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Environmental Contamination and Toxicology

Behavior of Activated Sludge with Dyes

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Many synthetic chemicals have been discharged into the environment in the form of herbicides, pesticides and industrial effluents. Regulatory agencies have focused their attention especially on dyeing and finishing wastes as pollutional discharges because of their high coloring and organic contents.

Dyes are included in the category of compounds difficult to degrade. A few reports have been presented concerning the biological degradation of azo dyes (Idaka et al. 1978; Kappeler et al. 1978; Meyer et al. 1979). The elimination of dye colors and organic matter by either chemical or biochemical treatment is not sufficient. A number of advanced waste water treatment methods have been proposed to achieve water quality improvement. Most of these methods are dependent on some combination of biological, chemical and physical processes (Netzer et al. 1976; Shelley. 1976). The activated sludge method is one of the widely used biological processes. However, in most of the wastewater treatment plants, toxicity of dyes in microorganisms causes a decline in the purification function.

The authors earlier measured the inhibition of growth and respiration of activated sludges by various dyes and also examined their inhibitory characteristics (Ogawa et al. 1978). But these tests were carried out with sludges unacclimated to dyes. In the case of practical wastewater treatment, sludges acclimated to dyes and having adaptability to dyes must be used (Ogawa et al. 1981). Inouye and Honda (1971) reported on the toxicity of wastewater to acclimated sludges (sulphur dyes and azo dyes). However, these reports described mixtures of various kinds of organic and inorganic compounds.

Congo Red, Orange II and Crystal Violet have each been added to acclimated activated sludges with medium, and their influence on the growth of activated sludge and also the removal rate of their dyes have been examined. In addition, the effects of dye concentrations on acclimated sludges have been studied.

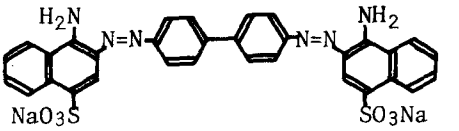
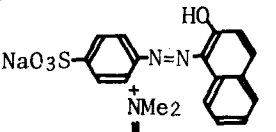
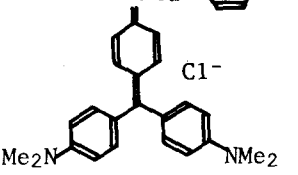
Common name	Color Index name	Structure
Congo Red	Direct Red 28 (C. I. 22120)	
Orange II	Acid Orange 7 (C. I. 15510)	
Crystal Violet	Basic Violet 3 (C. I. 42555)	

Figure 1. Dye structures

MATERIALS AND METHODS

The dyes used here included Congo Red, Orange II and Crystal Violet (Figure 1). They are products of Merck & Co., Inc. and were recrystallized with ethanol.

The following components were mixed as a standard medium (medium, 1): glucose, 0.7%; peptone, 0.35%; meat extract, 0.2%; Na_2HPO_4, 0.05%; K_2HPO_4, 0.01%; NaCl,0.02%; KCl, 0.01%; $CaCl_2$,0.01%; $MgSO_4$, 0.005%; $FeCl_3.6H_2O$, 0.005% (BOD 18,000 ppm, COD 13,200 ppm). They were dissolved in distilled water, adjusted to pH 7.0 and used after sterilization. Media 2, 3, 4 and 5 (BOD 3,000 ppm, COD 2,200 ppm) were, respectively, diluted medium 1 with 1.25, 1.7, 2.5 and 5 times water.

For the activated sludge, the return sludge obtained from Gifu municipal central sewage plant was used. Coarse debris in activated sludge was removed by passing the sludge through wire gauge (1 mm^2). After stirring, one loopful quantities of the activated sludge were added to medium 1 and incubated.

Every loopful of the return sludge was added to 100 ml of medium 1 in 500 ml shake flask and cultivated by shaking at 30°C with 120 rev. per min. The cultivation was first performed in medium 1 for 24 hours and then transferred successively to media 2, 3, 4 and 5. An aliquot of the culture was taken out every three hours and examined for pH, growth of cells and decreasing amount of dye. Activated sludge, acclimated for 40 or 60 days in medium 4 or 5, was cultivated continuously and their changes in dye decrease rate were checked. The acclimated sludge was also cultivated in medium 1 containing a higher concentration of dye

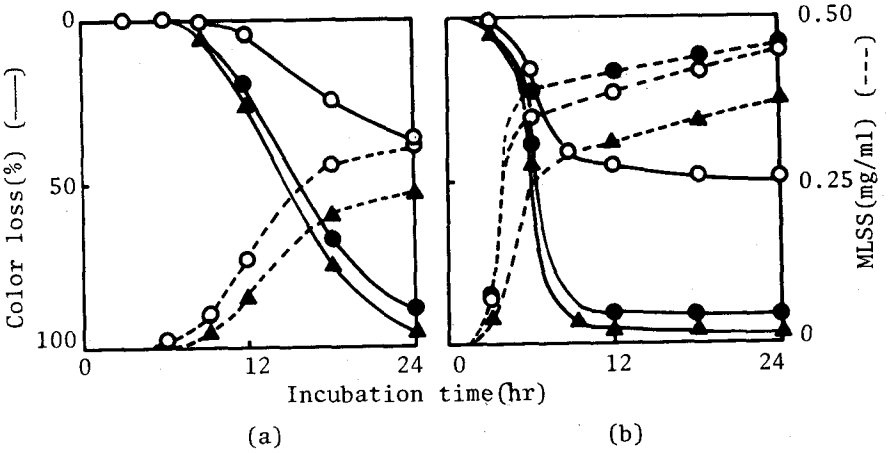


Figure 2. Time course of growth of activated sludge and color loss at the initial(a) and 15th(b) frequency in medium 1. Congo Red(●), Orange II(o), Crystal Violet(▲).

and the change in the elimination of dye was checked.

Color loss rate is represented as follows: Color loss rate (%)=[absorbance of dye (initial)]-[absorbance of dye (observed)]/[absorbance of dye(initial)] x 100. In the cultivation tests, cells were removed by centrifugation at 10,000 rpm for 15 min. to exclude absorption by cells. The wavelengths in the maximum absorption of Congo Red, Orange II and Crystal Violet were measured respectively at 488, 484 and 592 nm.

RESULTS AND DISCUSSION

A loopful of fresh return sludge taken from the sewage treatment plant was inoculated in medium 1 (BOD 18,000 ppm) and cultivated. The growth and the color loss in this process are shown in Figure 2a. The growth curve of activated sludges in medium 1 containing Congo Red or Orange II was similar to that without dye, but in the case of Crystal Violet, the growth was inferior to that of Congo Red or Orange II. After 48 hours, the broth was cleared up by floc formation. Precipitate of sludges was found to be connected to the decline of pH which reportedly occurred when a major carbon source was used (Ogawa et al. 1974).

To prevent the deactivation of the sludges by the toxicity of the dye, the BOD must be raised. In case of a concentration of 2×10^{-4} mol/l of dye, at a BOD of 18,000 ppm (equivalent to about 100 times that of municipal sewage) the color loss rate becomes highest and at a BOD of 3,200 ppm, the cell growth is completely inhibited (Ogawa et al. 1974).

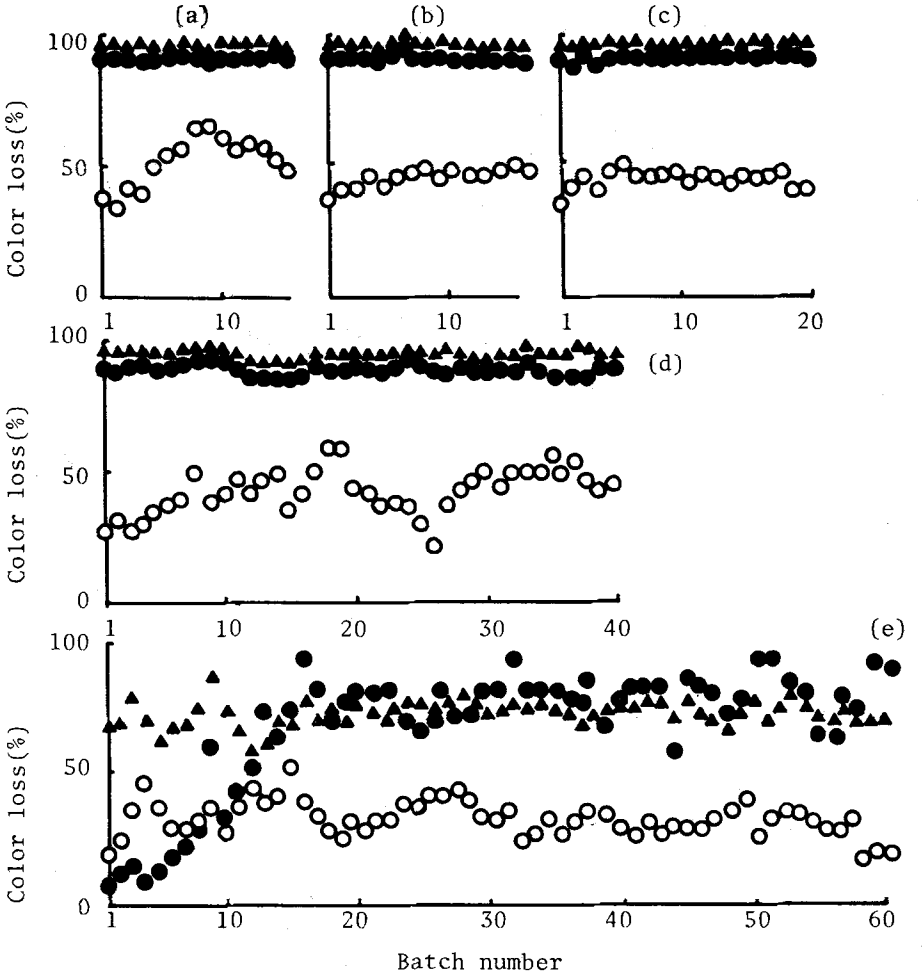


Figure 3. Relationship between batch number and color loss: (a) in medium 1, (b) in medium 2, (c)in medium 3, (d) in medium 4, (e) in medium 5.
Incubation was continuously carried out at 24 hr intervals for 150 days. Congo Red(●), Orange II(o), Crystal Violet(▲).

Then, altogether 150-fold batch incubations were continuously conducted from medium 1 to medium 5: in medium 1, 15 times, in medium 2, 15 times, in medium 3, 20 times, in medium 4, 40 times, in medium 5, 60 times, and at 24 hour intervals for a period of 150 days. The changes of color loss are shown in Figure 3.

In Table 1, the growth factors by continuous batch incubation are shown. Cells were acclimated and the eliminability of dyes was increased by repeated batch incubation. In the case of OrangeII, the more inoculation was repeated, the higher the color loss rate

Table 1. Growth factors by continuous batch incubation

Medium no.	Batch no.	Lag phase[1] T_l(hr)	Growth const[2] K(hr^{-1})	MLSS[2] (mg/ml)	Color loss of Orange II(%)[2]
1	1	8.0	0.15	0.30	36
	15	2.6	0.88	0.45	50
2	1	8.0	0.14	0.25	37
	15	3.0	0.45	0.30	47
3	1	8.0	0.14	0.18	35
	20	4.2	0.30	0.22	42
4	1	10	0.14	0.12	27
	40	6.0	0.20	0.15	45
5	1	12	0.13	0.09	18
	60	8.0	0.14	0.12	23

[1]These growth factors are referred to by Tempest (1970).
[2]After incubation for 24 hr with shaking culture.

became. As can be seen in Table 1, the growth of microorganisms was restricted by exchanging the medium. However, MLSS was again increased by repeated incubation. Figure 2b shows the changes of the growth with time and color loss after repeated cultivation (15 times). Comparing Figure 2a with Figure 2b, the lag phase is shortened and the growth constant is increased by repeated incubation in medium 1. Similar inclinations can be seen in media 2, 3, 4 and 5 as shown in Table 1. By exchanging the medium, the lag phase tended to grow longer, and the growth constant became smaller. The color loss rate can be observed in Table 1.

The activated sludge repeatedly cultured in medium 5 for 60 days was transferred to medium 4 and cultivated. When the activated sludge cultured in a comparatively high BOD value (medium 4) was transferred to a low BOD value (medium 5) after repeated cultivations, low color loss rate was observed (Figure 3e), but in a reverse transfer, a high color loss rate was again observed.

Figure 4 shows the change of color loss rate with the change in concentrations of Congo Red and Crystal Violet to $1x10^{-6}$-$1.2x10^{-2}$mol/l(medium 1). In the case of Congo Red, the color loss rate was moderate in every concentration range and any effect of dye concentration was not observed. However, in the case of Orange II and Crystal Violet, as the dye concentration increased, the color loss rate decreased. The inclination of color loss change by the dye concentration change coincided with those of the degree of inhibition when the dye concentration was changed (Ogawa et al. 1981).

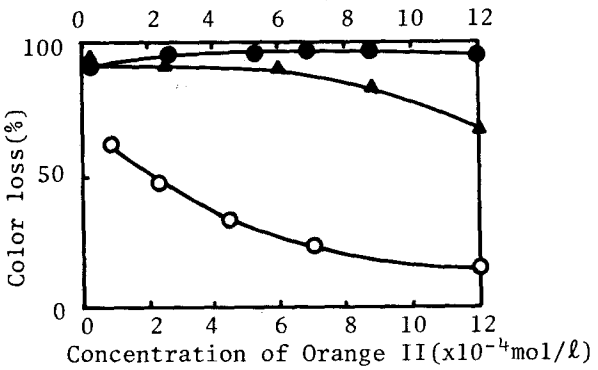


Figure 4. Relationship between the concentration of dyes and color loss. Orange II(○), Congo Red(●), Crystal Violet(▲).

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