On the modeling of microbiological hydrolysis of organic solids

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Abstract For the modeling basis of microbiological hydrolysis of organic solids, hydrolyzed quantity per unit time and unit surface area, namely hydrolytic flux was defined, and the hydrolytic models for estimating weight decrease of cubic and spherical organic-solids were derived. These models were verified and actual values of hydrolytic rate coefficients were obtained by the experiments, in which three sizes of cubic organic solid samples were made from each of rice cake, albumen congealed by heat, and beef tallow, submerged in reactors, and anaerobically hydrolyzed. Because the quantity of MLSS cannot correctly express that of the microbe in such reaction systems, the action of active anaerobe was estimated and evaluated based on ATP, in this study.

Keywords Hydrolysis; organic solid; anaerobe; model; ATP

Introduction

Microbiological decomposition of dissolved substance has long been investigated. However, microbiological hydrolysis of organic solids and especially its dynamics have not necessarily been studied fully, because form and quality of the solids are usually diverse and also it is difficult to separate the active microorganism concerned from the solids in such reaction systems.

Biological decomposition of organic solids and the process of their weight decrease have often been analyzed based on the first order reaction on the weight of respective solids. However in this case, the hydrolytic rate coefficient for an organic solid varies generally with the specific surface area of the solid and basically is not constant. In this research, therefore, the hydrolyzed quantity per unit surface area and unit time, namely hydrolytic flux was defined. Based on this, hydrolysis models for cubic and spherical organic solids were derived, and the models were verified by experimental results on the anaerobic microbiological hydrolysis using the three sorts of organic solids.

In anaerobic microbiological reaction systems including organic solids, it is not reasonable to regard MLSS as the microorganism participating in the reaction, because some dead and inactive bacteria and fine and small solid particles are usually contained. On the other side, adenosine triphosphate(ATP) which is an energy source for microbial growth, etc., seems to comprehensively reflect the activity of live bacteria. Therefore, the action of active microorganisms was evaluated based on ATP in this research.

Methods

The organic solids containing a large quantity of carbohydrate, protein and lipid respectively, namely rice cake, albumen congealed by heat and beef tallow were used as carbohydrate, protein and lipid solids respectively (Table 1). From each of these solids, large (1.5 cm cube), medium (0.75 cm cube) and small (0.3 cm cube) cubic samples were cut off. The ratio of specific surface area of large, medium and small samples was 1:2:5.

These samples were put into reactors together with the liquid mixture containing anaerobe and nutrient medium (Owen *et al*. (1979); Tong *et al*. (1990)) (Table 2), and decomposed anaerobically. The liquid mixture was made from 5% vol. supernatant of

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Table 1 Density ρ of organic solid samples

	ρ : wet basis	ρ: dry basis
Carbohydrate solid sample (rice cake)	1.20	0.70
Protein solid sample (albumen congealed by heat)	1.08	0.13
Lipid solid sample (beef tallow)	1.08	1.05

ρ; [g solid/cm^3]

Table 2 Composition of nutrient broth

$FeCl_3 \cdot 4H_2O$	0.37 g/L
$Na_2S \cdot 9H_2O$	0.50 g/L
$CaCl_2 \cdot 2H_2O$	0.25 g/L
NH_4Cl	0.40 g/L
$MgCl_2 \cdot 6H_2O$	1.8 mg/L
KCl	1.3 mg/L
H_3BO_3	5.7 mg/L
$CuCl_2 \cdot 2H_2O$	2.7 mg/L
$Na_2MoO_4 \cdot 2H_2O$	2.6 mg/L
$ZnCl_2$	2.1 mg/L
$NiCl_2 \cdot 6H_2O$	0.3 mg/L
$(NH_4)_2HPO_4$	80 mg/L
$MnCl_2 \cdot 4H_2O$	20 mg/L
$CoCl_2 \cdot 6H_2O$	30 mg/L

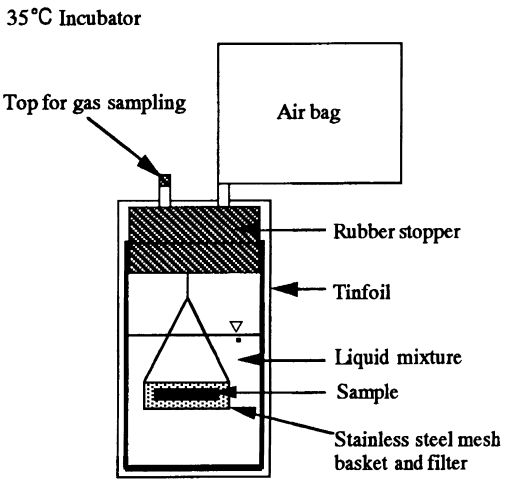


Figure 1 Experimental apparatus

anaerobically digested sludge which contains seeding anaerobe, 95% vol. nutrient medium of pH 7.0–7.1, and $NaHCO_3$ of 16 g/L for pH buffering. Concentrations of ATP and MLSS of the liquid mixture were 3×10^{-3} mg/L and 240 mg/L, respectively, and also it was confirmed that ATP conc. of the mixtures of each of the ground samples and distilled water were negligible.

A set of the experimental apparatus which consists of a reactor (500 mL capacity), an incubator, etc. is shown in Figure 1. At first, liquid mixture of 320 mL was put into the reactor, and then the organic solid samples were hung in the liquid mixture of the reactor by a stainless steel cage and a polyester filter. The air in the head space of the reactor was replaced with nitrogen gas, and then the reactor was stuffed with a rubber stopper with an air bag, wrapped with an aluminium sheet, and put in the incubator. Temperature of the reactor was kept around 35ºC.

In each experiment on the three sorts of organic solids, the reactors containing respectively 1 large, 5 medium, and 125 small samples were prepared, equalizing the sample weight in the reactors, and in all experiments on the combination of three sorts and three scales of organic solid samples, the reactors of the same number as the times of measurement in each experimental term were prepared and used.

At every certain time interval, the measurement was performed as follows: measurement and analysis of the gas, drying (105–110ºC) of the remaining organic solids and measurement of the weight, sampling of the liquid, measurements and analyses of ATP (extracted with tri-chloro acetic acid, measured by Ultraweak photoelectron detector UPD-8000), pH, ORP, MLSS, and volatile fatty acids (Sugisaki, 1989), etc.

Weight decrease of organic solids by anaerobic hydrolysis

The change of organic solid weight in biochemical hydrolytic processes can be expressed by Eq. (1) when the solids are colloids or comparatively small particles. And then Eq. (2) is obtained from Eq. (1).

$$-\left(\frac{1}{X}\right)\left(\frac{dW}{dt}\right) = k_W \cdot W \tag{1}$$

$$-\ln\left[\frac{W}{W_0}\right] = k_W \cdot \int X dt \tag{2}$$

X: conc. of microorganism [g cell/cm^3], W: weight of organic solid [g dry solid], k_W: hydrolytic rate coefficient on weight basis [1/day/(g cell/cm^3)].

However, such weight change should generally be analyzed based on solid surface area, because hydrolysis of solid proceeds exactly on its surface.

Then, hydrolyzed quantity per unit surface area and unit time, namely hydrolytic flux HS was defined here, and this was assumed to be proportional to the concentration of microorganism and the density of organic solid. In this case, the proportional coefficient is a hydrolytic rate coefficient which mainly depends on the solid's quality, microbial species, and environmental conditions of the system.

$$H_S = k_S \cdot \rho \cdot X \tag{3}$$

H_S: hydrolytic flux [g dry solid/cm^2/day], ρ: density of organic solid [g dry solid/cm^3], X: conc. of microorganism [g cell/cm^3], k_S: hydrolytic rate coefficient on the basis of surface area [(cm/day)/(g cell/cm^3)].

Based on the above equation, the weight decrease of organic solids by microbiological hydrolysis is shown as Eq. (4)

$$-\frac{dW}{dt} = H_S \cdot S = k_S \cdot \rho \cdot S \cdot X \tag{4}$$

S: surface area of organic solid [cm^2].

In the following, the weight changes of cubic and spherical organic solids were mathematically analyzed based on the Eq. (4)

Hydrolysis of organic cubic solids

Firstly, when the weight of an organic cubic solid is W, Eq. (6) is obtained by substituting Eq. (5) of the relation between W and surface area S of the solid into Eq. (4).

$$S = 6 \cdot \rho^{-2/3} \cdot W^{2/3} \tag{5}$$

$$-\frac{dW}{dt} = 6 \cdot k_S \cdot \rho^{1/3} \cdot X \cdot W^{2/3} \tag{6}$$

Furthermore, Eq. (7) is obtained by integrating the above equation, and can be utilized to analyze weight changes.

$$-\left(\frac{\rho^{-1/3}}{6}\right)\int W^{-2/3} dW = \left(\frac{1}{2}\right) \cdot \rho^{-1/3} \cdot \left(W_0^{1/3} - W^{1/3}\right) = k_S \cdot \int X dt \tag{7}$$

W_0: initial weight of an organic cubic solid [g dry solid].

Secondly, when the total weight of organic cubic solids of n pieces is W, the equation corresponding to Eq. (7) is obtained as Eq. (8).

$$\frac{1}{2} \cdot (\rho \cdot n)^{-1/3} \cdot \left(W_0^{1/3} - W^{1/3}\right) = k_S \cdot \int X dt \tag{8}$$

Hydrolysis of organic spherical solids

Firstly, when the weight of an organic spherical solid is W, Eq. (10) is obtained by substituting Eq. (9) into Eq. (4), and then Eq. (11) is obtained by integrating Eq. (10).

$$S = (36\pi)^{1/3} \cdot \rho^{2/3} \cdot W^{2/3} \tag{9}$$

$$-\frac{dW}{dt} = (36\pi)^{1/3} \cdot k_S \cdot \rho^{1/3} \cdot X \cdot W^{2/3} \tag{10}$$

$$\left(\frac{3}{4\pi}\right)^{1/3} \cdot \rho^{-1/3} \cdot (W_0^{1/3} - W^{1/3}) = k_S \cdot \int X dt \tag{11}$$

Secondly, when the total weight of organic spherical solids of n pieces is W, Eq. (12) can be applied to the weight change analysis of them.

$$\left(\frac{3}{4\pi}\right)^{1/3} \cdot (\rho n)^{-1/3} \cdot (W_0^{1/3} - W^{1/3}) = k_S \cdot \int X dt \tag{12}$$

The above analytical results indicate that hydrolytic rate coefficients on surface area basis can be determined as the gradients of linear relationships between the left-hand side value of Eq. (7), (8), (11), or (12) on the axis of ordinates and the right-hand side values on the axis of abscissas.

In this study, the concentration of ATP, C_{ATP} (g ATP/cm^3), was applied instead of X as stated in the introduction.

Results and discussion

The experimental results on the anaerobic hydrolysis of the carbohydrate, protein, and lipid solid samples are shown in Figures 2, 3 and 4, respectively. These results show the following: each sort of organic solids was gradually hydrolyzed after an initial inductive period, and finally decomposed almost completely. In the experiments using carbohydrate and lipid solid samples, the concentration of VFA increased with hydrolysis of solid samples. However, production of VFA from protein samples was retarded further than the hydrolysis of them. The concentration of ATP started to increase at a comparatively early stage. However, the increase was suppressed by the effect of accumulating VFA, and changed soon to slow decrease. The results also indicate that the decomposition by hydrolysis went on earlier in the following order of sample quality: carbohydrate, protein, lipid, and of cubic sample size: 0.3 cm, 0.75 cm, 1.5 cm.

The changes of active microorganism concentration X were evaluated by replacing with the changes of ATP concentration C_{ATP} measured. Figures 5 and 6 show the results of

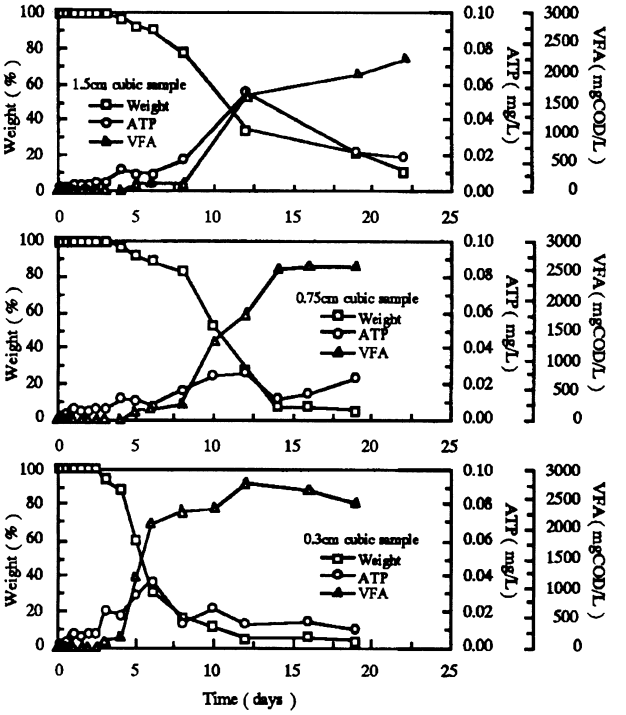


Figure 2 Changes of weight, ATP and VFA and concentrations in case of carbohydrate solid samples

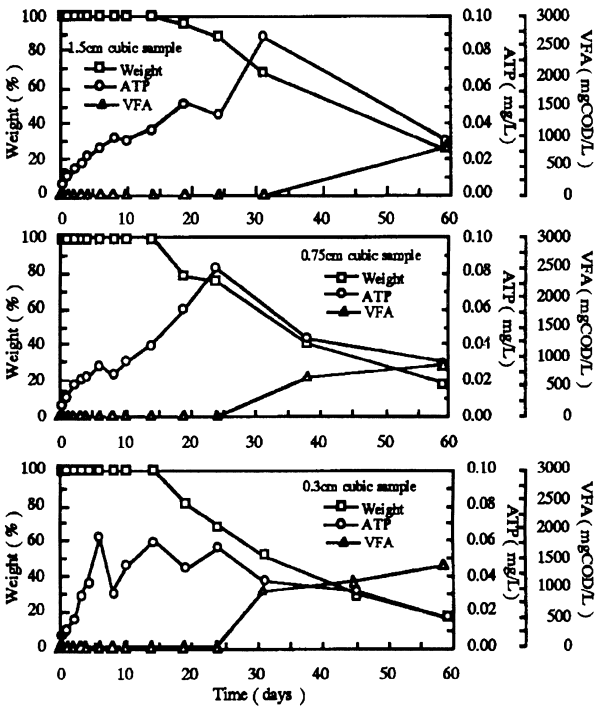


Figure 3 Changes of weight, ATP and VFA and concentrations in case of protein solid samples

applying Eq. (2) to the changes of C_{ATP} and sample weight W, and show that Eq. (2) was applicable in a comparatively wide range of weight decrease. In Table 3, numerical values of the hydrolytic rate coefficient k_W obtained are shown. Because this coefficient is defined

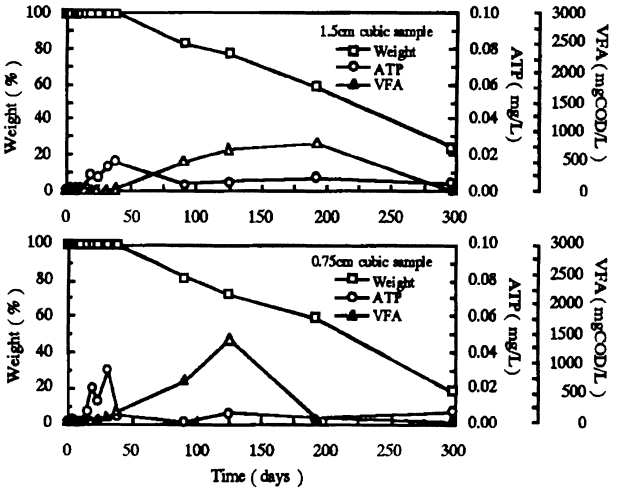


Figure 4 Changes of weight, ATP and VFA concentrations in case of lipid solid samples

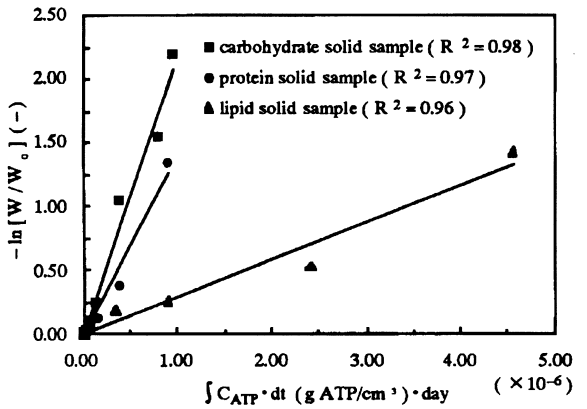


Figure 5 Analyses of the degradation of organic solid samples by the hydrolysis model based on solid weight

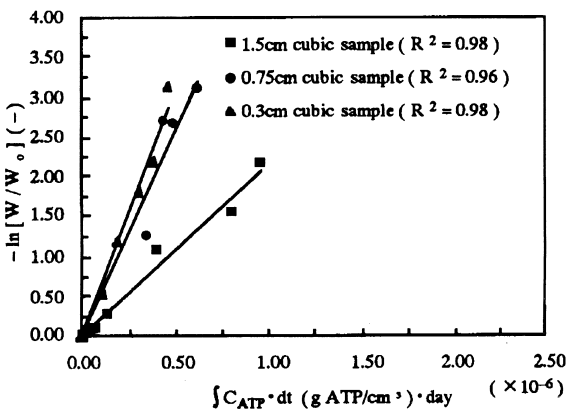


Figure 6 Analyses of the degradation of carbohydrate solid samples by the hydrolysis model based on solid weight

on the basis of the solid weight concerned, even the values obtained on the samples of the same quality varied in size, namely in specific surface area of them.

In each Figure A of Figures 7, 8 and 9, the hydrolysis model for cubic solids (Eq. 8) was applied to the results of measurement in every whole experimental term. Numerical values of the hydrolytic rate coefficient k_S of every size and every organic solid were derived from

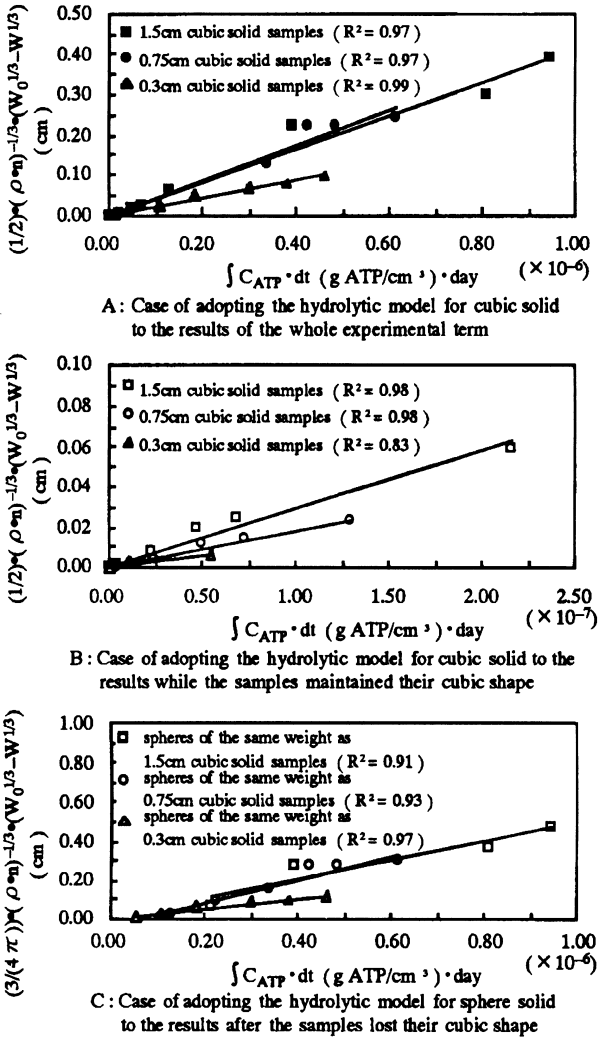
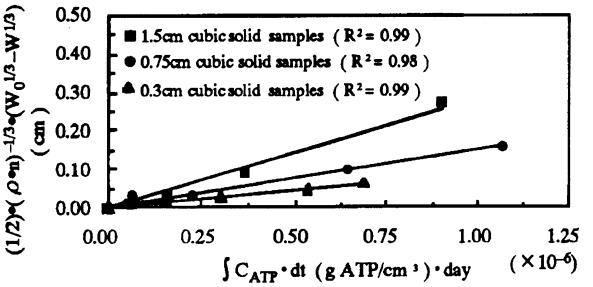


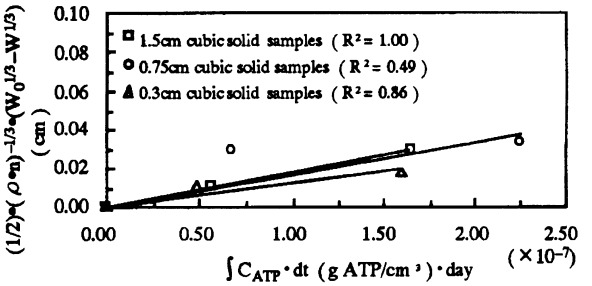
Figure 7 Analysis of the degradation of carbohydrate solid samples by the hydrolytic model on surface area basis

these analyses, and shown in Table 4-A. This coefficient was defined on the basis of the solid surface area concerned. As a result, the coefficient values of 1.5 cm and 0.75 cm cubic samples of the same quality are almost equal with each other or having comparatively small differences, in spite of the differences in their specific surface area. However, the values of 0.3 cm cubic samples were smaller. The values of the coefficient were different among the qualities of the solids, and larger in the following order: carbohydrate, protein, lipid.

It occurred that the cubic samples were deformed into indefinite shapes after which they were hydrolyzed to some extent, and that the smaller samples were deformed earlier. From these facts, in each Figure B of Figures 7, 8 and 9, the hydrolysis model for cubic solids (Eq. 8) was applied to the values measured while the samples maintained their cubic form. On the other hand, in each Figure C, the hydrolysis model for spherical solids (Eq.12) was applied to the values measured after the samples lost their initial cubic form, under the assumption that each of the cubic samples was deformed into a sphere of the same weight. Table 4-B and C show the hydrolytic rate coefficients obtained from the above Figures. The coefficients in Table 4-B were smaller than those in Table 4-A and C, and those in Table 4-C were larger than the others. However, the differences among the coefficients of every organic solid were not so large. It is considered that the hydrolytic rate coefficient values



A: Case of adopting the hydrolytic model for cubic solid to the results of the whole experimental term



B: Case of adopting the hydrolytic model for cubic solid to the results while the samples maintained their cubic shape

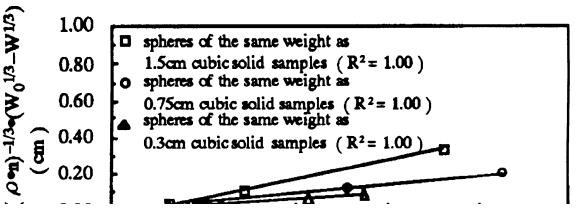
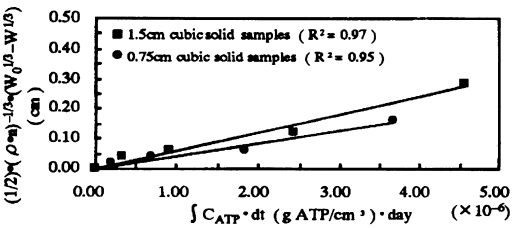


Figure 8 Analysis of the degradation of protein solid samples by the hydrolytic model on surface area basis



A: Case of adopting the hydrolytic model for cubic solid to the results of the whole experimental term

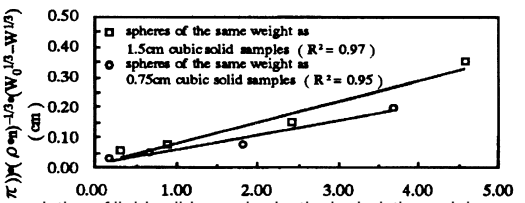


Figure 9 Analysis of the degradation of lipid solid samples by the hydrolytic model on surface area basis

obtained by applying the hydrolysis model for cubic solids to the data measured, on 1.5 cm cubic samples before they lost cubic form, are more reasonable and correct.

Table 3 Values of hydrolytic rate coefficient k_w (weight basis) for anaerobic degradation of organic solid samples

	Carbohydrate solid sample	Protein solid sample	Lipid solid sample
1.5 cm tube	2.2×10^6	1.4×10^6	0.3×10^6
0.75 cm tube	5.2×10^6	1.5×10^6	0.4×10^6
0.3 cm tube	6.1×10^6	2.4×10^6	–

k_w ; [1/day/(g ATP/cm^3)]

Table 4 Values of hydrolytic rate coefficient k_s for anaerobic degradation of organic solid samples (k_s; surface area basis [cm/day/(g ATP/cm^3)])

A. k_s obtained by applying the hydrolysis model for cubic solids to the results of the whole experimental term.

	Carbohydrate solid sample	Protein solid sample	Lipid solid sample
1.5 cm cubic sample	4.1×10^5	2.8×10^5	0.6×10^5
0.75 cm cubic sample	4.3×10^5	1.5×10^5	0.4×10^5
0.3 cm cubic sample	2.1×10^5	0.9×10^5	–

B. k_s obtained by applying the hydrolysis model for cubic solids to the results while samples maintained their cubic shape.

	Carbohydrate solid sample	Protein solid sample	Lipid solid sample
1.5 cm cubic sample	2.9×10^5	1.8×10^5	–
0.75 cm cubic sample	1.8×10^5	1.7×10^5	–
0.3 cm cubic sample	1.1×10^5	1.2×10^5	–

C. k_s obtained by applying the hydrolysis model for spherical solids to the results after samples lost their cubic shape.

	Carbohydrate solid sample	Protein solid sample	Lipid solid sample
Sphere of the same weight as 1.5 cm cubic solid sample	4.8×10^5	4.0×10^5	0.7×10^5
Sphere of the same weight as 0.75 cm cubic solid sample	6.0×10^5	1.9×10^5	0.5×10^5
Sphere of the same weight as 0.3 cm cubic solid sample	2.6×10^5	1.1×10^5	–

Conclusions

The rate process of organic solid hydrolysis by microorganism was reasonably modeled by defining the hydrolyzed quantity per unit time and unit surface area, that is a hydrolytic flux, which was assumed to be proportional to solid density and microorganism concentration and included a hydrolytic rate coefficient. Hydrolytic models, which were derived based on the definition, for estimating weight decrease of cubic and spherical organic solids explained reasonably the results of experiments on the anaerobic hydrolysis of different kinds of organic solids. From these experiments, reasonable values of the hydrolytic rate coefficient also were obtained. Furthermore, in case of the organic solid hydrolysis by anaerobes, it is useful to estimate and evaluate the action of active anaerobe based on ATP.

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