively, at the end (Fig. 1). Sucrose concentration, on the other hand, was decreased stepwise in correspondence to the increase of phenol, from an initial 2000 mg/l to become completely absent from the wastewater after day 130.

Phase 2 (days 131–386) was treated wastewater with increased concentrations of phenol and m-cresol without sucrose as co-substrate (Fig. 2). Phenol and m-cresol concentrations were kept at a near constant ratio of 2.5–3.0/l. Immediately after sucrose was absent from the wastewater on day 131, the removal efficiency of phenol dropped drastically. In order to reduce the phenolic toxicity to the biomass, starting on day 135, effluent recycle was introduced and lasted for the rest of the continuous experiment; the recycle flowrate equalled that of the incoming wastewater. In addition, on day 135 the concentrations of phenol and m-cresol were lowered 50% to 580 and 195 mg/l, respectively. Both concentrations were then gradually increased when phenol removal efficiency became improved. The concentrations were again lowered on day 328 when the removal efficiency deteriorated and increased once more 20 days later when the efficiency recovered.

Phase 3 (days 387–444) was conducted to examine the toxic effect of m-cresol in wastewaters containing a medium-strength (600 mg/l) phenol. The concentration of m-cresol was step-increased from 200 mg/l to 800 mg/l (Fig. 3).

**Batch experiments**

Biogranules were sampled on days 290, 293 and 396 for three series of batch tests in 157-ml serum vials at 37°C. In one series, degradations of phenol and m-cresol were closely monitored over a 240-h period. In a second series, the specific methanogenic activities (SMA) of biogranules degrading individual substrates, such as fatty acids, benzoate, phenol and cresols, were examined using the method developed by Owen *et al.* (1979) and Dolfing and Mulder (1985). In a third series, the effect of m-cresol concentration on the decrease in SMA of biogranules

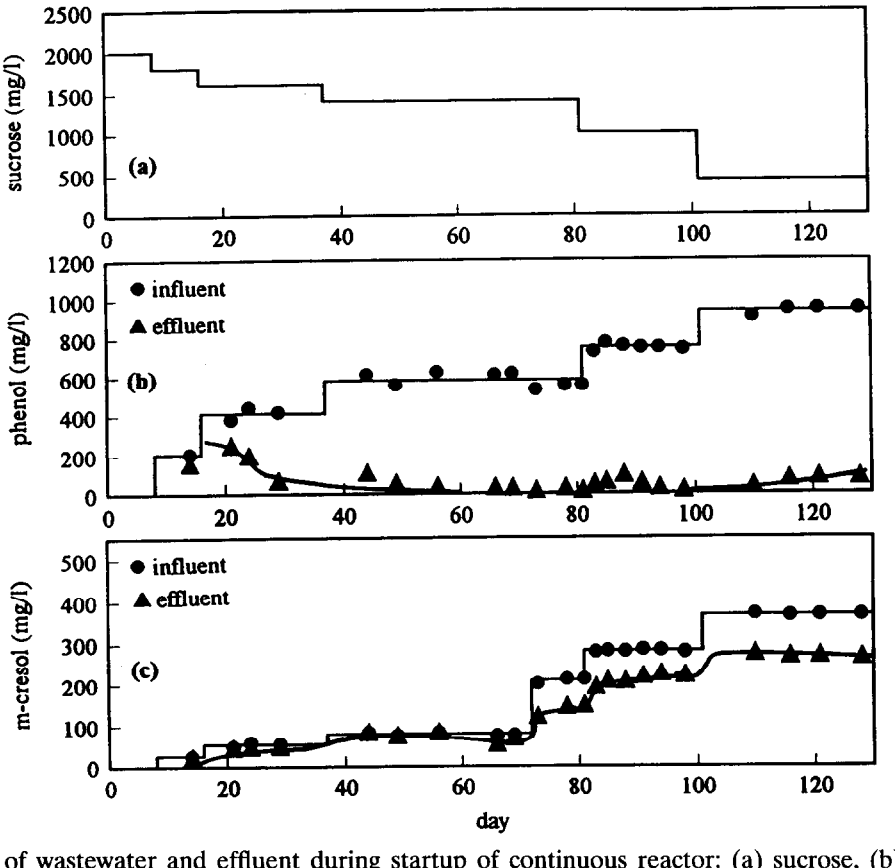


**Fig. 1.** Constituents of wastewater and effluent during startup of continuous reactor: (a) sucrose, (b) phenol and (c) m-cresol.

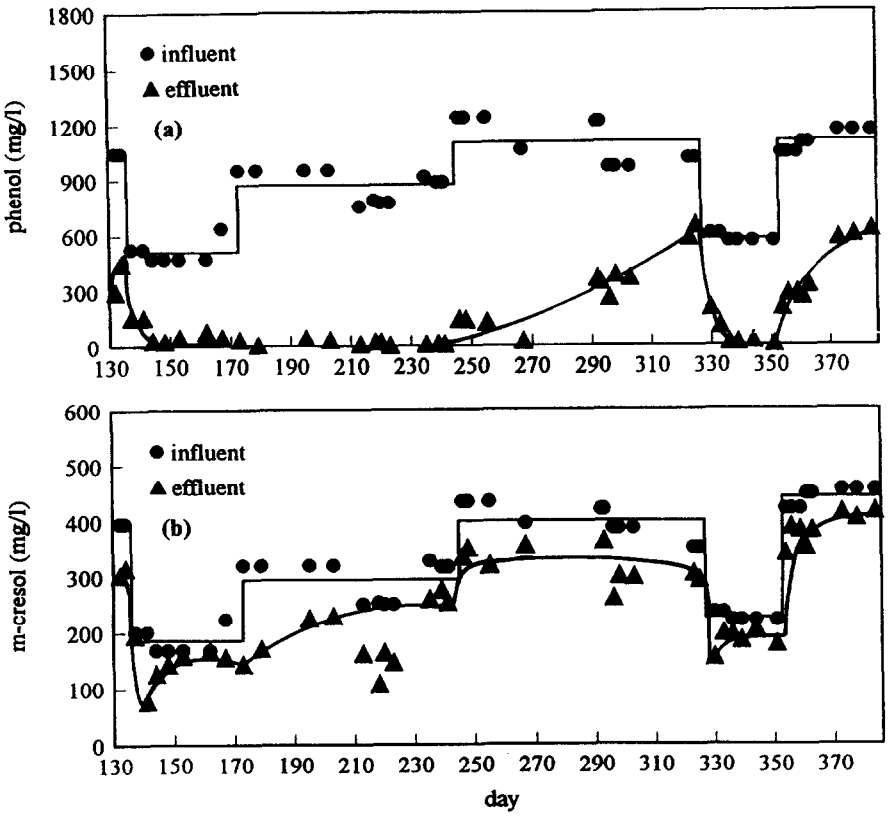


**Fig. 2.** Constituents of wastewater and effluent at increased concentrations of (a) phenol and (b) m-cresol.

treating phenol as substrate was investigated. All SMA tests were conducted in duplicate to confirm the reproducibility.

**Analytical methods**

In both continuous and batch experiments, concentrations of phenol, m-cresol and sucrose in both wastewater and effluent were constantly monitored, along with the methane production in the biogas. Methane content of the biogas was analyzed by a gas chromatograph (GC, Hewlett-Packard, Model 5890 Series II). Phenol, m-cresol and possible metabolic intermediates, such as benzoate and fatty acids (from acetic to heptanoic acids), were analyzed by a second gas chromatograph of the same model. Details of operational conditions followed those

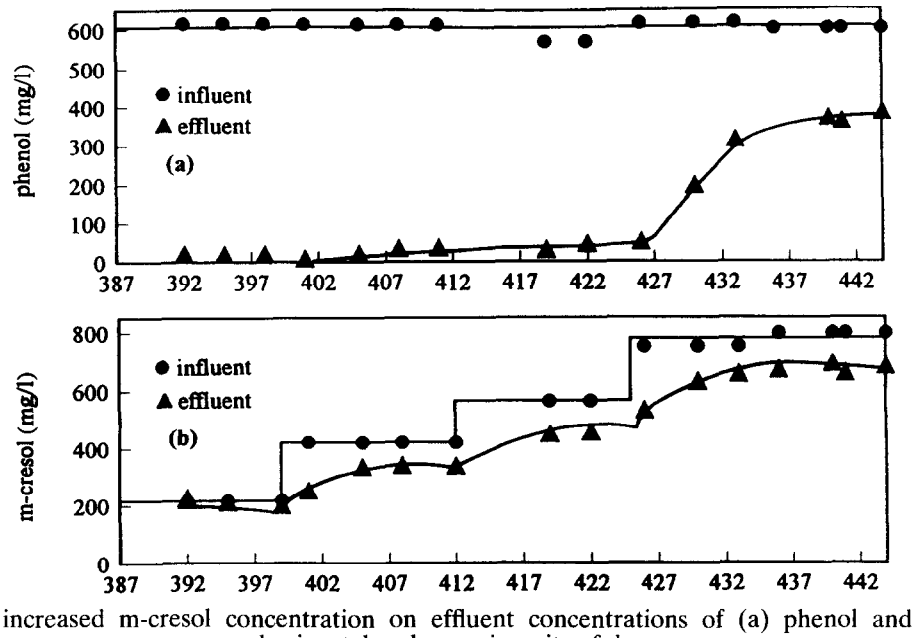


**Fig. 3.** Effects of increased m-cresol concentration on effluent concentrations of (a) phenol and (b) m-cresol. The horizontal scales are in units of days.

described in a previous study (Fang *et al.*, 1996). Sucrose was measured by a spectrophotometer at 625 nm (UV-160A, Shimadzu). Other parameters, such as VSS (volatile suspended solids) and soluble COD were measured according to the *Standard Methods* (APHA, 1985).

## RESULTS AND DISCUSSION

Throughout the 444-day operation, influent pH was kept at 8.1, while the effluent pH was stable, averaging 7.6. Degradation of phenols is a complex process involving a sequence of steps, each carried out by a specific group of bacteria. Phenols are first converted by acidogens to benzoate (Kobayashi *et al.*, 1989), which is then further converted by acetogens to acetate (Fina *et al.*, 1978; Keith *et al.*, 1978). Lastly, acetate is converted by methanogens to methane. In the continuous experiment, concentrations of possible intermediates, including benzoate and volatile fatty acids, were all below the detectable levels of 1 mg/l. This seems to suggest that the rate-limiting step for the degradations of phenol and m-cresol was likely to be the initial acidogenesis, instead of the subsequent acetogenesis and methanogenesis.

## CONTINUOUS EXPERIMENT

### Acclimation of methanogenic sludge to phenol and m-cresol

The seed sludge was a mixture of a common digester sludge and a UASB granular sludge treating sucrose-rich wastewater. It had not been exposed to phenolic pollutants prior to this experiment, and thus required a period of acclimation. Figure 1 illustrates that, during the 130-day acclimation phase, sucrose concentration was lowered stepwise from the initial 2000 mg/l to 425 mg/l, while concentrations of phenol and m-cresol were correspondingly increased from nil to 950 mg/l and 350 mg/l, respectively. All sucrose was completely degraded after day 40. Figure 1(b) illustrates that after 20 days of acclimation, biomass began to degrade phenol with increasing efficiency. After day 45, the phenol removal efficiency averaged 92%, and the residual phenol concentration in the effluent was consistently below 85 mg/l even when the wastewater contained 950 mg/l of phenol during days 101–130. Figure 1(c) illustrates that the removal of m-cresol was less satisfactory. Only 28% removal efficiency was achieved after 70 days of acclimation. During days 101–130, when the wastewater contained 350 mg/l of m-cresol, the residual m-cresol averaged 260 mg/l. These results showed that phenol was more biodegradable than m-cresol, and, after an acclimation period, degradation of phenol was relatively independent of the co-substrate concentration.

### Biomass capability of degrading phenol and m-cresol

Figure 2 illustrates the changes of phenol and m-cresol concentration in the sucrose-free wastewater and the corresponding residual concentrations in the effluent in Phase 2. Immediately after sucrose was absent from the wastewater, residual phenol concentration increased drastically from 87 mg/l to 450 mg/l by day 134. It appeared that biomass needed further acclimation to the sucrose-free condition. In order to lower the phenolic toxicity of wastewater, effluent recycle was introduced on day 135 and lasted for the rest of the experiment. The recycle flowrate equalled that of the incoming wastewater. In addition, on day 135 the phenol and m-cresol concentrations were lowered by 50% to 580 mg/l and 195 mg/l, respectively. Soon the residual phenol and m-cresol stabilized at 43 mg/l and 156 mg/l levels. As a result, phenol and m-cresols were further increased, respectively, to 900 mg/l and 320 mg/l on day 173, and later to 1100 mg/l and 400 mg/l on day 245.

Figure 2 further illustrates that residual phenol in the effluent gradually increased after day 245, reaching 650 mg/l by day 326, during which the residual m-cresol was increased to 300 mg/l. Action was taken on day 327 to lower phenol concentration in the wastewater to 600 mg/l and m-cresol to 220 mg/l. Within 10 days, the residual phenol was lowered to 17 mg/l. On day 353, phenol and m-cresol in the wastewater were again returned to 1100 mg/l and 400 mg/l, respectively. Residual phenol concentration increased correspondingly, reaching 625 mg/l on day 386 at the end of Phase 2.

Results of Phase 2 showed that the biomass was able to degrade over 98% of phenol and 20% of m-cresol when their concentrations were below a maximum tolerable level. Above such a level, the biomass activities were drastically impaired. But, the impairment was not permanent. Lowering the phenol/m-cresol concentrations in the wastewater enabled the biomass to fully regain its phenol-degrading capability in a few days. In a recent study, Fang *et al.* (1996) showed that UASB biogranules were also able to degrade over 97% of phenol at 37°C with 12 h retention time for phenol concentrations up to 1260 mg/l. On the other hand, the deterioration of bioactivities in this study during days 245–326 and 353–387 occurred when treating wastewater containing 1100 mg/l of phenol plus 400 mg/l of m-cresol. Compared with the results of the previous study, the deterioration of bioactivities in this study was likely to have been caused by the increased concentration of m-cresol, rather than phenol.

In Phase 3 (days 387–444), phenol was kept at a moderate concentration of 600 mg/l, while the concentration of m-cresol was step-increased from 200 mg/l to 800 mg/l. Figure 3 illustrates that phenol degradation was unaffected by m-cresol at concen-

trations up to 600 mg/l, as the residual phenol concentration in the effluent was consistently less than 40 mg/l. When m-cresol was raised to 800 mg/l on day 425, phenol-degrading activity began to deteriorate; the residual phenol concentration gradually increased to 380 mg/l by day 444 when the experiment was terminated. These results indicated that there was a threshold level between 600 and 800 mg/l for m-cresol toxicity toward the phenol-degrading activity of the biomass.

Figure 3(b) also illustrates that, on the other hand, the biomass was able to degrade 19.8% of m-cresol during days 412–424, corresponding to a m-cresol degradation rate of 82 mg/l.day, when the m-cresol concentration in wastewater was 400 mg/l. At 600 mg/l of m-cresol, the degradation efficiency became 19.7% and the degradation rate 111 mg/l.day. When m-cresol concentration was further increased to 800 mg/l during days 425–444, they became 15.3% and 118 mg/l.day. It thus appeared that the m-cresol degradation rate had reached a plateau near the end, and thus the removal percentage was lowered when the concentration in the wastewater was increased to 800 mg/l.

## BATCH TESTS

Biogranules were sampled from the UASB reactor on days 290 and 293 for two series of batch tests. During that period, the UASB reactor was treating wastewater containing 1100 mg/l of phenol and 400 mg/l of m-cresol, and the bioactivities of phenol-degradation were deteriorating.

*SMA using various individual substrates*

Table 1 summarizes the SMA data for the biogranules treating several individual substrates. The results in Table 1 show that SMA using acetate as substrate was 1.1 g-methane-COD/g-VSS.day, considerably higher than that with benzoate as substrate (0.51 g-methane-COD/g-VSS.day). The latter was yet higher than the SMA of 0.20-0.33 g-methane-COD/g-VSS.day when phenol was used as substrate. These results suggest that, in the three-step degradation of phenol, acidogenesis is likely to be the rate-limiting step, while methanogenesis appeared to be the fastest, followed by acetogenesis.

**Table 1. Specific methanogenic activity of biogranules**

| Substrate | Concentration (mg/l) | COD (mg/l) | SMA (g-COD/(g-VSS.day)) |
|---|---|---|---|
| acetate | 2300 | 2500 | 1.1 |
| benzoate | 1260 | 2500 | 0.51 |
| phenol | 840 | 2000 | 0.33 |
| phenol | 420 | 1000 | 0.26 |
| phenol | 210 | 500 | 0.20 |
| m-cresol | 159 | 400 | nil |
| o-cresol | 159 | 400 | nil |
| p-cresol | 159 | 400 | 0.07 |

At the time of sampling, the biogranules were able to degrade 15% of m-cresol in the UASB reactor. However, it is interesting to note from Table 1 that the biogranules were unable to degrade m-cresol at all in the SMA test. This was likely to be due to the absence of phenol in the feed solution in the batch test. Thus, it appeared that in the absence of sucrose, phenol served as a co-substrate for the degradation of m-cresol in the UASB reactor. Furthermore, it is also interesting to note that although they were unable to degrade m- and o-cresol, the biogranules were able to degrade p-cresol with a SMA of 0.07 g-methane-COD/g-VSS.day.

*Co-degradation of phenol and m-cresol*

Figure 4 illustrates the decreases of phenol and m-cresol concentration in the mixed liquor of the batch reactor. Phenol was degraded initially at a rate of 2.26 g/g-VSS.day, but the rate was gradually levelled off to 0.27 g/g-VSS.day during hours 72–240. The degradation of m-cresol was much slower; the concentration was lowered from the initial 300 mg/l to 200 mg/l after 240 h. It is interesting to note that benzoate, the key intermediate of phenol degradation, was detected in the mixed liquor. Benzoate concentration increased up to 66 mg/l after 15 h, and gradually fell after 150 h. No other fatty acids were detected in the batch reactor, because of the rapid rates of acetogenesis and methanogenesis.

*Toxicity of m-cresol to phenol degradation*

Biogranules sampled on day 396 were used to test the toxic effect of m-cresol on phenol degradation. SMA of the biogranules were measured for feed solutions containing phenol at 600 mg/l plus m-cresol at various concentrations (from nil to 1000 mg/l). The SMA decreased with the increase of m-cresol, indicating m-cresol was toxic to phenol degradation. Figure 5 illustrates the SMA relative to the control,

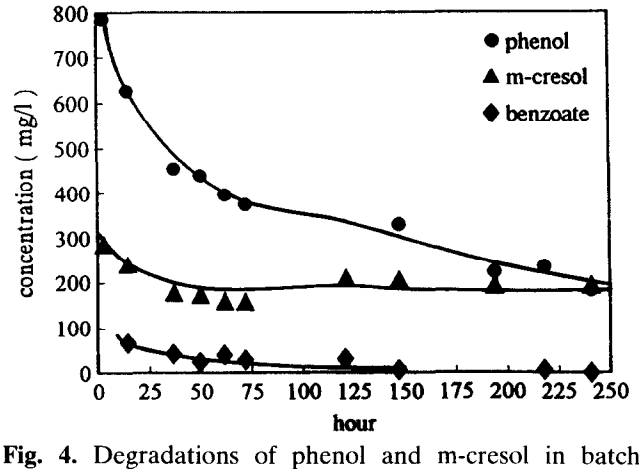


**Fig. 4.** Degradations of phenol and m-cresol in batch experiment.

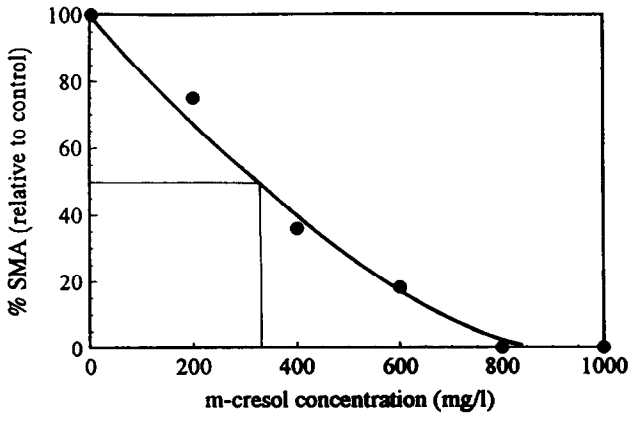


**Fig. 5.** Effect of m-cresol on SMA of biogranules using phenol as substrate.

which was measured on feed solutions free of m-cresol, at various m-cresol concentrations. Based on Fig. 5, the $IC_{50}$ value of the concentration of toxicant at which the SMA of the biogranules was reduced by 50% of m-cresol was 330 mg/l.

## CONCLUSION

Up to 98% of phenol and 20% of m-cresol were degraded in a UASB reactor for wastewaters containing up to 900 mg/l of phenol and 320 mg/l of m-cresol, and free of carbohydrate co-substrate when the reactor was operated at 37°C, with one-day hydraulic retention plus effluent recycle. Further increases of phenol and m-cresol concentration in wastewater impaired the phenol-degrading activity of the biomass. However, the biomass was able to regain activity once the phenolic concentrations were lowered. In treating a wastewater containing 600 mg/l of phenol, m-cresol had a threshold toxicity of 600–800 mg/l in a continuous reactor; but in a batch reactor the toxicity was progressive, with a $IC_{50}$ value of 330 mg/l. The absence of intermediate acids in the reactor effluent and other results of batch experiments suggested that the initial acidogenesis was likely to be the rate-limiting step for phenol degradation, instead of the subsequent acetogenesis or methanogenesis.

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