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A METHOD TO CLASSIFY BIODEGRADABILITIES OF ORGANIC COMPOUNDS

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Summary

Biodegradability is a most important characteristic of an organic compound for predicting its fate and life in the environment. Biodegradations of eleven principal organic compounds were tested with an electrolytic respirometer under various conditions, such as concentration of organic compound, concentration and acclimatization of microorganisms for seedings, and temperature. Five characteristic indexes of the biodegradations were obtained from the biochemical oxygen demand (BOD) curves and dissolved organic carbon (DOC) measurements, and standard conditions for testing biodegradabilities of organic compounds were recommended. Furthermore, a new method to classify the biodegradabilities of organic compounds into ten ranks was proposed by considering the biodegradation times and ratios.

Introduction

Synthetic organic compounds cause various environmental problems. Biodegradability is a most important characteristic of an organic compound for predicting its fate and life in the environment, and it is also important for anticipating treatability of wastewater containing the compound. Various testing methods of the biodegradabilities of organic compounds had been discussed and reported by the OECD committee [1], and several testing methods were set as standards or guidelines, as summarized by Urano and Kato [2]. However, the conditions of those testing methods differ from each other, and influences of the testing conditions on the results have not yet been clarified. Furthermore, the objects of those conventional testing methods were only to judge whether the organic compounds could be biodegraded or not. In this study, the biodegradations of principal organic compounds under various conditions are investigated, and characteristic indexes of the biodegradations are obtained. Furthermore, a method of classifying the biodegradabilities of organic compounds into 10 ranks is proposed.

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Materials and methods

Aqueous solutions of 11 organic compounds, whose names and properties are listed in Table 1, were used for the biodegradation tests in this study. The values of theoretical oxygen demand (ThOD) for each compound were calculated by the following oxidation equation from the molecular formulae.

$$C_aH_bN_cS_dO_e + (2a + b/2 + 2c + 2d - e)\,O \rightarrow a\,CO_2 + (b/2)\,H_2O + c\,NO_2 + d\,SO_2 \quad (1)$$

TABLE 1

Properties of organic compounds used

Compound name	Molecular formula	ThOD (g/g compound)	DOC (g/g compound)
D-Glucose	$C_6H_{12}O_6$	1.07	0.40
L-Glutamic acid	$C_2H_4(COOH)CH(NH_2)COOH$	1.37	0.41
Ethylalcohol	C_2H_5OH	2.08	0.52
Butyric acid	C_3H_7COOH	1.82	0.55
Ethylacetate	$CH_3COOC_2H_5$	1.82	0.55
Acetone	CH_3COCH_3	2.20	0.62
Monoethanolamine	$NH_2(CH_2)_2OH$	2.23	0.39
Phenol	C_6H_5OH	2.38	0.77
Aniline	$C_6H_5NH_2$	3.01	0.77
Benzenesulfonic acid	$C_6H_5SO_3H$	2.33	0.46
m-Aminophenol	$C_6H_4(NH_2)OH$	2.42	0.66

An electrolytic respirometer (Okura Riken Coulometer) whose system is shown in Fig. 1 was used for the biodegradation tests. This respirometer can continuously measure and automatically record the values of biochemical oxygen demand (BOD) for six samples simultaneously. Sample solutions of 300 ml were put into culture flasks, and 1 ml each of JIS inorganic mediums [3] and a fixed amount of activated sludge were added in the sample solutions. Then the flasks were set into the respirometer and BOD curves were measured for 14 days at a constant temperature. Compositions of JIS inorganic mediums are shown in Table 2. The activated sludge for the seeding had been semicontinuously cultured by the MITI method [4] with a synthetic sewage whose composition is shown in Table 3. In several tests, however, activated sludge which had been acclimatized for 2 weeks by the synthetic sewage containing 100 mg/l of the sample compound was used for the seeding. A part of the sample solution was extracted at fixed intervals of time for analyzing dissolved organic carbon (DOC) and concentration of biomass. DOC were analyzed with a total organic carbon analyzer (Beckman Model 102) after filtration with 0.45 μm membrane. The concentrations of

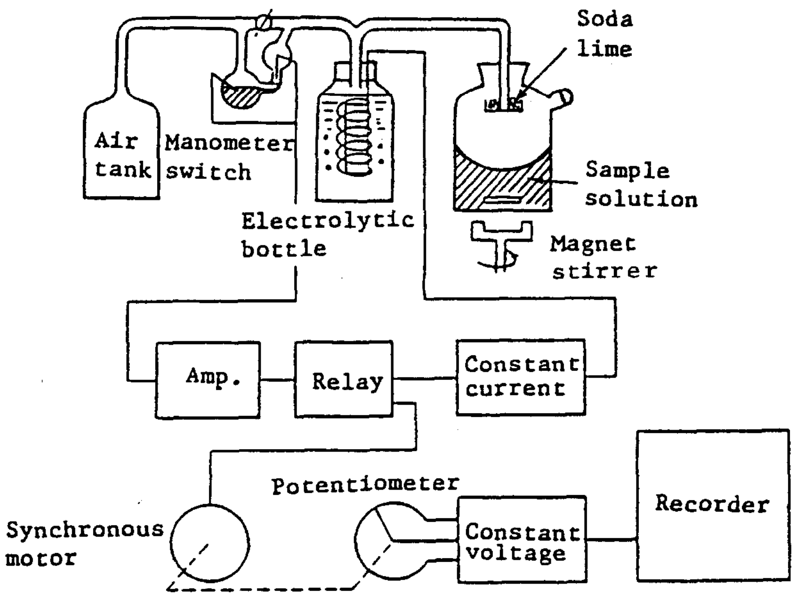

Fig. 1. System of electrolytic respirometer for biodegradation tests.

TABLE 2

Compositions of JIS inorganic mediums

Solution	Component per liter
A	K_2HPO_4: 25.75 g, KH_2PO_4: 8.5 g, $Na_2HPO_4 \cdot 12H_2O$: 44.6 g, NH_4Cl: 1.7 g
B	$MgSO_4 \cdot 7H_2O$: 22.5 g
C	$CaCl_2$: 27.5 g
D	$FeCl_3 \cdot 6H_2O$: 0.25 g

TABLE 3

Composition of synthetic sewage for culture of activated sludge

Component per liter	
Glucose	25 g
Peptone	25 g
Corn steep liquor	16 ml
KH_2PO_4	25 g
NaOH	16 g

biomass were obtained from absorbances at 660 nm (A_{660}) with a spectrophotometer (Hitachi Model 124). The BOD curve was revised for volume decrease of the solution with the sampling.

Results and discussion

Characteristic indexes of biodegradation of organic compounds

Changes of the biodegradation ratio (BOD/ThOD), DOC, and A_{660} with time (t) are shown representatively in Figs. 2 and 3 from the biodegradation tests of 100 mg/l solutions with seeding of 30 mg/l activated sludge at 20° C. After a lag time, BOD/ThOD and A_{660} increased geometrically, and DOC decreased inversely. Thereafter, BOD/ThOD increased gradually, but A_{660} decreased gradually by endogenous respiration of microorganisms, and DOC also decreased slightly.

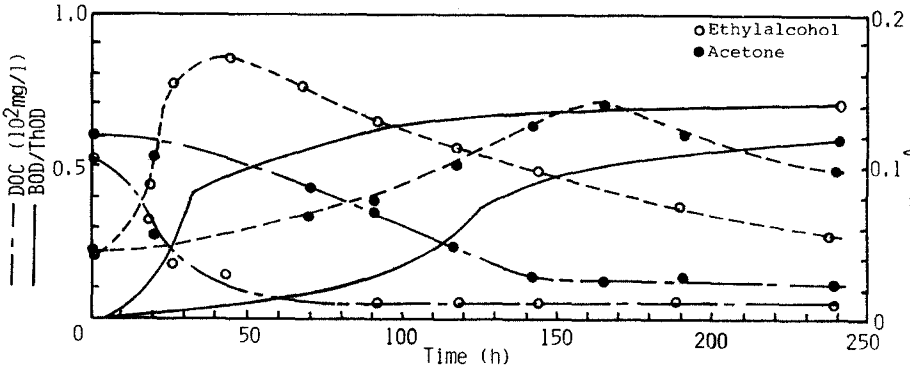

Fig. 2. Changes of biodegradation ratio (BOD/ThOD), dissolved organic carbon (DOC) and absorbance at 660 nm (A_{660}) with biodegradation time (t) for ethylalcohol and acetone. (Compound: 100 mg/l; activated sludge: 30 mg/l, 20° C.)

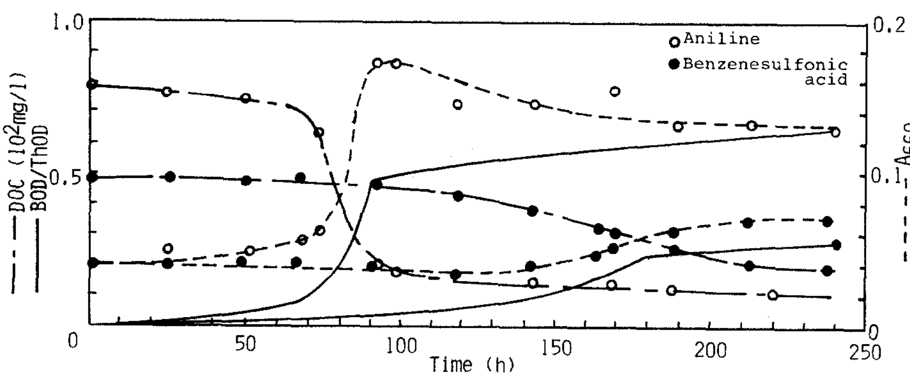

Fig. 3. Changes of BOD/ThOD, DOC, and A_{660} with t for aniline and benzenesulfonic acid. (Conditions are the same as in Fig. 2.)

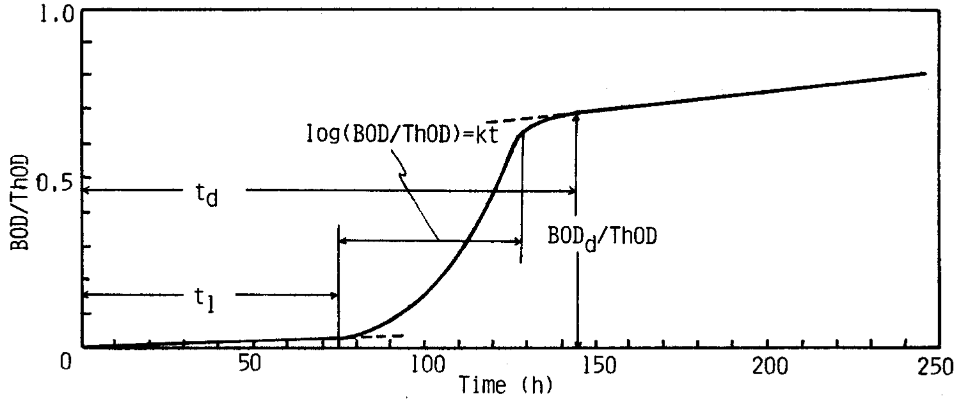

Fig. 4. Typical curve of BOD/ThOD and the characteristic indexes for biodegradation.

Therefore, it seemed that the BOD/ThOD curves could be characterized by four indexes, the lag time (t_l), the rate constant (k) for the geometrical biodegradation, the biodegradation time (t_d) before the endogenous respiration period, and the biodegradation ratio at t_d (BOD_d/ThOD) as shown in Fig. 4. The values of k can be obtained from the slope of the relationship between t vs. log (BOD) or log (BOD/ThOD), because the following first order eqn. (2) can be applied for the geometrical biodegradation period and eqns. (4) and (5) are obtained from eqn. (2).

$$d(\mathrm{BOD})/dt = k' (\mathrm{BOD}) \quad (2)$$

$$\ln (\mathrm{BOD}) = 2.3 \log (\mathrm{BOD}) = k't + \text{constant} \quad (3)$$

$$\log (\mathrm{BOD}) = (k'/2.3)\, t = kt + \text{constant} \quad (4)$$

$$\log (\mathrm{BOD/ThOD}) = \log (\mathrm{BOD}) - \log (\mathrm{ThOD}) = kt - \log (\mathrm{ThOD}) + \text{constant} \quad (5)$$

The value of t_l indicates the necessary time for adaptation, the value of k corresponds to the biodegradation rate, the value of t_d indicates the necessary time for biodegradation, and the value of BOD_d/ThOD indicates the ratio of biodegradation.

For evaluation of the biodegradabilities of organic compounds, the ratio of DOC at t_d to the initial value (DOC_d/DOC_0), which corresponds to the amount of intermediates, seemed also to be an important characteristic index of the biodegradation. Those characteristic indexes were obtained and are listed for the each compound in Table 4.

Influence of concentration of organic compounds on biodegradation

The representative BOD/ThOD curves for 10, 30 and 100 mg/l solutions of the organic compounds are shown in Fig. 5. The seeding concentrations of the activated sludge in these tests were 30% of the concentrations of the

TABLE 4

Characteristic indexes for each compound in various concentrations

	Concentration (mg/l)	t_l (h)	k (h^{-1})	t_d (h)	BOD_d / ThOD	DOC_d / DOC_0
D-Glucose	100	5	0.076	130	0.44	0.19
L-Glutamic acid	100	5	0.095	100	0.60	0.24
Ethylalcohol	100	5	0.049	100	0.55	0.13
	30	5	0.056	110	0.62	0.11
	10	5	0.057	110	0.65	0.11
Butyric acid	100	5	0.057	140	0.72	0.16
Ethylacetate	100	5	0.042	60	0.43	0.15
Acetone	100	20	0.016	155	0.42	0.22
Monoethanolamine	100	20	0.036	85	0.40	0.14
Phenol	100	20	0.028	100	0.62	0.09
	30	15	0.037	90	0.70	0.06
	10	15	0.041	90	0.60	0.07
Aniline	100	55	0.046	110	0.49	0.18
	30	55	0.035	120	0.49	0.15
	10	40	0.030	110	0.50	0.13
Benzenesulfonic acid	100	110	0.015	180	0.26	0.49
	30	90	0.015	175	0.42	0.28
	10	85	0.029	130	0.45	0.26
m-Aminophenol	100	>240	—	—	—	—
	30	>240	—	—	—	—
	10	>240	—	—	—	—

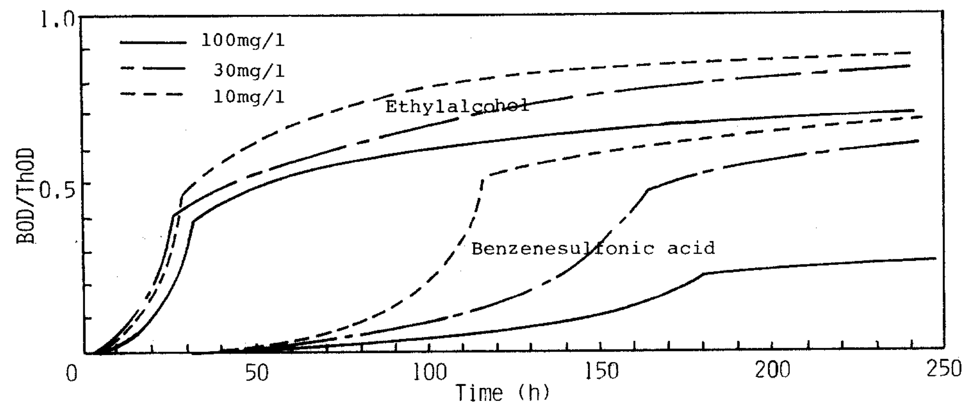

Fig. 5. Variation of BOD/ThOD curve with concentration of organic compound. (Activated sludge/compound = 0.3; 20° C.)

organic compounds with the same ratio as in the MITI method [4]. The characteristic indexes are also shown in Table 4. The values of the indexes for the high biodegradable compounds varied slightly with the concentrations, but for the low biodegradable compounds such as aniline and benzenesulfonic acid, t_l and t_d increased and k and BOD_d/ThOD decreased with increases of the concentrations. However, the concentration of 100 mg/l seemed to be better for the general biodegradation tests, because it was difficult to measure BOD curves and DOC accurately at lower concentrations.

Influences of concentration and acclimatization of microorganisms on biodegradation

The BOD/ThOD curves for 100 mg/l solutions of several organic compounds at 20°C with seedings of 10, 100, 1500 mg/l of activated sludge, or 30 mg/l of the acclimatized sludge are shown in Figs. 6 and 7. The character-

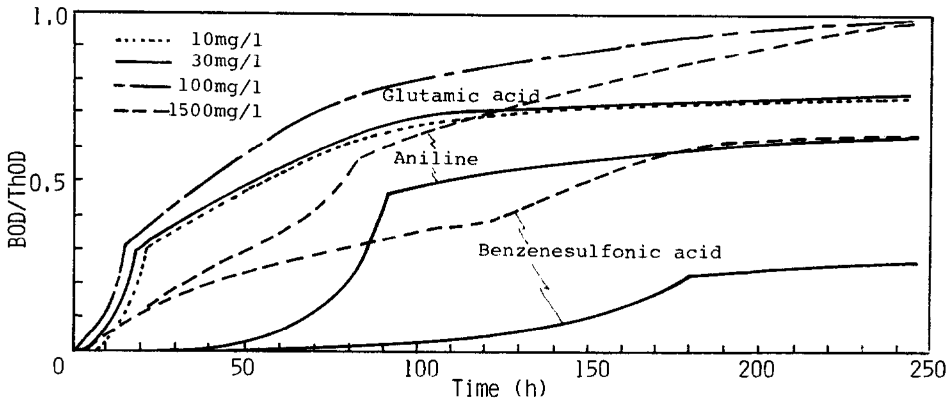

Fig. 6. Variation of BOD/ThOD curve for glutamic acid, aniline, and benzenesulfonic acid with concentration of activated sludge for seedings. (Compound: 100 mg/l; 20° C.)

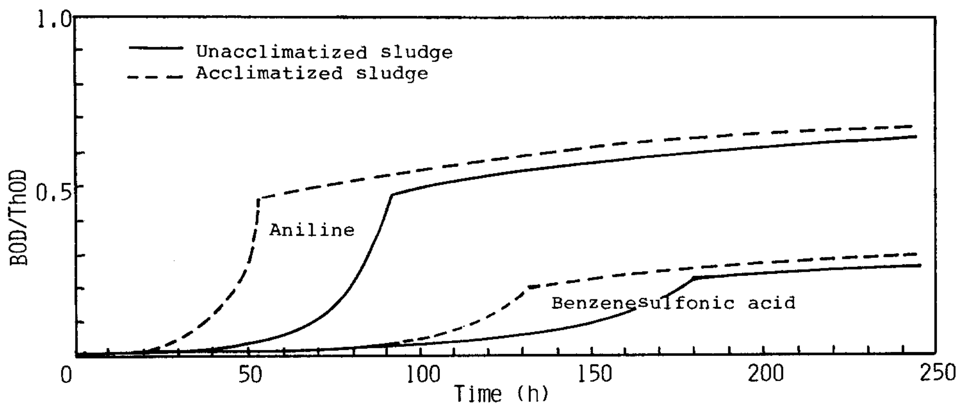

Fig. 7. Variation of BOD/ThOD curve for aniline and benzenesulfonic acid with acclimatization of activated sludge for seedings. (Compound: 100 mg/l; 20° C.)

istic indexes are shown in Table 5. The values of t_l and t_d decreased and BOD_d/ThOD increased with increase of the concentrations of the seeded microorganisms, but k varied slightly with the concentration of the microorganisms. Error in the BOD measurements for the organic compounds increased with increase of the concentration of the microorganisms, because the blank BOD by endogenous respiration of the microorganisms increased with increase of the concentration of the microorganisms. Reproducibility of the BOD measurement, however, decreased with decrease of the concentration of the microorganisms. Therefore, the seeding of 30 mg/l of activated sludge for 100 mg/l of organic compounds seemed to be better for the general biodegradation tests.

TABLE 5

Influence of concentration and acclimatization of microorganisms on biodegradation

Compound name	Concentration of activated sludge (mg/l)	t_l (h)	k (h^{-1})	t_d (h)	BOD_d/ThOD	DOC_d/DOC_0
L-Glutamic acid	100	5	0.071	85	0.77	0.21
	30	5	0.095	100	0.60	0.24
	10	10	0.092	90	0.58	0.25
Aniline	1500	20	0.044	75	0.59	0.24
	30	55	0.046	110	0.49	0.18
	30[a]	15	0.049	60	0.47	0.16
Benzenesulfonic acid	1500	90	0.030	160	0.51	0.26
	30	110	0.015	180	0.26	0.49
	30[a]	80	0.038	120	0.60	0.18

[a] Acclimatized sludge.

By the seeding of the acclimatized sludge, the values of t_l and t_d greatly decreased especially for the low biodegradable compounds, but k and BOD_d/ThOD were slightly increased. However, the necessary time for adaptation, t_l, is one of the important characteristic indexes for the evaluation of the biodegradabilities of organic compounds. Therefore, it seemed to be better to seed the unacclimatized activated sludge for the general biodegradation tests.

Influence of temperature on biodegradation

The BOD/ThOD curves at 10, 20 or 30°C for 100 mg/l solution of 5 organic compounds with seeding of 30 mg/l activated sludge are shown in Fig. 8, and the characteristics indexes are compared in Table 6. The values of t_l and t_d were smaller and k was larger at higher temperatures, but BOD_d/ThOD were slightly varied with the temperature. Namely, the temperature influenced the time required for adaptation and the biodegradation rates,

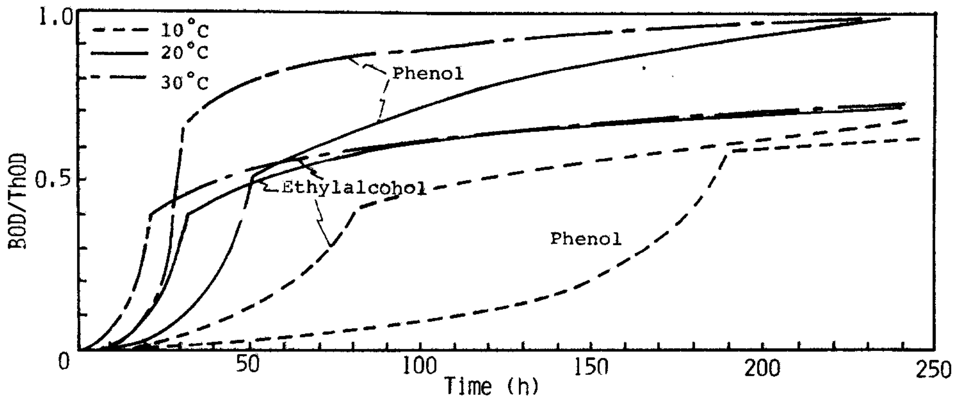

Fig. 8. Variation of BOD/ThOD curve with temperature for ethylalcohol and phenol. (Compound: 100 mg/l; activated sludge: 30 mg/l.)

TABLE 6

Influence of temperature

Compound name	Temperature (°C)	t_l (h)	k (h^{-1})	t_d (h)	BOD_d/ThOD	DOC_d/DOC_0
Ethylalcohol	30	5	0.082	60	0.61	0.13
	20	5	0.049	100	0.55	0.13
	10	10	0.019	130	0.52	0.15
Phenol	30	10	0.065	60	0.74	0.08
	20	20	0.028	100	0.62	0.09
	10	50	0.011	210	0.59	0.17
Aniline	30	45	0.061	90	0.55	0.12
	20	55	0.046	110	0.49	0.18
	10	>240	—	—	—	—
Benzenesulfonic acid	30	15	0.028	80	0.76	0.14
	20	110	0.015	180	0.26	0.49
	10	>240	—	—	—	—

but it influenced only slightly the biodegradation ratios. There are several equations for the relations between the rate constants of the biodegradation and the temperature as summarized by Eckenfelder [5] as follows

$$\log (k_{\theta_1}/k_{\theta_2}) = 0.0368\ (\theta_1 - \theta_2) \qquad (6)$$

$$\log (k_{\theta_1}/k_{\theta_2}) = 0.0315\ (\theta_1 - \theta_2) \qquad (7)$$

$$(k_\theta/k_{25}) \times 100 = 0.71\theta^{1.54} \qquad (8)$$

$$k_\theta/k_{20} = 1.065^{(\theta - 20)} \qquad (9)$$

where, θ shows the temperature in centigrade.

The values of k_{30}/k_{10} were calculated as 1.9 ~ 2.9 using eqns. (6)—(9), and they were nearly equal to the experimental values of k_{30}/k_{10}. Therefore, the above conventional equations are approximately applicable to the values of k in this study. Since a longer testing time is required at lower temperatures, and the water temperature in the environment may be lower than 30° C, the temperature of 20° C is reasonable for the general biodegradation tests.

Standard testing conditions and classification of biodegradabilities of organic compounds

From the above results, the standard testing conditions for the general biodegradation tests were determined as shown in Table 7. By using the results of the biodegradation tests with the electrolytic respirometer under these standard conditions, the organic compounds could be classified into 10 ranks by the biodegradation times and the biodegradation ratios as shown in Table 8. Upper case letters A, B, C, and D show the ranks of the biodegradation time, and lower case letters a, b, and c show the ranks of the biodegradation ratio. A classification procedure is proposed in Fig. 9 by considering the results of this study and conventional knowledge. The biodegradation

TABLE 7

Determined standard conditions for biodegradation tests

Concentration of compound	100 mg/l
pH of solution	7 ± 1
Concentration of activated sludge[a]	30 mg/l
Culture mediums	JIS inorganic mediums 1 ml/300 ml
Temperature	20 ± 1° C
Period	within 14 days

[a]Not acclimatized sludge.

TABLE 8

Biodegradation ranks

Rank	Biodegradation characteristics
A-a	Biodegraded completely in a short time by general microorganisms
A-b	Biodegraded almost completely in a short time by general microorganisms
A-c	Biodegraded partially in a short time by general microorganisms
B-a	Biodegraded completely in a middle time by general microorganisms
B-b	Biodegraded almost completely in a middle time by general microorganisms
B-c	Biodegraded partially in a middle time by general microorganisms
C-a	Biodegraded completely in a long time by acclimatized microorganisms
C-b	Biodegraded almost completely in a long time by acclimatized microorganisms
C-c	Biodegraded partially in a long time by acclimatized microorganisms
D	Not biodegraded within 240 hours

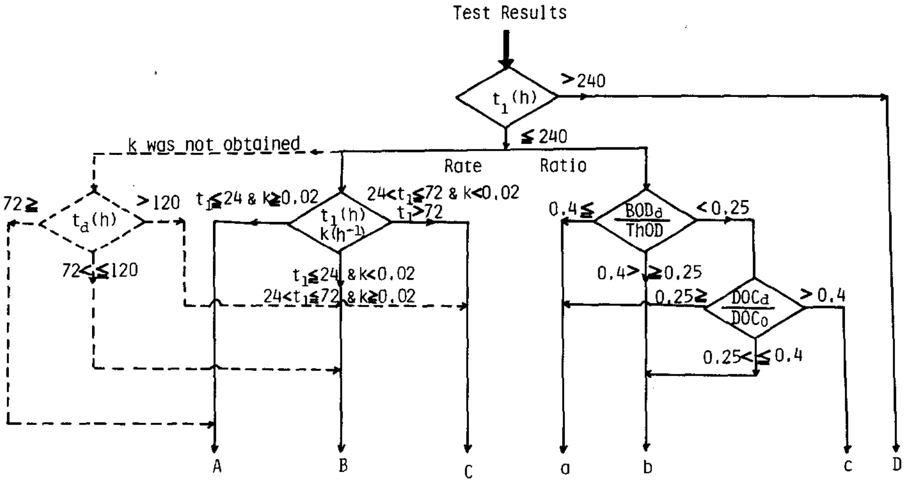

Fig. 9. Procedure for classification of biodegradation ranks.

ratio is evaluated by the values of BOD_d/ThOD and DOC_d/DOC_0, and the biodegradation rate is evaluated by the values of t_l and k. However, there are a few compounds whose values of k can not obtained correctly, because they are biodegraded by two or three steps or do not show the clear geometrical biodegradation period. For these specific compounds, the biodegradation rate is evaluated by the values of t_d. By this method, the biodegradabilities of organic compounds may be evaluated generally. Furthermore, they can be discussed in detail with the values of the characteristic indexes. The suitability of this method will be substantiated for many organic compounds in a subsequent paper.

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