Chemico-Biological Interactions in Biological Purification System
II. Biodegradation of Azocompounds by Activated Sludge

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In order to find the fate of azo compound in natural ecosystem and biological waste treatment process, the biodegradability of azo compound was determined by activated sludge which may contain most of the microorganisms contributing to self-purification process in aquatic environment. Pratt(1968-1970) reported the biodegradation of azo dyes by sewage microorganisms, but it was not systematic but individual. This paper deals with the relationship between the biodegradability of azo compounds and their molecular structures together with the factors affecting the biodegradability.

Materials and Methods

The microorganisms used in this study were sewage sludge maintained with glucose and peptone as carbon and nitrogen sources.

The chemicals used were commercial products of chemical grade and synthetic ones in our laboratory.

Azo compound was added to obtain a concentration of 50 mg/l in 200 ml of Erlenmyer flask containing 1000 mg/l of sludge and the culture medium*, based on a total volume of 100 ml and agitated with rotary shaker(175 rpm) at 25 ± 1 °C. The water soluble azo compounds(compounds 1 - 11) were added as solid state and slightly soluble ones(compounds 12 - 31) were added as ethanol solution, so that the concentration of ethanol in culture was 2 %.

Ten of such a reaction flask were provided for estimating the time course of the reaction. The samples were harvested with a period of 1 - 2 days. The concentrations of azo compounds were determined in terms of UV absorbance of the supernatant solutions of the cultures which were centrifuged for 15 min. at 13,000 g for water soluble ones and that ethanol extracts which were prepared by extraction with hot ethanol from freeze-dried cultures for slightly soluble ones.

Results and Discussion

The "biodegradability" of azo compound in this paper indicates the degree of primary biodegradation that the azo-bond is simply cleaved because the concentration of azo compound was determined only in terms of UV absorbance.

* same as that in the preceding paper

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Table I Biodegradability of Azo Compounds

No	Compound	Observed Wave Length (nm)	Type of Curve	Induced Period (days)	Rate Constant ($\times 10^2$/day)	Percent of Degradation
1	4'-SO_3Na	320				0 (20)***
2	4-OH,4'-SO Na	355	b,c	7	1.0	18 (20)
3	2,4-$(OH)_2$,4'-SO_3Na	429	a,c		2.6	60 (15)
4	4-NH_2,4'-SO_3Na	387	b,c	7	0.26	14 (20)
5	2,4-$(NH_2)_2$,4'-SO_3Na	448	b,c	6	6.0	55 (11)
6	4-Me,4'-SO_3Na	332				0 (20)
7	2,4-Me2,4'-SO_3Na	339				0 (20)
8	4-Cl,4'-SO_3Na	327				0 (20)
9	2,4-Cl2,4'-SO_3Na	333				0 (20)
10	4-MeO,4'-SO_3Na	346				0 (20)
11	4-NO_2,4'-SO_3Na	335				0 (20)
12	4-OH*	349	a		6.2	90 (15)
13	4-MeO	344	b	7	1.4	11 (11)
14	2-OH	370	b	11	5.5	61 (18)
15	2-MeO	350	b	7	1.8	15 (18)
16	4-COOH*	323				(18)
17	4-COOEt	323				(18)
18	2-COOH	318	b	7	0.28	10 (18)
19	2-COOEt	315	a		0.58	19 (18)
20	4-NH2*	387	a,a	7**	4.5, 11.2	89 (13)
21	4-OH,4'-NH2	383	a,a	9**	4.4, 16.6	91 (13)
22	4,4'-$(NH_2)_2$	399	a,a	7**	2.6, 8.1	78 (13)
23	4-AcNH	348	a,a	7**	1.5, 14.8	80 (11)
24	4-OH,4'-AcNH	363	a,a	9**	5.0, 17.7	94 (11)
25	4,4'-$(AcNH)_2$	369	b	8	9.9	74 (13)
26	2,4-$(NH_2)_2$	415	a		10.6	82 (7)
27	2,4,4'-$(NH_2)_3$	449	a		9.3	93 (11)
28	2,4-$(NH_2)_2$,4'-AcNH	445	a		10.3	88 (9)
29	4-Me_2N,2'-COOH*	406	a		200	99 (1)
30	4-Me_2N,3'-COOH*	409	a		10.0	50 (3)
31	4-Me_2N,4'-COOH*	423	a		36.6	92 (3)

* Commercial product
** The day when rate constant changes
*** The day observed

The reduction rate of the concentration of azo compound was approximated to first order equation for the elapsed time. The biodegradability of each azo compound was assessed by the rate constant of each equation. This rate constant,of course, was obtained by chance in this experiment and thus does not show the "true"value. However, we could discuss the relative biodegradability of each azo compound and the factors largely affecting the biodegradability.

The reaction courves were divided into three types: type a was decolorized without induced period, and type b was decolorized after some induced period, but as for type c, its decolorization proceeded only to some extent and kept constant so far as in our experiments.Some reaction curves showed two types simultaneously.

The experimental results of each azo compound were listed in Table I. Mecke(1957) showed that the azo compound with amino or hydroxy group was readily decolorized but that with methyl group was not decolorized by yeast. The results of the experiment from compounds 1 to 11 were similar to his results. Hydrogen-, chloro-, methoxy-, and nitro derivatives other than methyl derivatives were hardly decolorized, but only amino and hydroxy derivatives were decolorized.

Mecke(1957) and Walker(1971) suggested the effect of hydrogen bond with azo-bond and described that hydroxy or amino group situated at a position capable of forming hydrogen bond with azo nitrogen stabilized the azo-bond, while carboxy group situated at the same position as hydroxy group unstabilized it. The results from compounds 12 to 19 support the above idea. That is, the rate of 2-hydroxy derivative is less than that of 4-hydroxy one and the rate of 2-carboxy derivative is more than that of 4-carboxy one.

It seems to be a little strange that the rate of 2-hydroxy derivative is more than 2-carboxy one, although it may show the effect of hydrogen bond that the induced period of the latter is shorter than that of the former. Mecke(1957) described that 2'-carboxy-4-dimethylamino azobenzene was decolorized faster than 2'-hydroxy-4-dimethylamino azobenzene. From this, it is considered that the dimethylamino group makes an important contribution to determine the decolorizability of these compounds. Actually, compounds 29, 30 and 31 with dimethylamino group were decolorized extremely fast, while compound 16 without above group was not decolorized. Consequently, it might be considered that the carboxy group itself has a rate decreasing character similar to sulfonyl group because of its more hydrophilic character than hydroxy group,and the dimethylamino group compensates sufficiently the hydrophilic character of carboxy group.

The experiment from compounds 20 to 28 was undergone to determine the decolorizability of amino and its acetyl derivatives. In this series, the compound with amino or acetyl amino group at only 4- or 4,4'-position was decolorized in two steps. The periods of the rate change of compounds 23, 24 and 25 coincided with that of each original amino derivative and after the period, each of them was decolorized with much the same rate as each original amine.

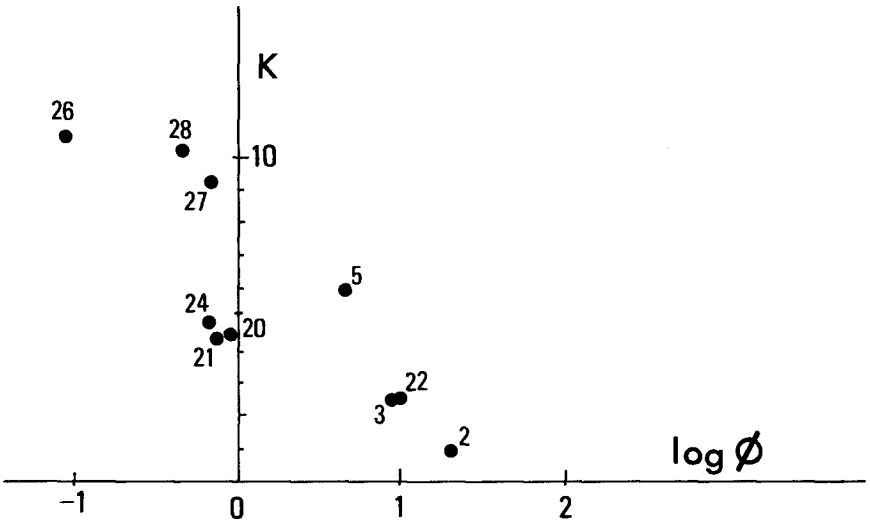

Figure I. The plots of the rate constants (K) versus 50 % growth inhibition concentrations* (Ø)

*from the preceding paper

Furthermore, compounds 23 and 24 were subjected to shift the maximum absorption bands of their original amines up to the time of the rate change; compound 23: 348→370→384→389 nm, compound 24: 363→378→385 nm. Compound 25 did not show the band shift. From this, it might be concluded that the compounds 23 and 24 are decolorized after or in parallel hydrolysis to free amine by activated sludge, while Sato(1962) described that 4-amino azobenzene was acetylized with liver homogenates.

The order of the initial rate constant of each amino derivative was compound 26 > 27 > 20,21 > 22 and the rate of each actylderivative was slightly higher than that of each original amine except 4-acetyl derivative.

Walker(1971) determined the rate of reduction on a series of analogous of Red 10B and Red 10G by cell-free extracts of Strep. faecalis and described that the predominant factor determining the reduction rate is electron density in the region of the azo group and the substitution of electron withdrawing group($-SO_3H$, $-SO_2NH_2$) in the para position resulted in a marked increase in reduction rate. The results of our experiment(not cell-free but whole cell) are quite different from that of Walker. That is, the contribution of the electron withdrawing group($-SO_3H$, -COOH, NO_2)is rather negative as shown in Table I.

In the whole cell condition we must search another factor as a predominant factor determining the decolorizability. Figure I shows the plots of the rate constant obtained in this experiment versus 50 % growth inhibition concentration*. There is an apparent correlation that the higher the 50 % growth inhibition concentration is, the lower the decolorizability. From this, the permeability through the cell menbrane that determined largely the 50 % growth inhibition concentration may be considered as one of the important factors determining the decolorizability of azo compound by activated sludge.

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