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Biodegradation of Azo Dyes in Multistage Rotating Biological Contactor Immobilized by Assimilating Bacteria

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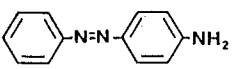
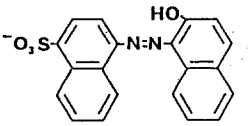
Wastewaters from dyeing and finishing processes contain a large amount of organic substances such as thickening agents as well as dyes. The activated sludge process has been frequently applied to the removal of organic substances. The elimination of the dye in the treatment results in the adsorption on the sludge, and the rate is not always high (Ogawa et al. 1974a,b, Hitz et al. 1978, Kimura 1980). Most plants utilize, therefore, a combined process of the activated sludge process for various organic substances and the coagulation or dissolved air floatation process for the dye. It is expected to develop a wastewater clarification technology for removing the dyes and the other organic substances in a single operation by use of the dye assimilating bacteria. The authors isolated and identified the azo dye assimilating bacteria from the drainage of a dyeing plant (Idaka et al. 1978a,b, 1980), and revealed the pathways of the degradation by the microbe (Idaka et al. 1982, 1987). p-Aminoazobenzene (Ogawa et al. 1981) and the acid azo dyes (Ogawa et al. 1986) in the model wastewater were effectively degraded by the continuous submerged culture of the microbe. The method had, however, a shortcoming that it was difficult to separate the microbe from the effluent due to a low rate of sedimentation. The model wastewater was treated, therefore, by a rotating biological contactor with disk on which *Pseudomonas cepacia* 13NA was immobilized with κ-carrageenan gel. It was known from the results that the dye-degradation activity was stable for a long time.



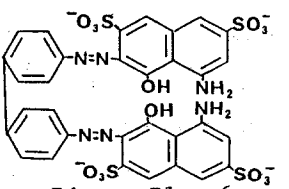
p-aminoazobenzene



Acid Red 88

MATERIALS AND METHODS

The dyes such as p-aminoazobenzene, Acid Red 88 and Direct Blue 6 shown at the right were used. The strain used was an azo dye assimilating bacterium, *Pseudomonas cepacia* 13NA, which was identified by us (Idaka et al. 1987b). The bacteria were shake-cultured in a sterilized medium composed of 2.0% molasses,



Direct Blue 6

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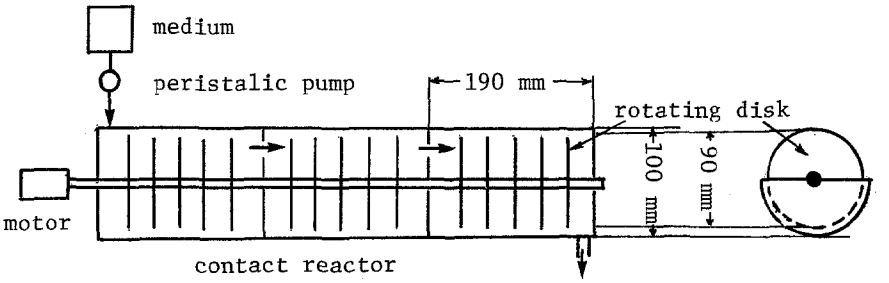

Figure 1. Apparatus of rotating biological contactor.

1.0% corn steep, 0.2% K_2HPO_4 and 0.1% NaCl for 16 hr and were collected by centrifugation. After washing, the microbes were added to a phosphate buffer (pH 7.0) and used for immobilizing. The cell concentration of suspension was determined spectrophotometrically by the measurement of transmittance at 660 nm.

Immobilization of bacetria on rotating disk: Each rotating disk made of polymethyl methacrylate 90 mm in diameter and 1.0 mm thick was covered with a sheet of gauze to readily immobilize the gel and the circumference was fixed with rubber tape 10 mm wide. κ-Carrageenan (2 g) was added to 0.8% KCl solution (80 mL) and dissolved at 120°C for 5 min in an autoclave. The cell suspension (5.0 mL) 2.4% aqueous solution (5.0 mL) of polyethyleneimine, crosslinking agent (Takata et al. 1982), were added to the solution cooled down to 60°C. After stirring, the solution was run on disks. The volume and surface area of the gel adhered to each disk were 112.7 cm^3 and 125.7 cm^2, respectively.

Apparatus: It is shown in Figure 1. The reactor vessel made of polyvinyl chloride was divided into three compartments with partition plates of polymethyl methacrylate. Each compartment had five disks fixed to the brass rotating shaft. The rotational speed of the disk was 15 rpm. The apparatus was placed in a cabinet kept at 30°C.

Clarification treatment: The model wastewater was prepared in such a way that the dye was added to the above-mentioned sterilized molasses corn steep medium, which was diluted with a phosphate buffer (pH 7.0), and was 300 BOD. The solution was put into a flask which was stoppered with cotton and cooled with ice to prevent the contamination by extraneous bacteria. The solution was continuously fed into the 1st reactor with a peristalic pump and successively sent to the 2nd and 3rd reactors through small holes in the partition plates. The dye concentration in each reactor was periodically determined in such a way that a small amount of suspended cells were removed by centrifugation and the absorbance at wavelength (λmax) showing the maximal absorption was measured. The values of λmax of p-aminoazobenzene, Acid Red 88 and Direct Blue 6 were 375, 505 and 600 nm, respectively.

RESULTS AND DISCUSSION

Clarification efficiency: The model wastewater containing 10 ppm p-aminoazobenzene was treated at immobilized cell concentration of 200 ppm by use of the apparatus. The results are shown in

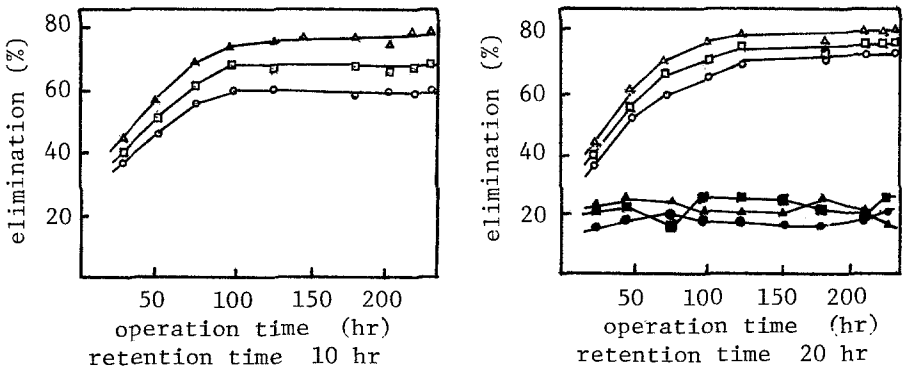

Figure 2. Relationship between elimination of p-aminoazobenzene and operation time.
contact reactor:
first ○ (●), second □ (■), third △ (▲)
( ): control experiment

Figure 2. The control experiment was done similarly with gel containing no cell. The retention time was defined as the period from the time when the solution enters the 1st reactor to the time when it leaves the 3rd reactor through the 2nd reactor. The rate of elimination of p-aminoazobenzene was almost constant after approximately 100 hr because the elimination ability was kept stable under the open conditions. The rate increased as the solution moves from the 1st reactor through the 2nd reactor to the 3rd reactor. The rates in the 1st and 2nd reactor were low in the system of retention times of 10 hr compared to that of 20 hr. And the rates in the 3rd reactor were approximately 80% in both the system. It is desirable that the retention time in the treatment of the wastewater is as short as possible because the water can be treated in quantity. The final rates in both systems were approximately equal, and hence the amount of elimination in the system of retention times of 10 hr was nearly double as much as that of 20 hr. It was known from the result that the operation of retention times of 10 hr was better.

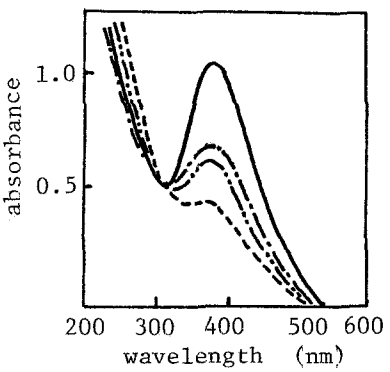

Figure 3. Absorption spectra of the fluids containing p-aminoazobenzene.
operation time 50 hr
——— influent
—·—·— first reactor
—··—··— second reactor
------ third reactor

It is thought that the elimination is attributed to either the adsorption on the gel or the biodegradation. In order to confirm this, the UV spectrum of the supernatant liquid in each reactor was measured. The result is shown in Figure 3. The absorption at 375 nm were decreased by the treatment, while those at shorter wavelength were increased by it, because conjugated compounds were produced by the biodegradation of p-aminoazobenzene. The authors

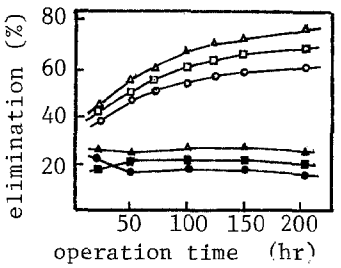

Figure 4. Relationship between elimination of Acid Red 88 and operation time.
retention time 20 hr
contact reactor:
first ○ (●)
second □ (■)
third △ (▲)
( ): control experiment

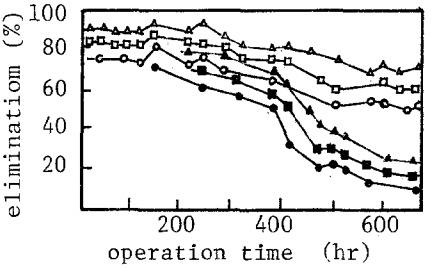

Figure 5. Relationship between elimination of Direct Blue 6 and operation time.
retention time 20 hr
contact reactor:
first ○ (●)
second □ (■)
third △ (▲)
( ): control experiment

have already elucidated the metabolic process of the azo compounds by the microbe as follows: (1) an azo bond is cleaved by reduction to produce amino and acetylamino compounds; (2) amino compounds are converted into aminohydroxy compounds, and then are metabolized through the Krebs cycle after the ring opening reaction (Idaka et al. 1987a,b). Degradation products in the effluent were extracted with methylene chloride. p-Phenylenediamine and acetanilide were detected by thin-layer chromatography and gas chromatography, but o-aminophenol was not detected. The results indicate that the degradation of p-aminoazobenzene in the operation is the reductive cleavage of the azo bond (step (1)).

The results obtained by the treatment of Acid Red 88 and Direct Blue 6 are shown in Figure 4 and 5, respectively. The solutions were treated in the system of retention times of 20 hr, because the dyes were difficult to degrade. The rate of elimination of Direct Blue 6 was high for the early period of operation, but it decreased with an increase in the operation time. The results of the control experiments indicated that most of Direct Blue 6 was eliminated by the adsorption on the gel during first 360 hr, and since then the adsorption sharply decreased because the adsorption reached saturation. An amount of the biodegradation expressed by the difference between the elimination and the adsorption, increased. The biodegradation of Direct Blue 6 was, however, lower than those of p-aminoazobenzene and Acid Red 88. The rate of biodegradation of azo compounds is affected by permeability of cell membrane depending upon the molucular weights and the intramolecular hydrogen bond between the azo and hydroxy groups (Yatome et al. 1981a). It is considered that the low biodegradation of Acid Red 88 and Direct Blue 6 is attributed to those factors. Longer retention time may be, therefore, required to enhance the rate of elimination of those compounds.

Relationship between biodegradation and nutrient: The wastewater from the dyeing and finishing processes generally contains a large amount of natural organic substances. Microbes mainly grow by the assimi-

Table 1. Chemical oxygen demand (COD) of the fluid in the reactor.

retention time (hr)	COD (ppm)			
	influent	contact reactor		
		first	second	third
10	318	159	134	102
20	324	123	90	71

Table 2. Rate constant of biodegradation of the dye in each reactor.

	r.t. (hr)	(min^{-1})	p-aminoazo-benzene	Acid Red 88	Direct Blue 6
influent		k_o	9.5×10^{-4}	5.8×10^{-4}	2.2×10^{-4}
contact reactor					
furst	10	k_1	1.4×10^{-3}	9.9×10^{-4}	4.4×10^{-4}
		k_1/k_o	1.5	1.7	2.0
	20	k_1	1.9×10^{-3}	1.3×10^{-3}	8.4×10^{-4}
		k_1/k_o	2.0	2.2	3.8
second	10	k_2	1.8×10^{-3}	1.2×10^{-3}	7.9×10^{-4}
		k_2/k_o	1.9	2.0	3.6
	20	k_2	2.3×10^{-3}	1.7×10^{-3}	9.9×10^{-4}
		k_2/k_o	2.3	2.9	4.5
third	10	k_3	2.2×10^{-3}	1.6×10^{-3}	9.2×10^{-4}
		k_3/k_o	2.3	2.7	4.2
	20	k_3	2.5×10^{-3}	1.9×10^{-3}	1.1×10^{-3}
		k_3/k_o	2.7	3.2	5.0

r.t.: retention time

lation of those organic substances in the biological treatment, while dyes are preferably degraded by starved cells (Yatome et al. 1981b). It is necessary, therefore, to reconcile the incompatible conditions of the growth of the microbe and the degradation of the dye in the biological treatment of wastewater. The effect of the nutrients in the medium on the degradation of the dye was investigated to evaluate the performance of the experimental apparatus. After continued operation for a period of 150 hr using a medium without dye, the medium in each reactor was centrifuged to remove the microbes, and COD was measured on the supernatant liquid. The results are shown in Table 1. The COD decreased as the medium moves from the 1st reactor through the 2nd reactor to the 3rd reactor. The decrease in the operation of retention times of 20 hr was larger than that in the operation of retention times of 10 hr. Each supernatant liquid was sterilized in the autoclave, and then kept at 30°C after the addition of the dye and the microbe. An amount of the degradation of the dye was measured spectrophotometrically at regular intervals. The rate constants were calculated assuming that the dye was degraded according to the first order kinetic equation. Table 2 shows the degradation rate constants of dyes in the influent and the solutions in the 1st, 2nd and 3rd reactor, k_o, k_1, k_2 and k_3. The comparision with Table 1 indicated that the poorer the nutrient, the higher the degradation rate constants of the dyes were. The tendency was more conspicuous in low degradative dyes. Those results indicate that the treatment in the 1st reactor is suited for the growth of the microbes because of rich nutrient quality, while that

in the 3rd reactor is suited for the degradation of the dye because of poor nutrient quality. It may be possible to keep both the growth of the microbe and the degradation of the dye high by periodically changing the path of solution in the wastewater treatment plant.

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