Chemico-Biological Interactions in Biological Purification System
I. Growth Inhibition Effect of Azo Compounds on Activated Sludge Microorganisms

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There are enormous discharge of synthetic organic compounds into the environment by industrial production. These compounds give large influences not only in natural ecosystem but directly in humanlife and industrial production itself. Then it is important for maintenance and development of production to clarify those behaviors in the environment and to reduce those discharge into the environment.

The biological waste treatment method has rather lower running cost and is the most popular method. It is necessary to determine the behavior of those compounds in biological waste treatment process to establish more effective system. Furthermore this method has been developed by modifying the natural self-purification process and the system of the microorganisms in this may be considered as a model of natural micro-ecosystem. Then the behavior of those compounds in this process gives significant information about the fate of synthetic organic compounds in the environment.

Azo compound is one of the oldest industrially synthesized organic compounds and widely used even now. It has been also noted for its high biological activity, i.e., carcinogenic activity. Then there are many works on its effect on higher animals, but there are few works on its effect on natural ecosystem and waste treatment plant.

In order to evaluate the effect of azo compound on the biological waste treatment, the growth inhibition effect of it on activated sludge microorganisms was studied.

Materials and Methods

Chemicals Azobenzene, 4-aminoazobenzene, o-, m-, and p-methyl red used in these experiments were chemical reagent grade. Other azo compounds used were prepared by authors.

Evaluation of growth inhibition effect Growth inhibition effect was evaluated by the concentration of azo compounds in which logarithmic phase growth rate constant was decreased to 50 % of that of the culture without azo compound. Growth rate constant was determined with turbidity of that of the culture at 660 nm. The

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culture medium and all azo compounds used did not absorb the light at that wave length. The mixed microorganisms were obtained from supernatant of activated sludge and pre-incubated with the medium without azo compounds for 2 days. The microorganisms were inoculated to the experimental medium to give the optical density of the culture at 0.02-0.05. Incubation temperature was at 25 ± 1 °C. The culture medium was shown in Table I.

Table I Culture Medium

Glucose	500	ppm
Peptone	500	
KH_2PO_4	270	
K_2HPO_4	100	
Na_2HPO_4	660	
$MgSO_4\ 7H_2O$	2.00	
$CaCl_2$	0.0200	
$FeSO_4\ 7H_2O$	0.0200	

in (i) aqueous solution
(ii) 2 % aqueous ethanol solution

Typical example of the relation between growth rate constant and azo compound concentration was represented in Figure I. This cor-

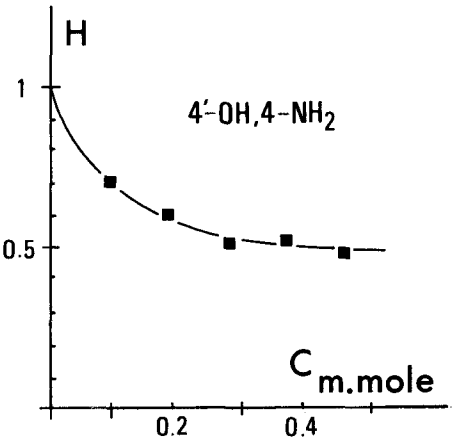

Fig. I. Relative growth rate constant (H) as a function of 4-amino-4'-hydroxyazobenzene concentration (C).

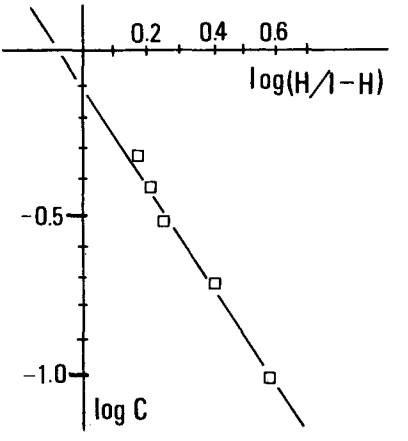

Fig.II. Logarithms of 4-amino-4'-hydroxyazobenzene as a function of log(H/1-H).

relation could be appoximated by Equation (1).

$$H = \phi^n / (c^n + \phi^n) \qquad \text{.....(1)}$$

where H ; relative growth rate constant
H = 1 in the culture without azo compound
C ; azo compound concentration (m,mole/l.)
Ø ; azo compound concentration of 50 % growth inhibition (m.mole/l.)
n ; constant

Equation (1) was rearranged to the form

$$\log C = -\frac{1}{n} \log(H/1-H) + \log Ø. \quad \text{.....(2)}$$

Then log Ø was obtained by plotting log C vs. log(H/1-H) as shown in Figure II.

Partition coefficient between octanol and water Partition coefficient was calculated with HANSCH's method (LEO et al. 1971) by using observed data of 4-aminoazobenzene and 4-dimethylamino-azobenzene in their review. Contributions of functional groups to the partition coefficient were calculated from that of aniline derivatives.

Results and Discussion

In Table II, 50 % inhibition concentrations for 19 azo compounds are shown. As 4-dimethylaminoazobenzene (butter yellow), azobenzene, and 4-aminoazobenezene-4'-sulfonic acid had very low solubility in the culture, they could not be provided for experiments. Any growth inhibition effects were not shown by 4-dimethylaminoazobenzene-3'-carboxylic acid (m-methyl red, 9), 4-methyl-azobenzene-4'-sulfonic acid (12), 2,4-dimethylazobenzene-4'-sulfonic acid (13), and 4-nitroazobenzene-4'-sulfonic acid (18) in maximum exprimental concentration.

Except 4,4'-diaminoazobenzene (3) and m-methyl red (9), aminoazobenzenes showed strong growth inhibition effect. And azobenzenesulfonic acids showed weak effect. Methyl orange (4-dimethylaminoazobenzene-4'-sulfonic acid) and 2,4-diaminoazobenzene-4'-sulfonic acid (19), which have both amino and sulfonic acid groups, had relatively low solubility in culture medium, then 50 % growth inhibition concentration of them could not be determined. But in the preliminary experiments with whole activated sludge other than supernatant, 50 % growth inhibition concentration of those compounds could be determined at the concentration over their solubility in the culture medium. They had medium log Ø values between aminoazobenzenes and azobenzenesulfonic acids, although this result can not be directly compared with that result. (Each log Ø value of them was 0.742 and 0.649.)

The growth inhibition effect of amino derivatives increased irrespective of the number of amino function, which might depend on their substitution site.

HANSCH et al. presented a model of appearance of biological activity of organic compound (HANSCH et al. 1963, HANSCH and

Table II. 50 % Growth Inhibition Concentrations (Ø) of azobenzene Derivatives

	Compound	Medium	log Ø	log C	log P_{oct}
1.	4-NH_2	e	-0.100	-0.595	2.89
2.	2,4-$(NH_2)_2$	e	-1.056	-0.752	2.19
3.	4,4'-$(NH_2)_2$	e	0.982	-0.423	1.78
4.	2,4,4'-$(NH_2)_3$	e	-0.192	-0.356	0.99
5.	2,4-$(NH_2)_2$,4'-AcNH	e	-0.332	-0.731	1.21
6.	4-NH_2,4'-OH	e	-0.118	-0.328	2.00
7.	4-AcNH,4'-OH	e	-0.188	-0.407	2.22
8.	4-Me_2N,2'-COOH	e	-1.102	-0.430	4.88
9.	4-Me_2N,3'-COOH	e	—	-0.435	3.85
10.	4-Me_2N,4'-COOH	e	-0.397	-1.25	4.32
11.	4-SO_3Na	w	2.72	0.674	-0.61
12.	4-Me,4'-SO_3Na	w	—	0.646	-0.16
13.	2,4-Me_2,4'-SO_3Na	w	—	0.620	0.30
14.	4-OH,4'-SO_3Na	w	1.31	0.556	-1.51
15.	2,4-$(OH)_2$,4'-SO_3Na	w	0.933	0.624	-1.88
16.	4-Cl,4'-SO_3Na	w	1.89	0.529	0.28
17.	2,4-Cl_2,4'-SO_3Na	w	1.36	0.250	1.17
18.	4-NO_2,4'-SO_3Na	w	—	0.590	-0.10
19.	2,4-$(NH_2)_2$,4'-SO_3Na	w	—	-0.277	-2.24

Ø ; concentration of 50 % growth inhibition (m.mole/1.)
C ; maximum experimental concentration (m.mole/1.)
P_{oct} ; partition coefficient between octanol and water
e ; 2 % aqueous ethanol solution
w ; aqueous solution

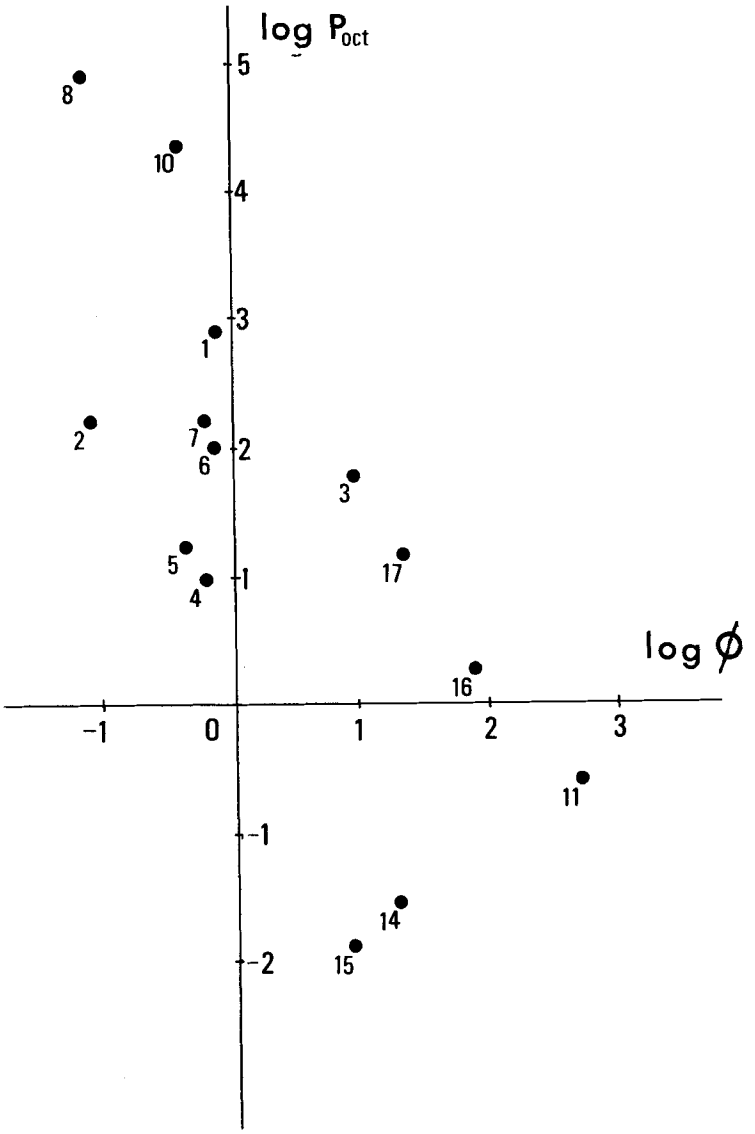

Table III. The relation of 50 % growth inhibition concentration (∅) of azobenzenes with their partition coefficient between octanol and water (P_{oct})

FUJITA 1964), which was constituted with permeation of organic compound through the cell menbrane and their function in the cell. In their model the partition coefficient between octanol and water (P_{oct}) was used to evaluate the permeability of organic compound through the cell menbrane, and HAMMETT's constant (σ) was used to evaluate their activity in the cell.

Figure III shows the relation between the P_{oct} and 50 % growth inhibition concentrations ($\emptyset$). As the azobenzene derivatives used in our experiments are not definite in their active site, HAMMETT's constant could not be applied. However, it was found that the higher the partition coefficient was, the weaker the growth inhibition effect was. This relation could be expressed in the form

$$\log \emptyset = 1.00 - 0.417 \log P_{oct} . \quad \ldots\ldots\ldots (3)$$

The correlation coefficient of Equation (3) was 0.716. Then the 50 % growth inhibition concentration of azobenzenes had significant correlation over 0.995 confidence level with their partition coefficient between octanol and water.

This result shows that the inhibition effect of azo compounds on activated sludge microorganisms depends on the cell menbrane permeability of them, and permeation through the cell menbrane may be a rate determinant in growth inhibition process of azo compounds.

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