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A test method for determining biodegradability of organic substances

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Abstract—In this paper, a rational test method for determining biodegradability of organic substance under aerobic conditions was established. Index of biodegradation was elaborated through analysis for biodegradability of organic substances. 23 organic substance were studied with this test and their indexes of biodegradation were calculated. A classification of biodegradability of the organic substance according to the indexes of biodegradation was suggested.

Keywords: biodegradability; biodegradation test; production of carbon dioxide; organic substance.

1 Introduction

A number of tests for determining biodegradability of organic substance have been elaborated (Mausner, 1969; Pitter, 1990). Each of them has certain advantages and shortcomings. Different, even contradictory results are usually obtained for a certain organic substance in various tests (Gerike, 1979; Means, 1981). This gives chaos in the study of biodegradation and the results are not quite useful and comparable, thus, it is very important and urgent to establish a rational test for determining biodegradability (Paiter, 1986).

Using PCD (PCD stands for production of carbon dioxide) as measuring index for biodegradability has no effect to nitrification and cell adsorption. Furthermore, in relation to pollution problems, organic substances converting to CO_2 is more meaningful. Ludzack *et al.* (Ludzack, 1959) used PCD as the measuring index of biodegradation and investigated the biochemical oxidation of some organic cyanides in river water. Ludzack's test was modified in two ways by Sturm in the study of nonionic surfactants biodegradation (Sturm, 1973): using BOD dilution water as biological medium in place of river water and acclimated sewage as inoculum. In these tests, because of lower concentration of the inoculum, testing time is longer, cell synthesis may be significant and affect the result. In addition, evaluation of biodegradability in terms of cumulative CO_2 produced as a percent of theoretical value is not reliable. In order to overcome these shortcomings, a rational PCD test was established and an index of biodegradation was elaborated.

2 Experimental

2.1 Experimental apparatus for PCD test

Diagram of apparatus for PCD test is shown in Fig. 1.

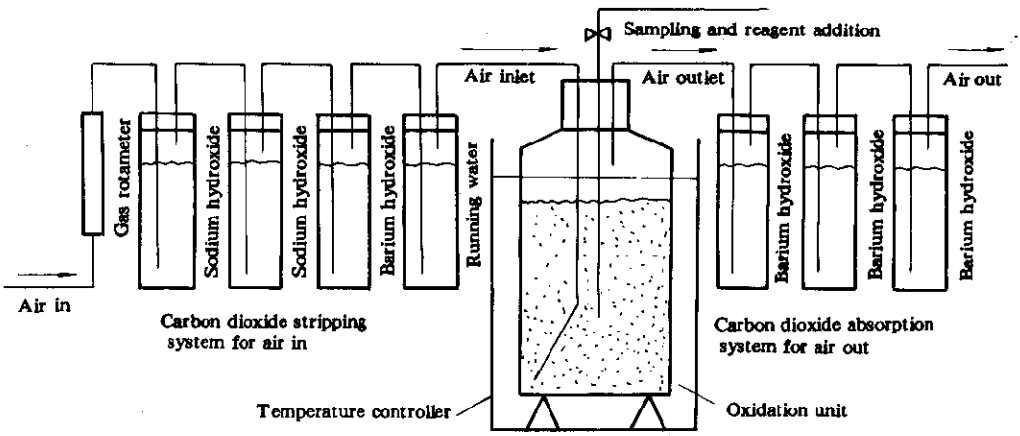

Fig. 1 Diagram of apparatus for PCD test

As shown in Fig. 1, entering air is stripped of carbon dioxide with 10 mol/L sodium hydroxide and 0.05 mol/L barium hydroxide. The running water bottle is placed before oxidation unit to avoid the base solution going into the oxidation unit (the running water often changed). CO_2 produced is absorbed with 0.05 mol/L barium hydroxide and measured by HCl titration method.

2.2 Experimental condition for PCD test

2.2.1 Biological medium

In the oxidation unit, solutions of calcium chloride (27.5g $CaCl_2$/L), magnesium sulphate (22.5g $MgSO_4 \cdot 7H_2O$/L) and ferric chloride (0.25g $FeCl_3 \cdot 6H_2O$/L) are added in 4 ml portions each, then 10 ml of a phosphate buffer of pH 7.2 (8.5g KH_2PO_4/L, 21.8g K_2HPO_4 and 44.7g $Na_2HPO_4 \cdot 12H_2O$/L) and moderate trance elements are added, the total amount of the solution in the oxidation unit is 2000 ml (including the tested substance and the inoculum).

2.2.2 Inoculum

The activated sludge taken from the Beixiaohe Sewage Treatment Plant of Beijing was used as the inoculum.

2.2.3 Testing conditions

The tested substance concentration in the biological medium is 100 mg DOC/L, the tested substance is sole source of organic carbon for the microbes of the inoculum, the amount of the inoculum in the biological medium is 500 mg MLSS/L, the temperature is 25℃, the test time is 14 days, the gas flow rate is 20 L/h, the CO_2 production is measured in different test time. In the test, the oxidation unit is of two types, one is biochemical oxidation unit, the other is endogenous oxidation unit (tested substance not added), the carbon dioxide evolution in the blank unit (tested substance and inoculum not added) subtracting from the carbon dioxide evolution in the

oxidation unit is the reality production of carbon dioxide in the oxidation unit.

3 Results and discussion

3.1 Results

The results obtained for 23 organic substances are listed in Table 1.

Table 1 The testing results for 23 organic substances

Unit: mmol/L

Substances	PCD (mmol/L) in different testing time, d						
	2	4	6	8	10	12	14
Inoculum(1)[a]	3.58	6.36	7.03	7.08	7.11	7.13	7.15
Domestic sewage	8.36	12.62	13.71	13.88	14.06	14.14	14.22
Ethylenediamine	3.34	6.31	7.68	8.84	9.26	9.53	9.94
Urea	12.23	14.74	15.31	15.34	15.37	15.38	15.40
Ethylenediamine tetra acetic acid	2.68	4.46	6.03	6.70	6.75	6.78	6.82
Glucose	11.81	14.35	15.13	15.18	15.26	15.28	15.31
Hexamethylene tetramine	2.01	3.68	4.58	4.81	5.07	5.12	5.14
Acetic acid	10.93	14.21	15.21	15.28	15.37	15.43	15.45
Oxalic acid	10.11	13.87	15.14	15.35	15.41	15.48	15.52
Inoculum (2)	3.68	6.31	7.05	7.14	7.18	7.20	7.25
Phenol	8.52	13.80	14.93	15.13	15.16	15.24	15.37
Benzoic acid	10.46	15.38	15.21	15.28	15.36	15.38	15.41
Sulfobenzene	4.42	6.63	8.87	9.21	-	10.76	11.21
Aniline	4.46	6.37	7.65	8.83	9.88	10.22	10.61
Nitrobenzene	4.24	6.13	7.82	8.96	9.78	10.26	10.78
4-Methylphenol	5.36	11.28	13.63	-	15.21	15.26	15.34
4-Chlorophenol	4.78	8.98	13.05	14.86	15.15	15.32	15.41
4-Bromophenol	4.21	8.06	11.92	14.14	14.94	15.20	15.31
4-Sulfophenol	2.81	7.72	-	14.03	14.20	14.20	14.32
4-Nitrophenol	2.43	6.02	10.14	13.41	14.27	-	14.29
4-Aminophenol	2.46	6.13	9.89	13.12	13.98	14.17	14.21
3-Hydroxyphenol	6.11	12.13	14.12	14.84	15.18	15.33	15.47
3-Sulfotoluene	2.73	7.15	10.87	13.59	-	14.12	14.23
3,4-Dimethylphenol	2.87	6.95	10.08	11.78	12.50	13.24	13.35
1,3-Dinitrobenzene	1.86	3.16	3.27	3.32	3.43	3.45	3.45

a: PCD from the inoculum is called endogenous PCD

3.2 Discussion

3.2.1 Evaluation for biodegradability of organic substance

From the testing results, the typical PCD curves can be drawn (Fig. 2). The evaluation of biodegradability will be discussed through the analysis of these curves.

As shown in Fig. 2, tested substance (a) has no course of acclimation, its rate of reaction and degree of biodegradation are higher, as a result, the biodegradability is greater and the area between PCD curve and abscissa is also greater. The tested substance (b) has a course of acclimation, its rate of reaction and degree of biodegradation are lower, as a result, the biodegradability is smaller and the area between PCD curve and abscissa is also smaller. Tested substance (c) can not be degraded, and is toxic to the inoculum, the PCD curve is under the endogenous PCD

curve, the area between PCD curve and abscissa is the smallest. Therefore, the area between PCD curve and abscissa gives biodegradability of organic substance. Hence, the biodegradability in PCD test can be expressed in terms of index of biodegradation as:

$$IB=\frac{A_s}{A_0}\times 100\%, \tag{1}$$

where IB is index of biodegradation; A_s is the area of biochemical PCD curve; A_0 is the area of endogenous PCD curve.

The acclimation is usually completed within 7 days and the ultimate degree of biodegradation may be attained in 3 days. Therefore, a testing time in 10 days is selected in the evaluation of biodegradability.

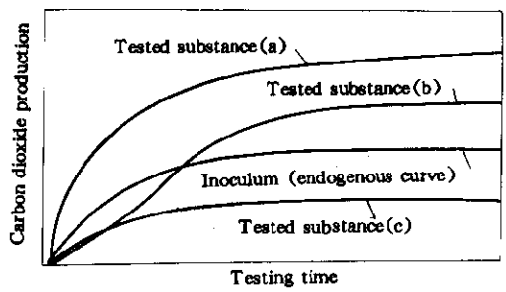

Fig. 2 Typical PCD curves

3.2.2 Calculation for the index of biodegradation

The relationship between the PCD and testing time can be described by the following mathematical model:

$$\mathrm{PCD}=\mathrm{PCD_u}\frac{(1-e^{-kt})}{(1+Be^{-kt})}, \tag{2}$$

where PCD is production of carbon dioxide; $\mathrm{PCD_u}$ is ultimate production of carbon dioxide; t is testing time; k is reaction rate constant; B is constant (it has relation with inoculum).

By integration:

$$A=\mathrm{PCD_u}\{t+(1+\frac{1}{B})\frac{1}{k}\ln[(e^{kt}+\frac{1}{B})\frac{B}{(B+1)}]\}. \tag{3}$$

Based on the testing results, using FORTRAN programs in the computer, k, B and $\mathrm{PCD_u}$ in Equation (2) can be obtained. Using testing time 10 days, the area A can be calculated from Equation (3), then, the IB can be obtained from Equation (1). The calculating results are shown in Table 2.

3.2.3 Classification for biodegradability of organic substance

Evidently, when the value of IB is greater, the biodegradability of the organic substance is greater, and when the value of IB is smaller, the biodegradability of the organic substance is also smaller. When the value of IB is smaller than 100%, it can be seen from Fig. 2 that the PCD curve of tested substance is under the endogenous PCD curve, showing that the substance has toxic affection on the inoculum under the testing condition. Therefore, when the value of IB of the organic substance is smaller than 100%, it may be considered as refractory substance. The domestic sewage is a typical representative of easy biodegradation substance and its value of IB is about 200%. Thus, when the value of IB of the tested substance is greater than 200%, the substance may be considered as readily degradable substance, when the value of IB is greater than 100% but smaller than 200%, the substance can be considered as degradable substance with medium extent. As a result, according to the index of biodegradation, the biodegradability of or-

ganic substances may be classified as follows: $IB > 200\%$: readily degradable; $100\% < IB < 200\%$: degradable with medium extent; $IB < 100\%$: refractory.

Table 2 The calculating results for indexes of biodegradation

Substances	k	B	PCD_u	A^a	IB, %
Inoculum(1)	0.843	3.131	7.152	55.65	100.0
Domestic sewage	0.780	1.576	14.263	114.37	205.5
Ethylenediamine	0.337	0.792	10.13	63.45	114.0
Urea	0.792	0.001	15.39	134.47	241.6
Ethylenediamine tetra acetic acid	1.038	9.031	6.821	51.38	92.3
Glucose	0.604	0.001	15.41	128.63	231.1
Hexamethylene tetramine	0.525	1.849	5.124	35.64	64.0
Acetic acid	0.685	0.205	15.42	129.55	232.8
Oxalic acid	0.650	0.420	15.49	126.70	227.7
Inoculum(2)	0.675	0.933	7.220	56.00	100.0
Phenol	0.888	2.825	15.437	122.80	219.3
Benzoic acid	1.325	5.062	15.396	128.88	230.1
Sulfobenzene	0.220	0.001	11.43	68.08	121.6
Aniline	0.211	0.001	11.08	64.64	115.4
Nitrobenzene	0.196	0.001	11.168	62.66	111.9
4-Methylphenol	0.616	3.196	15.236	106.00	189.3
4-Chlorophenol	0.577	4.814	15.456	98.10	175.2
4-Bromophenol	0.595	7.450	15.23	90.90	162.3
4-Sulfophenol	0.601	8.158	14.495	85.52	152.7
4-Nitrophenol	0.487	8.069	15.488	78.22	139.7
4-Aminophenol	0.531	9.945	14.941	76.80	137.1
3-Hydroxyphenol	0.721	3.717	15.549	113.11	202.0
3-Sulfotoluene	0.614	9.894	14.313	82.37	147.1
3,4-Dimethylphenol	0.592	9.094	13.347	76.22	136.1
1,3-Dinitrobenzene	0.921	3.229	3.467	27.56	49.2

a: A for organic substance stands for A_s; A for inoculum stand for A_0.

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