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Scale-Up of Water-Oil Hydrolysis System

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Abstract Scale-up experiments for hydrolysis of beef tallow, fat, and palm kernel with lipase derived from *Candida cylindracea* were carried out in 1-l, 100-l, and 10,000-l reactors. The optimum agitation speed for the hydrolysis of the 1-l reactor was investigated and found to be 350 rpm, and this was a basis for the scale-up of agitation speed. The hydrolysis system in this work was the oil-water system in which the hydrolysis seems to process a heterogeneous reaction. An emulsion condition was the most important factor for determining the reaction rate of hydrolysis. Therefore, the scale-up of agitation speed was performed by using the power n = 1/3 in an equation of the rules of thumb method. The geometrical similarity for scaling-up turned out to be unsatisfactory in this study. Thus, the working volume per one agitator was used for the scale-up. In the case of scale-up from a 1-l reactor to a 100-l reactor, the hydrolysis of palm kernel was very much scaled-up by initiating the rules of thumb method. However, the hydrolysis of fat and beef tallow in a 100-l reactor was a little higher than that of the 1-l reactor because of the difference of geometrical similarity. The scale-up of hydrolysis from the 100-l reactor to the 10,000-l reactor was improved compared to that of the 1-l to 100-l reactor. The present results indicated that the scale-up of hydrolysis in the oil-water system by the rules of thumb method was more satisfactory under the condition of geometrical similarity. Even in the case where geometrical similarity was not satisfactory, the working volume per one agitator could be used for the scale-up of a heterogeneous enzyme reaction.

Key words: Scale-up, geometrical similarity, rules of thumb, emulsion state, small-scale system

Various scale-up methods have been used in a field of chemical engineering. These methods are generally categorized into the fundamental method, semi-fundamental method, rules of thumb, dimensional analysis, and the regime analysis [1, 2, 4, 6]. Fermentation and enzyme reaction systems in heterogeneous fluids are complicated and make boundary conditions extremely difficult. Therefore, a fundamental method using all the micro balances for momentum, mass, and heat-transfer in the system cannot be easily applied to the scale-up of biological processes, nor can the semi-fundamental method be applied to simplifying the flow model as a plug flow with dispersion or a well-mixed system [6, 7]. In biological engineering, however, the rules of thumb method is commonly used as a scale-up procedure. Scale-up criteria used for rules of thumb is power per volume ratio, oxygen transfer rate, impeller tip speed, and oxygen tension [1, 2, 4, 6]. If the geometrical similarity between a bench-scale system and a full-scale system is satisfied in a scale-up problem, the following equation is frequently used to determine the agitator speed, N_2, for utilizing to duplicate the small-scale results using N_1

$$N_2 = N_1 \left(\frac{D_{T1}}{D_{T2}}\right)^n \quad (1)$$

where D_T is the diameter of its reactor, n = 1 for equal liquid motion, n = 3/4 for equal suspension of solids, and n = 2/3 for equal rate of mass transfer which is equivalent to equal power per unit volume [1, 2, 4, 5, 11].

Generally, in the enzymatic reactions of a water-soluble substrate system, enzyme concentration, substrate concentration, temperature, pH, and metal ions are known to be the important factors to determine the rate of reaction. In the lipase catalyzed reaction of organic solvent system, water activity has a great effect on the kinetics [12]. However, in heterogeneous reactions such as hydrolysis of oil in the water-oil system, the emulsion conditions have a larger effect on the rate of reaction. The factors that determine the state of emulsion are the water-oil ratio, stirring method, shape of reactor and the presence of surfactants [3, 9, 13]. Therefore, the scale-up in this work for the hydrolysis of oil was based on the same emulsion conditions between the bench-scale, the pilot-scale, and the full-scale systems.

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Table 1. The composition of fatty acids for various oils.

Oil	C_{10}	C_{12}	C_{14}	$C_{14:1}$	C_{15}	C_{16}	$C_{16:1}$	C_{17}	$C_{17:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}	$C_{20:1}$	C_{22}
Beef tallow	-	0.10	2.80	0.58	0.48	26.03	3.85	1.32	0.69	17.96	40.92	3.89	0.42	0.15	0.81	-
Fat	0.16	1.20	2.26	0.29	0.19	23.46	2.53	0.60	0.36	12.88	42.54	11.58	0.74	0.30	0.76	0.16
Palm kernel	0.34	51.04	15.66	-	-	8.74	-	-	-	2.19	15.83	2.68	-	0.23	-	-

MATERIALS AND METHODS

Materials

Lipase-OF 360,000 derived from *Candida cylindracea* was the product of MEITO SANGYO Corporation. It was kept in a dried state at 4°C in a refrigerator. It had a very high activity, as much as 360,000 unit/g, and interestingly did not lose its activity even in the acidic solution. Therefore, it was not necessary to include acid or alkali to maintain the enzyme activity in the process of hydrolysis. The optimum temperature was between 35 to 45°C and activity was measured before hydrolysis. The oils used in this work were beef tallow, fat, and palm kernel.

Table 1 shows the composition of fatty acids which formed triglycerides of oils used in this work, and represented by the area percentage of the analysis of gas chromatography [HP 5890, U.S.A.].

Hydrolysis and Analysis

Figure 1 shows a typical enzyme reactor. The dimensions of three different reactors used in this work are summarized in Table 2. Bench-scale hydrolysis was performed in a 1-l working volume stirred tank reactor. Pilot-scale was carried out in a 100-l working volume and full-scale in a 10,000-l working volume.

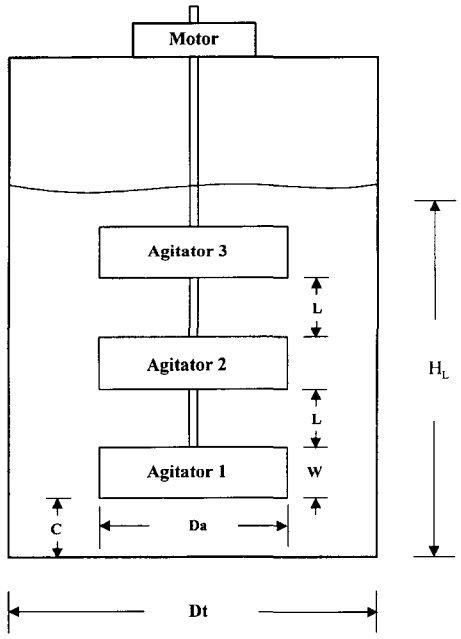

Fig. 1. Typical enzyme reactor.

A mixture of oil and distilled water (1:1 vol/vol) was agitated for 48 h. The rpm of the bench-scale system was 350, but the rpm of the pilot-scale and that of full-scale were determined by the scaling-up process. Samples for the measurement of hydrolysis were taken into test tubes at 1, 2, 4, 6, 20, and 48 h of hydrolysis. The sample tubes were immediately put into boiling water to stop the enzyme activity. The compositions of triglyceride (TG), diglyceride (DG), monoglyceride (MG), and free fatty acid (FFA) were analyzed by TLC-FID. One hundred μl sample was taken from the upper layer of the reaction products, which were dissolved in 100 μl chloroform. One μl of the solution was spotted on a thin layer chromatography [CHROMROD-S] and developed with a solvent that was composed of benzene, chloroform, and acetone [70:30:2, vol/vol/vol]. Hydrogen gas velocity was 160 ml/min, air velocity was 20 l/min, and scan speed was 30 sec. The results were treated with IATROCODER TC-21 [IATRON, Japan].

RESULTS AND DISCUSION

Hydrolysis of 1-l Reactor

Figure 2 shows hydrolysis of the fats listed in Table 1 according to the rpm of the agitator of the 1-l reactor in Fig. 1. The reaction temperature was fixed at 42°C because the melting point of the fat was 40.5°C and the optimum

Table 2. Dimensions of bench, pilot, and full-scale enzyme reactors.

Reactor	Bench-scale	Pilot-scale	Full-scale
Total volume (l)	1	100	10,000
Working volume (l)	1	2	3
Agitator 1			
Blade type [number of blade]	Flat (2)	Propeller (3)	Flat (2)
Agitator 2			
Blade type [number of blade]	-	Flat (2)	Flat (2)
Agitator 3			
Blade type [number of blade]	-	-	Flat (2)
Da (m)	0.065	0.26	1.8
Dt (m)	0.11	0.5	2.72
W (m)	0.011	0.0520	0.15
H_L (m)	0.108	0.51	1.72
C (m)	0.03	0.14	0.30
L (m)	-	0.35	0.84

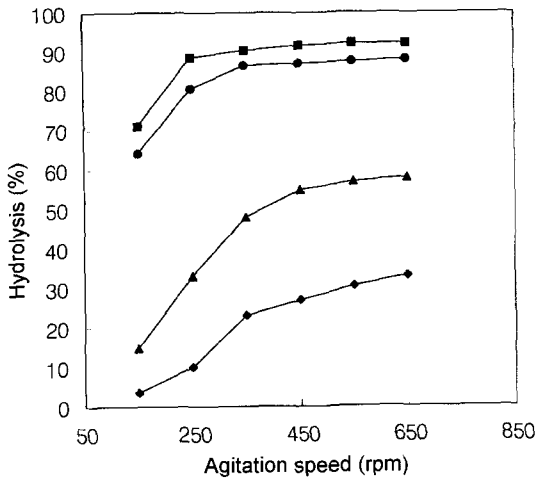

Fig. 2. Hydrolysis of fat according to agitation speed at fixed hydrolysis times, 1 h (◆), 4 h (▲), 24 h (●), and 48 h (■).

temperature for the activity of Lipase-OF 360,000 was 37°C [8, 10]. The hydrolysis became intense as the rpm increased at a fixed reaction time. However, the rate of increase of hydrolysis reached to nearly zero with an agitation speed of above 450 rpm. In particular, in the case where the hydrolysis time exceeded 24 h and the agitation speed was higher than 350 rpm, the hydrolysis did not change with the increase of agitation speed. Thus, the agitation speed of the agitator of the 1-l reactor was fixed at 350 rpm in this work by taking into consideration both energy consumption of agitation and the hydrolysis rate according to agitation speed.

Figure 3 represents hydrolysis of the fat, the beef tallow, and the palm kernel. Their melting points were different from each other. Therefore, hydrolysis temperatures were fixed at 37°C for the palm kernel, 42°C for the fat, and 42°C for the beef tallow. The hydrolysis of these substrates

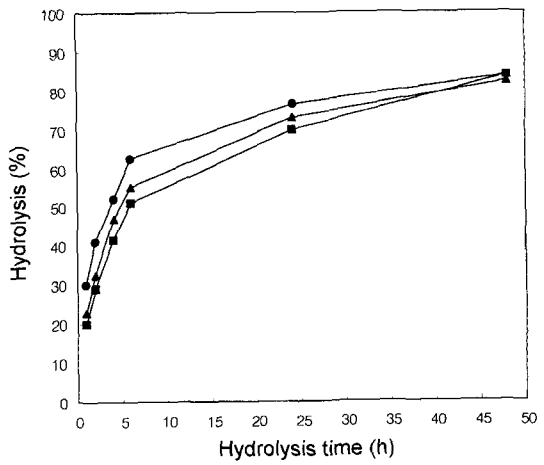

Fig. 3. Hydrolysis of beef tallow (●), fat (▲), and palm kernel (■) according to the reaction time.

showed practically the same trend in all the reaction times ranging from 0 to 50 h. The hydrolysis of all these lipids progressed very slowly after 24 h and became nearly unchanged after 48 h. The maximum hydrolysis was 83.5% for the beef tallow, 82.1% for the fat, and 83.5% for the palm kernel.

Scale-Up

The hydrolysis system in this work was the oil-water system in which the hydrolysis seemed to process a heterogeneous reaction. Therefore, an emulsion condition was the most important factor for determining the reaction rate of hydrolysis. If the other factors such as pH, enzyme concentration, metal ions, and water-oil ratio remain the same between small-scale and large-scale systems, the scaling-up of agitation speed must depend on the equal suspension, that is, the equal emulsion state [1, 2, 4, 5, 11].

Generally, the diameter of the tank reactor is designed to be equal to the height of the reactant solution. Therefore, a relationship between the diameter of tank reactor and the working volume becomes the following Eq. (2)

$$\frac{V_2}{V_1} = \frac{D_{T2}^3}{D_{T1}^3} \tag{2}$$

where V is the working volume. When Eq. (2) is substituted to Eq. (1), then Eq. (3) is obtained.

$$N_2 = N_1\left(\frac{V_1}{V_2}\right)^{\frac{n}{3}} \tag{3}$$

The reactors in Fig. 1 and Table 1 did not have the same geometrical design. That is, the 1-l reactor had one agitator, the 100-l had two, and the 10,000-l contained three. Therefore, a working volume of each reactor must be converted into a working volume per one agitator (Table 3).

The power n of Eq. (3) was used as 3/4 because hydrolysis of this work was a heterogeneous reaction and the reaction rate was determined by the emulsion state [1, 2, 4, 5, 11]. The fifth column of Table 3 shows the scaled-up agitation speeds actually used for the hydrolysis of each reactor.

Hydrolyses of beef tallow, fat, and palm kernel were carried out in both 100-l and the 10,000-l reactors. The agitation speeds were 150 rpm for the 100-l reactor and 46

Table 3. Working volume and agitator speeds scaled-up and actually used.

Reactor	Working volume (l)	Number of agitator	Working volume per one agitator (l)	Agitation speed actually used
1-l	1	1	1	350 rpm
100-l	60	2	30	150 rpm
10,000-l	10,000	3	3,330	46 rpm

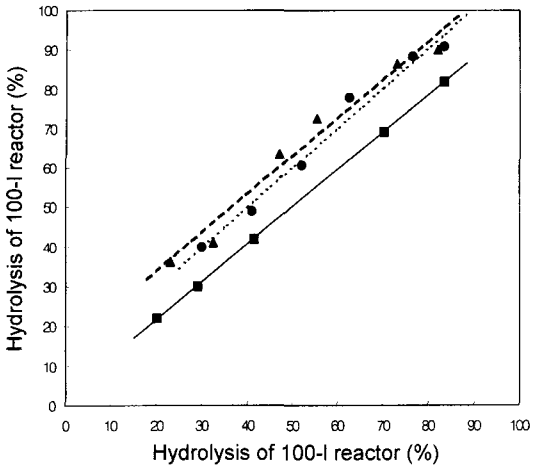

Fig. 4. Correlations between the hydrolysis of the 1-l reactor and that of the 100-l reactor for beef tallow (●; · · ·), fat (▲; - -), and palm kernel (■; —) at the same time of hydrolysis.

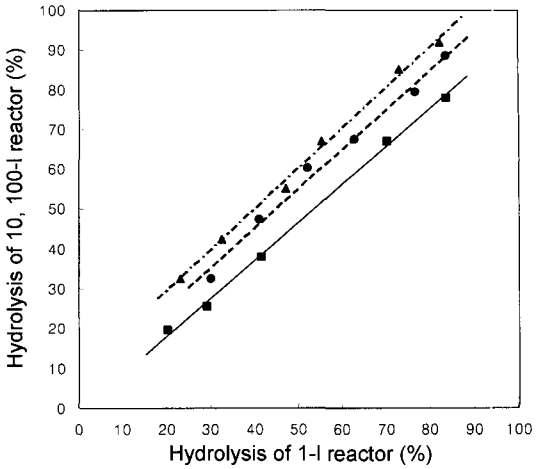

Fig. 5. Correlations between the hydrolysis of the 1-l reactor and that of the 10,000-l reactor for beef tallow (● ; · · ·), fat (▲ ; - · -), and palm kernel (■ ; —) at the same time of hydrolysis.

rpm for the 10,000-l reactor, which were scaled-up by the Eq. (3). Samples were taken at 1, 2, 4, 6, 24, and 48 h and were analyzed for the hydrolysis at the same times of hydrolysis as the 1-l reactor.

Figure 4 shows the correlation between the hydrolysis of the 1-l reactor and that of the 100-l reactor whose agitation was scaled-up by the Eq. (3). Every point forming the correlation was based on the same hydrolysis time between the two reactors. The functional relationship between the two hydrolysis systems were linear for all three of the beef tallow, fat and palm kernel. Thus, a dynamic scale-up by this Eq. (3) was proven to be reasonable. However, the results of the scale-up for the three materials were different from each other. The hydrolysis of beef tallow in the 100-l reactor was more deviated from that in the 1-l reactor, compared to the hydrolysis of others at the same time of the process. This could be explained by both the high melting point of 42°C and the difference of its geometrical design. The points of correlation for the palm kernel were located very close to the diagonal. This presumed that the emulsion states between the two reactors were almost the same because the melting point was low enough to make the same size distribution of water drops in the reactant [13]. Similar results were obtained for the scale-up from 1-l reactor to 10,000-l reactor (Fig 5). The points of correlation were much closer to the diagonal than those for the correlation between 1-l and 100-l reactors.

Figure 6 shows the results of correlations of hydrolysis between the 100-l reactor and 10,000-l reactor. The geometrical design was much more similar between the two reactors and the points of correlations were distributed close to the diagonal. This result indicated that the scale-up of hydrolysis of the heterogeneous system by the Eq. (3) was more satisfactory under the condition with the geometrical similarity between the small and the large-scale systems.

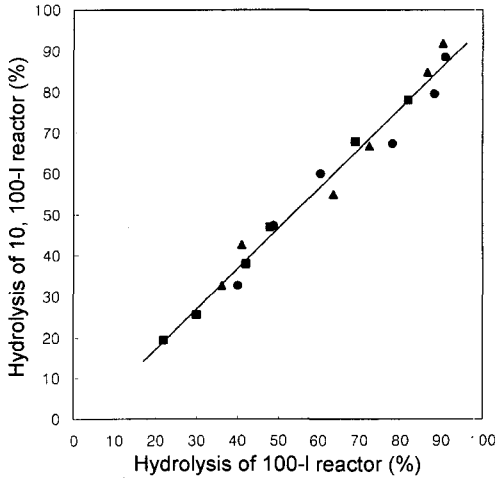

Fig. 6. Correlations between the hydrolysis of 100-l reactor and that of 10,000-l reactor for beef tallow (●), fat (▲), and palm kernel (■) at the same time of hydrolysis.

However, even in the case where the geometrical similarity was not satisfactory, the working volume per agitator could be used for the scale-up of a heterogeneous enzyme reaction.

Acknowledgments

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REFERENCES

1. Aiba, S., A. E. Humphrey, and N. F. Millis. 1973. *Biochemical Engineering*, pp. 202-216. 2nd ed. Academic Press Inc., New York, U.S.A.

2. Bailey, J. E. and D. F. Ollis. 1986. *Biochemical Engineering Fundamentals*, pp. 508-512. 2nd ed. McGraw-Hill Book Company Inc., New York, U.S.A.
3. Boswinkel, G., J. T. P. Derksen, K. V. Riet, and F. P. Cuperus. 1996. Kinetics of acyl migration in monoglycerides and dependence on acyl chain length. *J. Am. Oil Chem. Soc.* **73:** 707-711.
4. Crueger, W. and A. Crueger. 1990. *A Textbook of Industrial Microbiology*, pp. 92-96. 2nd ed. Sinauer Associates Inc., Sunderland, MA, U.S.A.
5. Geankoplis, C. J. 1983. *Transport Processes and Unit Operations*, pp. 157-159. 2nd ed. Allyn and Bacon Inc., Newton, MA, U.S.A.
6. James, J. W. 1992. *Bioreactor Design and Product Yield*, pp. 7-16. 1st ed. Butterworth-Heinemann Ltd., Oxford, England.
7. Johnstone, R. E. and M. W. Thring. 1957. *Pilot Plants, Models, and Scale-up Methods in Chemical Engineering*, pp. 12-26. 1st ed. McGraw-Hill Book Company Inc., New York, U.S.A.
8. Jung, J. Y., H. S. Yun, and E. K. Kim. 1997. Hydrolysis of olive oil by lipase, immobilized in hydrophobic support. *J. Microbiol. Biotechnol.* **7:** 151-156.
9. Kierkels, J. G. T. *et al.* 1994. *Pseudomonas fluorescens* lipase adsorption and the kinetics of hydrolysis in a dynamic emulsion system. *Enzyme Microb. Tech.* **16:** 513-521.
10. Kim, I. H. and T. H. Park. 1991. Enzymatic hydrolysis of beef tallow. *Kor. J. Appl. Microbiol. Biotechnol.* **19:** 377-382.
11. Margaritis, A. and J. E. Zajic. 1978. Mixing, mass transfer, and scale-up of polysaccharide fermentations. *Biotechnol. Bioeng.* **20:** 939-1001.
12. Rhee, J. S. and S. J. Kwon. 1998. Water activity control in lipase-catalyzed reaction system. *J. Microbiol. Biotechnol.* **8:** 191-196.
13. Tanaka, Y., J. H. Hirano, and T. Funada. 1992. Concentration of docosahexaenoic acid in glyceride by hydrolysis of fish oil with *Candida cylindracea* lipase. *J. Am. Oil Chem. Soc.* **69:** 1210-1214.