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Phenolic Wastewater Treatment by a Mixed Culture GE2 Immobilized on Activated Carbon

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The biological treatment by a mixed culture GE2 immobilized on activated carbon was investigated with a phenolic resin industrial wastewater containing 41,000 mg/l of phenol and 2,800 mg/l of formaldehyde. At a dilution of 20 times with aerated tap water, influent and effluent COD_{Mn} were 4,587 mg/l and 46 mg/l, that is, COD_{Mn} removal efficiency was 99.0%. At this time, phenol and formaldehyde concentration of the effluent were 1.24 and 6.80 mg/l, indicating removal efficiencies of 99.9 and 94.1%, respectively.

Phenol is a wide-spread environmental pollutant found in the effluent of refinery plants, petroleum chemical plants and phenolic resin plants. Wastewaters from phenolic resin plants and coking plants often contain very high concentrations of phenol, which cannot be treated by normal activated sludge systems (5). Therefore, phenolic resin wastewaters containing high concentrations of both phenol and formaldehyde have been treated by incineration, which also tends to produce secondary pollutants. Formaldehyde also has microbiacidal effects and can cause severe symptoms in humans exposed to high concentrations (1).

The treatment of phenolic wastewater using both pure and mixed cultures has been investigated (4, 9, 10). Ambujom and Manilal (2) have reported that the combinations of two, four and six different isolates of phenol-degrading bacteria showed progressively increasing rates of degradation. Also, versatile materials to immobilize microorganisms have been developed: calcium-alginate or chitosan-alginate (13), alginate beads (12), active carbon (5, 9), sintered glass (10) and polyethylene cilia (11). However, most research has been concentrated on the study of the physiological and ecological characteristics of strains and the treatment of synthetic wastewaters. There are few reports focused on the treatment of phenolic industrial wastewater using microorganisms.

Therefore, this study is mainly focused on the development of biological technologies for the treatment of phenolic industrial wastewater. In this study the wastewater from a phenolic resin plant was collected, analysed and biologically treated by with a mixed culture GE2 using granulated active carbon as a carrier.

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Key words: Phenolic wastewater, activated carbon, mixed culture, removal efficiency

MATERIALS AND METHODS

Microorganisms

The mixture of microorganisms used was composed of 12 bacteria including *Flavobacterium* sp. BEN2, *Acinetobacter* sp. GEM63, *Acinetobacter* sp. GEM2 and 1 yeast *Candida* sp., and named as GE2 (11).

Carrier

Granulated active carbon (Junsei Chemical Co.; size, 1-2 mm) was mixed with some granulated Styrofoam (size, 1-2 mm) by a ratio of 19:1 (w/w) to maintain buoyancy in the bioreactor. The mixture was put into a plastic frame with a netted cylindrical structure (diameter, 3 cm; length, 8 cm) filling the reactor to a volume 20 g/l.

Characteristics of Wastewater

The phenol concentration of the wastewater collected from the phenolic resin plant was 41,000 mg/l. Formaldehyde concentration was 2,800 mg/l and pH was 3.0. COD_{Mn} and COD_{Cr} were 89,000 mg/l and 150,000 mg/l, that is, the COD_{Cr}/COD_{Mn} ratio was 1.66. BOD was only 210 mg/l.

The original wastewater was diluted step by step to 80, 40 and 20 times with tap water aerated for 1 day to exclude dissolved chlorine. Mineral nutrient was a mixture of $(NH_4)_2HPO_4$, $NH_4H_2PO_4$ and $CaCl_2$ at the ratio of 1:1:0.1. 0.2 g/l of the mixture was added to the diluted wastewater and 0.2 g/l of yeast extract was also added as an organic nutrient.

Design and Operation of Wastewater Treatment Process

The wastewater treatment process was designed on the basis of an activated sludge system. The system was divided into three 10-liter reactors composed of two 5-liter subreactors made of transparent plexiglass. Each reactor had a cover in order to catch volatile gases through a pre-installed tube.

Air was supplied by an air blower to keep DO in the range of 2-4 mg/l. Temperature was controlled at 25± 1°C and hydraulic retention time (HRT) was 48 h. All 3 sets of reactors were acclimated for 30 days with the papermill activated sludge and then, in GE2 treatment a mixed culture GE2 was added to the control. For activated carbon treatment, activated carbon was added to the GE2 treatment. GE2 was innoculated at a concentration of 2% (v/v) everyday for 5 days at the beginning and for 2 days after lowering the dilution. MLSS was controlled in the range of 2,000-3,000 mg/l.

Analytical Measurements

The influent and effluent were sampled every other day and analyzed for the following: COD_{Mn}, MLSS, phenol and formaldehyde concentration. When the removal efficiency reached a stable value, COD_{Cr}, COD_S and BOD were measured. COD_{Cr}, BOD and MLSS were analyzed according to standard methods (3). COD_{Mn} and COD_S after centrifugation were assayed according to Golterman *et al.* (6).

Phenol concentrations in the samples were determined spectrophotometrically (3). Formaldehyde content was measured by a slightly modified method of Matsuda *et al.* (8).

RESULTS

Fig. 1 shows influent and effluent COD_{Mn} for each treatment at three consecutive dilutions of 80, 40 and 20 times. When the average of influent COD_{Mn} was 1,050 mg/l by a dilution of 80 times, effluent COD_{Mn} became stable at 15 days after GE2 innoculation. There was no significant difference in effluent COD_{Mn} among the treatments. At 40-times dilution, the effluent COD_{Mn} of Control increased temporarily up to 180 mg/l, but GE2 treatment showed a stable COD_{Mn}, ca. 50 mg/l. Activated carbon treatment showed 84 mg/l in the beginning and then immediately decreased to a low level of 22 mg/l. At 20-times dilution, influent COD_{Mn} increased up to 5,360 mg l, but the effluent COD_{Mn} of activated carbon treatment was under 50 mg/l after stabilization.

Fig. 2 shows phenol concentrations of the influent and effluent at each dilution of 80, 40 and 20 times. At 80-times dilution, the average of phenol concentration of the influent was 487 mg/l and those of the effluent were stable after 15 days and were under 1.0 mg/l. At 40-times dilution, the phenol concentration of the control increased temporarily up to 86 mg/l and then decreased to 2.1 mg/l. As a whole, GE2 and activated carbon treatments resulted in relatively low phenol concentrations in the effluent. At 20-times dilution, the phenol concentration of the effluent from the activated carbon treatment showed an initial increase up to 4.3 mg/l, much lower than that in the other treatments, and in stabilizing was near 1.0 mg/l.

Formaldehyde concentration of the influent gradually increased from 26.0 to 114.5 via 47.1 mg/l after consecutive dilutions of 80, 40 and 20 times and those of the effluent of the activated carbon treatment reached to stable values of 1.92, 3.18 and 6.80 mg/l. The pH of the

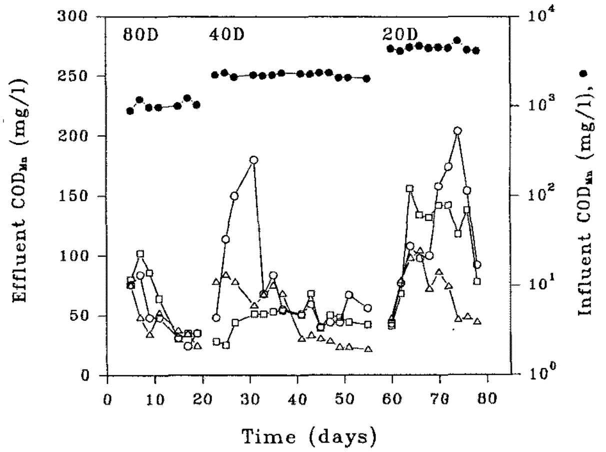

Fig. 1. COD_{Mn} of the influent and effluent at each dilution of 80, 40 and 20 times.

Symbols are: ●, Influent; ○, Control; □, GE2 treatment; △, Activated carbon treatment.

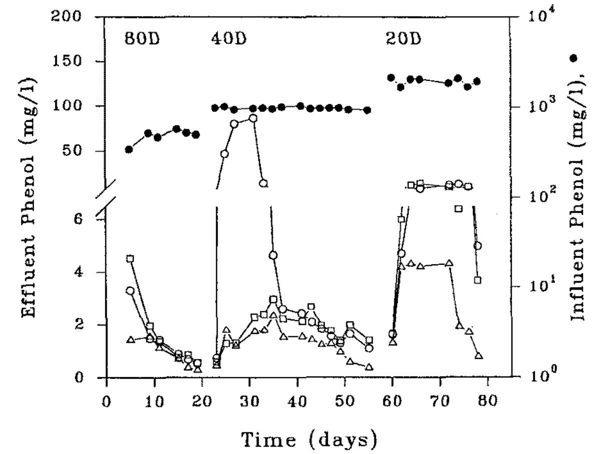

Fig. 2. Phenol concentration of the influent and effluent at each dilution of 80, 40 and 20 times.

Symbols are: ●, Influent; ○, Control; □, GE2 treatment; △, Activated carbon treatment.

Table 1. COD_{Mn}, phenol and formaldehyde concentrations of the influent and effluent at each dilution of 80, 40 and 20 times. The removal efficiencies were in parentheses.

Item	Dilution times	Influent	Effluent of each treatment		
			Control	GE2	Activated carbon
COD_{Mn}[a] (mg/l)	80	1,093	30 (97.3)	33 (97.0)	32 (97.1)
	40	2,067	56 (97.3)	45 (97.8)	22 (98.9)
	20	4,587	150 (96.7)	111 (97.6)	46 (99.0)
Phenol[b] (mg/l)	80	510	0.61 (99.9)	0.71 (99.9)	0.34 (99.9)
	40	935	1.36 (99.9)	1.69 (99.8)	0.48 (99.9)
	20	1,805	7.21 (99.6)	6.56 (99.6)	1.24 (99.9)
Formal-dehyde (mg/l)	80	26.0	2.91 (88.8)	2.87 (89.3)	1.92 (92.6)
	40	47.1	4.24 (91.0)	3.40 (82.8)	3.18 (93.3)
	20	114.5	7.50 (93.4)	6.50 (94.3)	6.80 (94.1)

[a]mean value of the last 3 measurements. [b]mean value of the last 2 measurements.

Table 2. COD_{Mn}, COD_S, COD_{Cr} and BOD in the influent and effluent at a dilution of 20 times. The ratios of COD_S, COD_{Cr} and BOD to COD_{Mn} were in parentheses.

Treatment	COD_{Mn} (mg/l)	COD_S (mg/l)	COD_{Cr} (mg/l)	BOD (mg/l)
Influent	4,240	4,160 (0.98)	7,045 (1.66)	3,400 (0.80)
Control	154	126 (0.82)	168 (1.09)	10.1 (0.07)
GE2	138	110 (0.80)	164 (1.19)	5.2 (0.04)
Activated car-	48	43 (0.90)	144 (3.00)	1.1 (0.02)

influent at 20-times dilution was 6.5 and, after stabilization, those of the effluent were in the range of 7.25-7.42.

The removal efficiencies of COD_{Mn}, phenol and formaldehyde in each treatment are listed in Table 1. Effluent COD_{Mn} was greatly decreased from 150 mg/l to 46 mg/l by adding GE2 with activated carbon as a carrier. COD_{Mn} removal efficiency was the highest one at 99.0% at 20-times dilution with activated carbon treatment. Phenol removal efficiencies of all treatments were in the range of 99.6-99.9% which were higher than those of COD_{Mn} as a whole. Formaldehyde removal efficiency showed an increasing tendency with decreasing dilution times. At 20-times dilution, formaldehyde removal efficiencies were all over 93.4% and the highest was 94.3% with GE2 treatment.

Effluent BOD_S in the Control, GE2 and activated carbon treatment were 10.1, 5.2 and 1.1 mg/l which met the standard of wastewater discharge for BOD, 30 mg/l (Table 2). The $COD_{Cr}:COD_{Mn}$ ratio in the activated carbon treatment was the highest at 3.00. The BOD: COD_{Mn} ratios of the effluents were much lower than that of the influent.

DISCUSSION

At 80-times dilution, the effects of GE2 and activated carbon on the decrease of effluent COD_{Mn} and phenol were not great (Fig. 1-2). That is, the effect of GE2 and activated carbon increased with decreasing dilution times. If the dilution is lower, treatment efficiency is higher. As the dilution decreased, the removal efficiency of COD_{Mn} and phenol did not change much, but the COD_{Mn} and phenol concentrations of the effluent increased. Considering that the standards of wastewater discharge for COD_{Mn} and phenol are 50 and 5 mg/l, GE2 treatment could meet the standard in the case of 40 times dilution and activated carbon treatment can meet the standard even at 20-times dilution.

The phenol-degrading strain, *Pseudomonas putida* EKII, was isolated from a soil enrichment culture and utilized phenol up to 10.6 mM (1.0 g/l) as the sole source of carbon and energy (7). It has been known that immobilization and mixing cultures could increase tolerance and degradation for the target compounds. In reality, Mörsen and Rehm (9) had reported that a mixed culture system immobilized on active carbon was able to degrade phenol up to 17 g/l using synthetic wastewater. In this experiment, it was shown that phenolic industrial wastewater containing 1,805 mg/l of phenol and 114.5 mg/l of formaldehyde by 20-times dilution could be biologically treated using GE2 and activated carbon as a carrier. Also, it should be considered that the wastewater was real industrial wastewater containing not only high concentrations of phenol but also high concentrations of formaldehyde which is also known to have microbiacidal effects. Notably, activated carbon treatment was more effective in the removal of COD_{Mn} and BOD than COD_{Cr} (Table 2).

In conclusion, it is believed that a phenolic wastewater treatment system using GE2 with activated carbon as a carrier has the following advantages: (1) the COD_{Mn}, phenol and formaldehyde concentration of the effluent are considerably lower; (2) the effluent is more stable against sudden increases in the concentration of pollutants; (3) the water quality of the effluent at 20-times dilution is clean enough to meet the standard of wastewater discharge.

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