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Acclimation of Activated Sludge to Dye

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Dye industries generally use an activated sludge process to purify waste water. However, some dyes are toxic to microbes and lessen their purifying function. We investigated the proliferous and respiratory inhibition of the unacclimated sludge by the dyes and elucidated the relationship between the structure of the dyes and the inhibition of the microbial growth (OGAWA *et al* 1974, 1978).

In the practical process of purification, the microbes contact dyes for a long time and must adapt to such surroundings. The toxicity of sulphur and azo dyes was reported (INOUYE and HONDA 1970, 1971), but the latter were in a mixture in which the components were unknown and the toxicity of a sole dye was not proven. Dye factories very frequently change the kind of dyes, so it is important for the purifying process by the acclimated microbes to maintain their adaptation to different kinds of dyes. Thus, in the present study the oxygen uptake rates of microbes acclimated through continuous culture in a medium containing dyes were obtained for the same and different kinds of coexisting dyes, and the influence of these dyes on the respiratory inhibition of the microbes was investigated.

EXPERIMENTAL

Dye

Dyes are shown in the table.

Activated sludge

The return sludges from the North Treatment Plant of Gifu City, Japan were used as the microbial source. The plant was the conventional activated sludge process and the waste water from industry is not much flowed in. Seed microbes were inoculated into the 100ml aerator as shown in Fig. 1 and cultured continuously in a medium (BOD_5 300ppm) containing glucose 200ppm, pepone 100, meat extract 50, $Na_2HPO_4 \cdot 12H_2O$ 15, KH_2PO_4 2, NaCl 5, KCl 2, $MgSO_4$ 2, $CaCl_2$ 1, $FeCl_3 \cdot 6H_2O$ 1 and dye 5.0×10^{-5}mol/ℓ. The inflow rate, the residence time and the residence volume of the medium were 12ml/hr, 5.8hr and 70ml, respectively. The sedimentary cell volume was kept to 50% of the residence volume (70ml) at 25° C in the aerator. The microbes were acclimated for 2 months, taken from the separator, washed with phosphate buffer, centrifuged and collected. The harvested cells were kept at 4°C. The oxygen uptake of the cells was completely recovered within 24hr.

Oxygen uptake rate

The cells were suspended in the medium, and oxygen uptake

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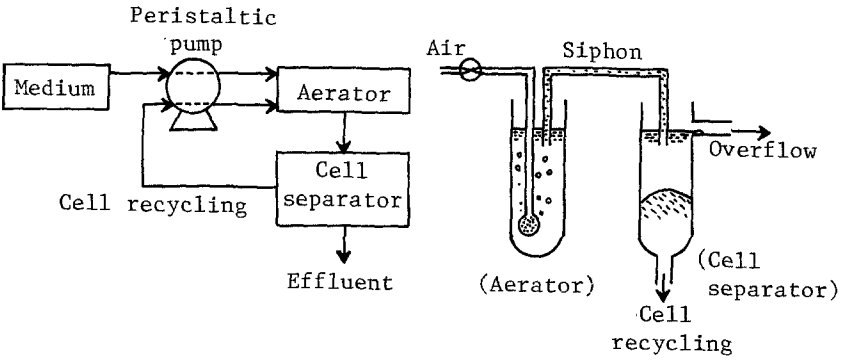


Fig. 1 Flow diagram of continuous culture

was measured with a DO meter in a 20ml rounded bottom flask after stirring for 3min. The degree of inhibition was obtained from the following formula:

$$\text{Degree of inhibition} = (1-\frac{Vd}{Vo}) \times 100 \ (\%)$$

Vd: oxygen uptake rate containing dye
Vo: oxygen uptake rate without dye

RESULTS AND DISCUSSION

Eliminability of dye by continuous culture

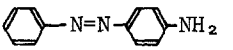
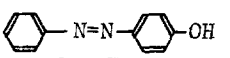
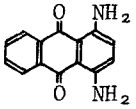
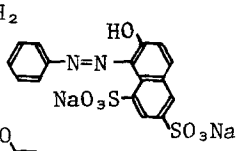
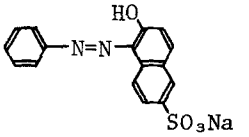
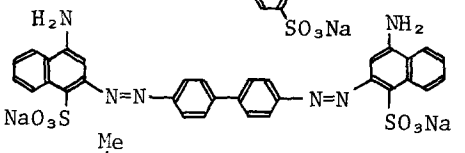
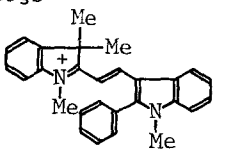
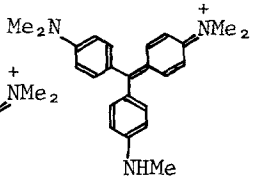
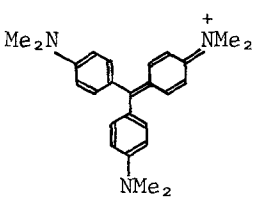
MLSS and pH in each aerator varied respectively in the range of 80-100ppm and 6.5-7.5 in the continuous culture. The eliminability of the dyes was as listed in the table. The results according to the classification of the dyes were as follows: the disperse dyes showed 30-75%, the acid dyes 5-50%, the basic dyes 50-100%, *i.e.*, compared with the disperse dyes (non-ionic), the acid dyes (anionic) showed a somewhat low eliminability, but the basic dyes (cationic) revealed a high one. The microbial cells were charged negative in appearance at pH 7. Thus, one cause of the low eliminability of the acid dyes is attributable to the ionic repulsion between dyes and cells. Hence, the high eliminability of the basic dyes results from the bioflocculation stimulated by ionic inducement between dyes and cells (OGAWA *et al* 1978c).

Respiratory inhibition by dye

The oxygen uptake rate of microbes acclimated to a dye by the continuous culture was measured with the same coexisting dye. The results of the disperse dyes were shown in Fig. 2. PAAB and PHAB were measured at below 5×10^{-5}mol/ℓ owing to low solubilities. The inhibitive degree of microbes unacclimated to PAAB became greater as its concentration increased. However, both PAAB and PHAB showed little inhibition of acclimated microbes in the concentration range, 1×10^{-6}-5×10^{-5}mol/ℓ. The inhibition of the former became rather negative in the low range concentration.The results revealed that the microbial cells underwent respiratory inhibition by disperse dyes, but their acclimation made the degree low. Concerning the negative inhibition, the following possibility can be considered. Provided that dye assimilating mi-

TABLE

Elimination of dye by continuous culture of activated sludge

Dye	Formula	Rate of elimination (%)
p-Aminoazobenzene (PAAB)		30-38
p-Hydroxyazobenzene (PHAB)		50-75
Disperse Violet 1		50-75
Acid Orange 10		5-20
Acid Orange 12		15-50
Direct Red 28		15-25
Basic Orange 22		50-70
Basic Violet 1		90-100
Basic Violet 3		80-100

crobes are in the sludge, their proliferation is greater in the continuous culture when these dyes coexist and thus make for a distribution different from that in the initial group of microbes. The oxygen uptake rates of the groups became high with coexisting dyes.

We previously undertook screening, isolation and identification of azo dye assimilating bacteria from soil in the drainage ditches of dyestuff factories, and clarified their metabolic pathways (IDAKA *et al* 1978, 1979, HORITSU *et al* 1977, OGAWA *et al* 1978c). As activated sludges are microbial populations of very many kinds of microbes, microbes assimilating azo dyes can be considered to be among them. Colonies with a proliferation of

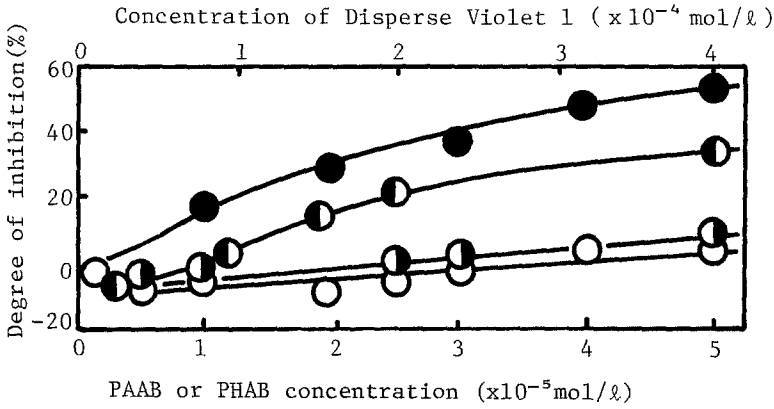


Fig. 2 Relationship between concentration of disperse dye and degree of inhibition
BOD_5 300ppm, MLSS 100ppm, temperature 25°C, pH 7.0,
● PAAB (non-acclimated), ○ PAAB, ◑ PHAB, ◐ Disperse Violet 1

certain bacteria were found as the result of the plate culture of activated sludges in the agar culture containing a disperse dye as a organic nutrition. Every dye was added to the cell suspension, left for 12hr, extracted with CH_2Cl_2, concentrated and developed with t.l.c. As a result, a few spots different from dyes at Rf value were identified and they were regarded as metabolites of dyes. From the results, an assimilating bacteria for every kind of dye was known to exist in the activated sludges. Accordingly, one of the causes of negative inhibition can be considered to be the contributions of the bacteria.

The results of the acid azo dyes and the direct dye are shown in Fig. 3. Compared to the unacclimated microbes, the inhi-

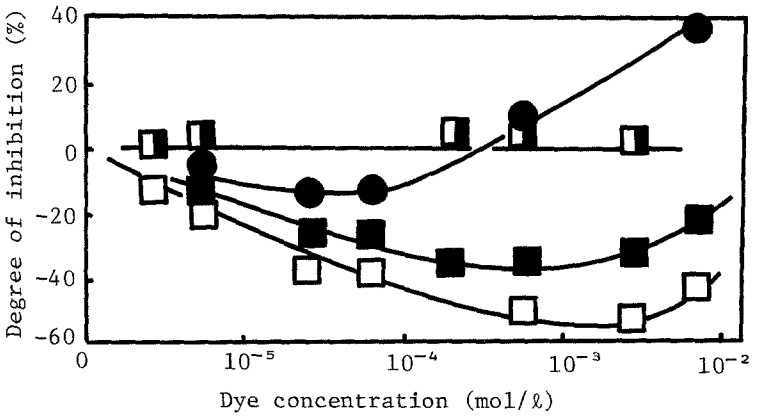


Fig. 3 Relationship between concentration of acid or direct dye and degree of inhibition
BOD_5 300ppm, MLSS 100ppm, temperature 25°C, pH 7.0,
● Acid Orange 10 (non-acclimated), □ Acid Orange 10,
■ Acid Orange 12, ◨ Direct Red 28

bition of acclimated ones is low and the inhibition by the acid azo dye is negative in the concentration range, $1x10^{-6}$-$1x10^{-2}$mol/ℓ. The inhibition of the acclimated microbes by the direct dyes is greater than by the acid dyes. However, in the case of unacclimated microbes, the inhibition of both the acid dyes and the direct dyes was about the same level (OGAWA *et al* 1978b). From this, the diminution of inhibition by acclimation to the acid dyes was known to be greater than that of the direct dyes. As one cause, the molar volume of the acid dyes is smaller than that of the direct dyes, so the permeability of the cell wall is greater and the acclimated microbes consequently have a much higher assimilative capability. The isolated azo dye assimilating microbes degraded the acid dyes more than the direct dyes.

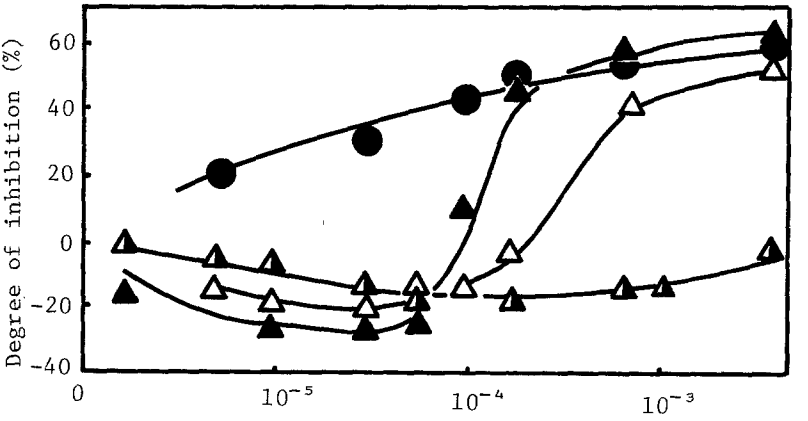


Fig. 4 Relationship between concentration of basic dye and degree of inhibition
BOD_5 300ppm, MLSS 100ppm, temperature 25°C, pH 7.0,
● Basic Violet 1 (non-acclimated), ▲ Basic Violet 1,
▲ Basic Violet 3, ▲ Basic Orange 22

The inhibition levels of the basic dyes is shown in Fig. 4. That of the unacclimated microbes by Basic Violet 1 increases as the dye concentration increases. Compared to the other dyes in Figs. 2 and 3, Basic Violet 1 shows a greater inhibitive action. This coincides with the previous report showing the relationships of respiratory inhibition: basic dye > acid dye ≃ direct dye ≃ disperse dye. The acclimated microbes showed negative inhibition at a low dye concentration and a positive one at a high concentration. The tendency of them was remarkable from triphenylmethanes (Basic Violet 1 and Basic Violet 3).

To ascertain the cause of the negative inhibition, we tested the biodegradability of the dyes. There are reports on the biodegradability of the azo dyes (YONEZAWA and URUSHIGAWA 1977, KAPPELER *et al* 1978, MEYER *et al* 1979) besides ours, but none on the biodegradability of the basic dyes. Therefore, the degradation of Basic Violet 1 was tested. A suspension of microbial cells acclimated with Basic Violet 1 was incubated in the medium containing Basic Violet 1 for 12hr, then extracted with *n*-butanol and devel-

oped on t.l.c. As a result, some spots corresponding to metabolites of the dye were observed, and microbes degrading Basic Violet 1 were known to exist. Isolation, identification and biodegradability of the microbes are under way.

Respiratory inhibition by a different dye used for acclimation

The respiratory inhibition of the microbes acclimated to the dyes was measured in the medium containing Acid Orange 10 as shown in Fig. 5. The microbes acclimated by Acid Orange 10 show

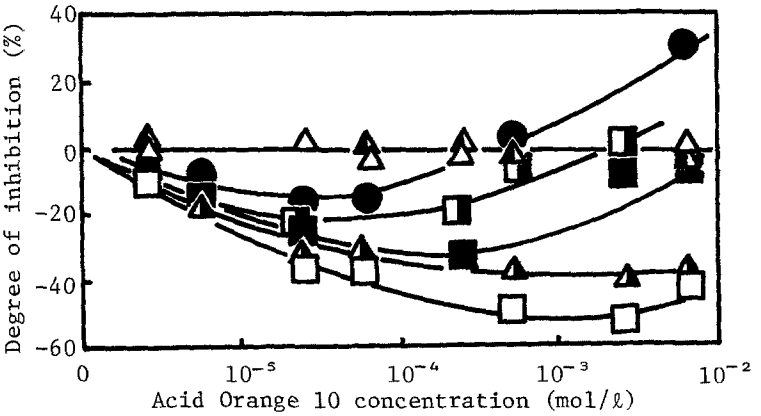


Fig. 5 Relationship between Acid Orange 10 concentration and inhibition of microbes acclimated by a dye different from Acid Orange 10
BOD_5 300ppm, MLSS 100ppm, temperature 25°C, pH 7.0,
● non-acclimated, □ Acid Orange 10, ■ Acid Orange 12,
△ Basic Violet 1, ▲ Basic Orange 3, ◭ Basic Orange 22,
◨ Direct Red 28

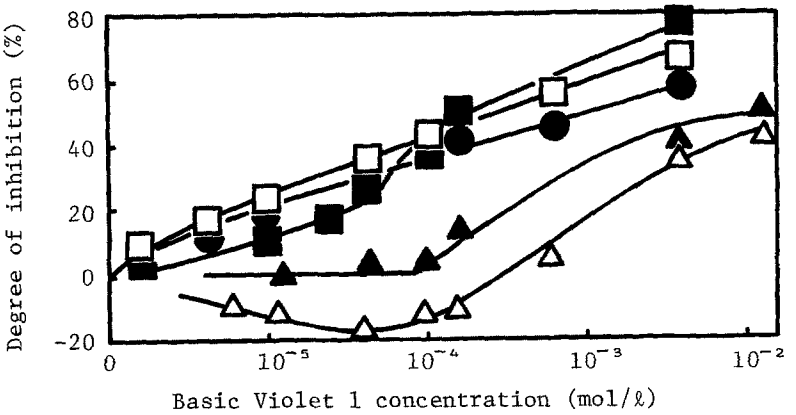


Fig. 6 Relationship between Basic Violet 1 concentration and inhibition of microbes acclimated by a dye different from Basic Violet 1
BOD_5 300ppm, MLSS 100ppm, temperature 25°C, pH 7.0,
● Basic Violet 1 (non-acclimated), △ Basic Violet 1,
▲ Basic Violet 3, □ Acid Orange 10, ■ Acid Orange 12

the least inhibition. Direct Red 28, which is the same acid azo dye with Acid Orange 10 but has a great molar volume, showed a low inhibition compared with unacclimated microbes. On the other hand, the microbes acclimated by Basic Violet 1 (triphenylmethanes) evidenced a higher inhibition than unacclimated microbes at a concentration below 5×10^{-5} mol/ℓ.

The respiratory inhibition of the microbes acclimated by Basic Violet 1 was measured as shown in Fig. 6. The microbes acclimated by Basic Violet 1 showed the lowest inhibition. The microbes acclimated by Basic Violet 22 showed the next lowest. The levels of inhibition of the microbes acclimated to Acid Orange 1 and Acid Orange 12 were about the same as by the unacclimated one. From the results, it was thought that the tolerance of the microbes to the dyes is adaptable to the same strain of dyes used for the acclimation but failed to display any adaptability to a different strain of dyes. This suggests that in the practical treatment of waste water by microbes, the mixing of different kinds of dyes is the cause of the decline in the purification capability.

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