

# Scale-up of flat-plate reactors for enzymatic hydrolysis of fats and oils

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*To determine the feasibility of continuous enzymatic fat-splitting, immobilized lipase reactors were constructed from alternating layers of enzyme support material and separator screens. Partially purified lipase from Thermomyces lanuginosus was loaded onto the support material at pH 5.5 by irreversible adsorption. Melted edible tallow at 51°C was pumped through the immobilized enzyme layers and swept from the downstream separator screens by buffer recycled from a continuous oil/water separator. Results from continuous operation of 10-layer reactors were compared with data from single-layer reactors. The activity per square centimeter of 10-layer reactors was nearly as much as that of single-layer reactors at the same enzyme loading and oil feed rate. Data were fitted to an empirical mathematical model.*

**Keywords:** Lipase; immobilized enzyme; activity; glass fiber; empirical model; acrylic; tallow; *Thermomyces lanuginosus*; fermentation; separator; screens

## Introduction

Improved product quality and reduced consumption of nonrenewable resources will result from industrial hydrolysis of tallow by enzymatic catalysis if it can be shown to be economically competitive against traditional and current methods of fat-splitting. There have been many different proposals for the use of immobilized lipase to catalyze reactions between a natural oil or melted fat and an immiscible aqueous or alcohol phase.<sup>1-5</sup> Reactor types include packed beds with solvent<sup>1</sup> or without solvents,<sup>5</sup> continuous stirred reactors,<sup>2,3</sup> and reversed micelles.<sup>4</sup> The benefits of increased mass transfer and reaction rates provided by solvents or reversed micelles may be offset by additional costs of product recovery. When a reaction mixture is stirred, an emulsion is readily formed. Rapid stirring generally results in a finer emulsion and more rapid reaction, but energy costs for stirring and separation of the emulsion may become prohibitive.<sup>2</sup> To overcome this problem, membrane reactors have been proposed in which the aqueous and oil phases are on opposite

sides of a membrane containing immobilized lipases.<sup>6-8</sup> Both hydrophobic<sup>6</sup> and hydrophilic<sup>7</sup> microporous membranes, as well as an ultrafiltration (dialysis) membrane,<sup>8</sup> have been used. In such cases the cost of the membrane itself often becomes the controlling factor. We now report that reactors built with a much cheaper glass fiber prefilter had about the same activity as those with the membrane. The design was also scaled up by a factor of 10.

## Materials and methods

### Reactors

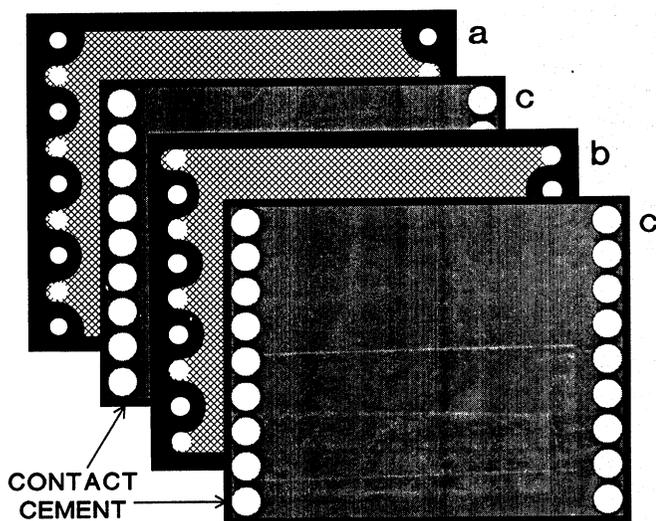
The membrane or support layer for lipase immobilization was an acrylic microporous membrane cast on nonwoven nylon fabric, Versapor, AP-200 or AP-450, Gelman (Ann Arbor, MI) or a glass fiber prefilter with acrylic binder, Presep, Micron Separations (Westboro, MA). The material was cut to 16 × 19 cm with 18 holes and a 3-mm bead of contact cement was applied to the edge, as shown in *Figure 1*. The polypropylene screens, 17 × 20 cm, with vinyl borders, Tetko (Lancaster, NY), also shown in *Figure 1*, were of two types corresponding to the oil side and buffer side of the reactor. The 1-inch stainless top and bottom plates, 23 × 28 cm, were held together with four studs at the corners. Holes in the plates were aligned with alternate holes on each edge of the screens so that each plate had one connection to the oil side and one connection to the buffer side. Membranes or prefilters were always dou-

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**Figure 1** Repeating unit of multilayered flat-plate reactor for immobilized lipase. (a = buffer side screen, b = oil side screen, c = double layer of enzyme support material)

bled. Thus single-layer reactors consisted of two sheets of support material between one oil-side screen and one buffer-side screen. Ten-layer reactors were assembled from six buffer-side screens and five oil-side screens with 10 double layers (20 sheets) of support material. In the repeating unit of *Figure 1*, double layers are shown as one. Five such repeating units were assembled with one additional buffer-side screen on the top of the stack to make a 10-layer reactor. The effective area was 220 cm<sup>2</sup> for single-layer and 2,200 cm<sup>2</sup> for 10-layer reactors.

### Lipase

Quantity of lipase was expressed in International Units (IU), defined as the amount of lipase that produces one micromole per minute of free fatty acid from olive oil at pH 8 and 37°C as measured by a flow-through pH-stat method.<sup>9</sup> Thermostable, nonspecific lipase was prepared by fermentation of a thermophilic fungus, *Thermomyces lanuginosus*, QM 225, NRRL (Peoria, IL), as previously described.<sup>10</sup> The crude culture filtrate was adjusted to pH 3.8 with HCl, and the acid precipitate was collected by centrifugation and freeze-dried. The lipase was dissolved as needed in 20 mM Tris (tris hydroxymethyl aminomethane) buffer, pH 8.0, containing 40 mM KCl, 0.02% sodium azide, and 0.01% Triton X-100, Rohm and Haas (Philadelphia, PA). The dark-brown solution was clarified by centrifugation both before and after adjusting the pH to 5.5 with acetic acid. Single-layer reactors were loaded by recycling through the reactor overnight about 200–300 ml of the pH 5.5 supernatant containing about 200–300 IU ml<sup>-1</sup>. For 10-layer reactors, 1 l containing about 300 IU ml<sup>-1</sup> was used. In most experiments, the amount of lipase to use for loading was chosen so that about half of the lipase originally in solution was adsorbed by the

support material. After rinsing the unadsorbed lipase from the reactor, the adsorption was measured by difference.

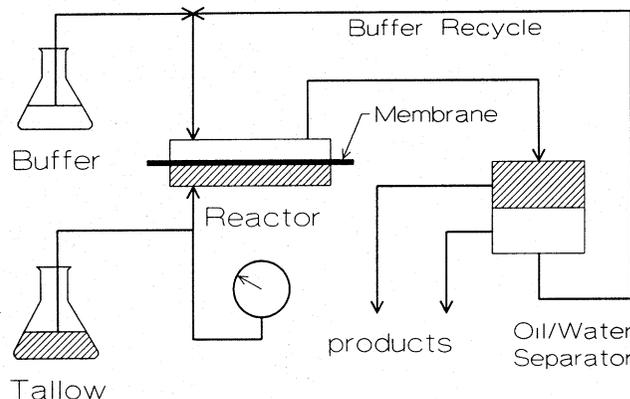
### Continuous operation

After loading, reactors were placed in a 51°C chamber containing reservoirs of melted edible tallow, Ed Miniat (Chicago, IL), and 40 mM sodium acetate buffer, pH 5.5, with 0.02% sodium azide, oil and buffer feed pumps, pressure gauge, continuous oil/water separator, buffer recycle pump, and Teflon tubing connected as shown in *Figure 2*. For single-layer reactors, the oil feed rate varied from 0.03 to 0.2 g min<sup>-1</sup>, and the buffer feed and recycle rates were fixed at approximately 0.04 and 1 g min<sup>-1</sup>, respectively. The oil feed pressure was approximately 0.3–1.0 kg cm<sup>-2</sup>, depending on the oil feed rate. For 10-layer reactors, the oil feed rate was approximately 0.7 g min<sup>-1</sup>, the buffer feed rate was approximately 0.2 g min<sup>-1</sup>, and the buffer recycle rate was approximately 10 g min<sup>-1</sup>. Faster buffer recycle rates caused excessive pressure and emulsification of the reactor product stream.

The adsorption was irreversible at pH 5.5, and there was no leakage of immobilized enzyme from the reactor.<sup>7</sup> Free fatty acid in the oil-phase product from the reactors was measured by mixing a weighed sample (about 1 g) with 8 ml of 33% hexane, 67% ethanol (95%) in a 1-ounce vial and titrating to pH 10.4 with 0.1 M NaOH. The reference electrode was filled with methanol saturated with KCl to prevent clogging of the reference junction with insoluble soaps. The acid concentration was multiplied by the oil flow rate to give the activity of the reactor. The initial activity was found as the zero-time intercept of the best straight line through a semilogarithmic plot of the activity against time (first-order model for deactivation).

### Results and discussion

*Table 1* shows the loading, flux (oil flow rate per square centimeter of effective enzyme support layer), and ini-



**Figure 2** Continuous operation of flat-plate immobilized lipase reactor, 51°C

**Table 1** Initial activity of single-layer acrylic-membrane reactors and agreement with the empirical model:  $(\text{INIT ACT}) = 0.0232 (\text{FLUX}) / [15.9 + (\text{FLUX})/(\text{LOAD})]$ 

Lipase loading (IU cm <sup>-2</sup> )	Oil flux (μg min <sup>-1</sup> cm <sup>-2</sup> )	Initial activity (μmol min <sup>-1</sup> cm <sup>-2</sup> )		Percent error <sup>a</sup>
		Measured	Predicted	
169	144	0.173	0.200	15
102	131	0.184	0.176	-4
308	156	0.201	0.221	10
83	161	0.201	0.209	4
63	153	0.202	0.193	-4
127	144	0.205	0.196	-4
123	161	0.207	0.217	5
104	170	0.211	0.225	7
98	175	0.215	0.230	7
120	187	0.223	0.248	11
68	188	0.229	0.234	2
96	158	0.229	0.209	-9
71	186	0.232	0.233	0
125	173	0.232	0.232	0
86	170	0.232	0.220	-5
116	170	0.238	0.227	-5
334	173	0.245	0.244	0
107	181	0.258	0.238	-8
372	166	0.259	0.235	-9
73	305	0.349	0.352	1

<sup>a</sup> Percent error =  $100 \times (\text{predicted activity} - \text{measured activity}) / \text{measured activity}$

tial activity of a number of single-layer acrylic-membrane reactors. Data were fitted by nonlinear least squares to the empirical model:

$$\text{INIT ACT} = A * \text{FLUX} / (B + \text{FLUX} / \text{LOAD})$$

where INIT ACT = initial activity (μmol min<sup>-1</sup> cm<sup>-2</sup>), FLUX = oil flux (μg min<sup>-1</sup> cm<sup>-2</sup>), LOAD = lipase loading (IU cm<sup>-2</sup>), and A and B have units consistent with the other variables and values to be determined by least squares.

This model was chosen because of the following qualitative features. Initial activity is higher at higher flux or higher loading, but exhibits saturation with respect to both. That is, a point is reached beyond which

further increase in loading or flux does not produce additional activity. Also, the effect of loading is more noticeable at higher fluxes, and the effect of flux is more noticeable at higher loading. *Table 1* also shows the percent difference between the measured initial activity and that predicted by the model for each reactor, generally less than 10%.

*Tables 2* and *3* show similar results for single-layer glass fiber and 10-layer glass fiber reactors, respectively. As in *Table 1*, all values are calculated per square centimeter to facilitate comparison between single-layer and 10-layer reactors. Again, agreement of the data with the empirical model is within 10%. Comparison of the models in *Tables 1, 2, and 3* reveals that

**Table 2** Initial activity of single-layer glass fiber reactors and agreement with the empirical model:  $(\text{INIT ACT}) = 0.0264 (\text{FLUX}) / [15.1 + (\text{FLUX})/(\text{LOAD})]$ 

Lipase loading (IU cm <sup>-2</sup> )	Oil flux (μg min <sup>-1</sup> cm <sup>-2</sup> )	Initial activity (μmol min <sup>-1</sup> cm <sup>-2</sup> )		Percent error <sup>a</sup>
		Measured	Predicted	
125	262	0.414	0.402	-3
134	344	0.495	0.514	4
203	342	0.527	0.537	2
44	918	0.671	0.676	1
55	895	0.733	0.750	2
98	873	0.989	0.961	-3

<sup>a</sup> Percent error =  $100 \times (\text{predicted activity} - \text{measured activity}) / \text{measured activity}$

**Table 3** Initial activity of 10-layer glass fiber reactors and agreement with the empirical model:  $(INIT\ ACT) = 0.13 (FLUX) / [117 + (FLUX) / (LOAD)]$ 

Lipase loading (IU cm <sup>-2</sup> )	Oil flux (μg min <sup>-1</sup> cm <sup>-2</sup> )	Initial activity (μmol min <sup>-1</sup> cm <sup>-2</sup> )		Percent error <sup>a</sup>
		Measured	Predicted	
81	320	0.307	0.344	12
137	334	0.331	0.364	10
79	325	0.334	0.349	4
112	313	0.343	0.340	-1
132	315	0.348	0.343	-2
116	340	0.352	0.368	5
86	334	0.386	0.360	-7
101	335	0.395	0.362	-8
123	367	0.426	0.398	-7

<sup>a</sup> Percent error =  $100 \times (\text{predicted activity} - \text{measured activity}) / \text{measured activity}$

the initial activity of single-layer glass fiber reactors was a little higher than that of single-layer acrylic membranes, while the 10-layer glass fiber reactors had nearly 10 times the initial activity of single layers. For example, at representative values of 100 IU cm<sup>-2</sup> loading and 350 μg min<sup>-1</sup> cm<sup>-2</sup> flux, the models predict initial activities of 0.497, 0.419, and 0.378 μmol min<sup>-1</sup> cm<sup>-2</sup> for single-layer glass fiber, single-layer acrylic membrane, and 10-layer glass fiber, respectively. One may expect reduced activity upon scale-up due to uneven flow distribution among the layers. Higher oil flux and resulting higher pressure drop across the enzyme support layer will give better flow distribution, but can also result in excessive leaking of the stack. Because the glass fiber support material can be purchased for only about one-third the cost of the acrylic membranes, this substitution should be a significant improvement in the overall economics of the process. For example, preliminary cost analysis indicates that the support material cost will be reduced from approximately \$0.15 per pound of fatty acid produced to approximately \$0.05 per pound with this substitution.

### Conclusions

Single-layer flat-plate reactors built from glass fiber enzyme support layers had slightly greater initial activities than acrylic membrane reactors of similar con-

struction at significantly lower cost. Reactors that were scaled up by a factor of 10 showed nearly 10 times the initial activity of the single-layer design. The cost of lipase is also estimated to be approximately \$0.05 per pound of fatty acid produced, assuming large-scale operation. Other costs, including capital costs, will also depend on the scale of operation. For large-scale operation, an operating cost savings of \$0.10 per pound of product is estimated to be highly significant compared to the total cost.

### References

- 1 Wisdom, R. A., Dunnill, P., and Lilly, M. D. *Enzyme Microb. Technol.* 1985, **7**, 567-572
- 2 Buhler, M. and Wandrey, C. *Fat Sci. Technol.* 1987, **89**, 598-605
- 3 Brady, C., Metcalfe, L., Slaboszewski, D. and Frank, D. J. *Am. Oil Chem. Soc.* 1988, **65**, 917-921
- 4 Kim, T. and Chung, K. *Enzyme Microb. Technol.* 1989, **11**, 528-532
- 5 Kosugi, Y. and Tanaka, H. *Biotechnol. Bioeng.* 1990, **36**, 617-622
- 6 Yamane, T., Hoq, M. M., Itoh, S. and Shimizu, S. *J. Japan Oil Chem. Soc.* 1986, **35**, 632-636
- 7 Taylor, F., Panzer, C. C., Craig, J. C., Jr. and O'Brien, D. J. *Biotechnol. Bioeng.* 1986, **28**, 1318-1322
- 8 Pronk, W., Kerkhof, P. J. A. M., van Helden, C. and van't Riet, K. *Biotechnol. Bioeng.* 1987, **32**, 512-518
- 9 Taylor, F. *Anal. Biochem.* 1985, **148**, 149-153
- 10 Taylor, F. *J. Ferment. Bioeng.* 1989, **68**, 141-143