ORIGINAL

Continuous Glycerolysis of Fat by Lipase in Microporous Hydrophobic Membrane Bioreactor*

Tsuneo YAMANE**, Mohammad Mozammel HOQ, Sumiyo ITOH, and Shoichi SHIMIZU

Laboratory of Bioreaction Engineering, Department of Food Science and Technology,
School of Agriculture, Nagoya University, (Chikusa-ku, Nagoya)

The continuous glycerolysis of olive oil by *Pseudomonas fluorescens* lipase was performed at 40°C in a microporous hydrophobic membrane bioreactor previously developed by the authors. Two kinds of flat membranes, one made of polypropylene and the other made of poly (tetrafluoroethylene) (Teflon), were used. The olive oil was fed into the fat compartment on one side of the membrane and the product was obtained from the outlet in a single phase. The glycerol containing small amounts of water and enzyme was fed in to the opposite side of the membrane in the bioreactor and recycled to the glycerol reservoir. After 9~20 hours of a transient state, steady states were attained. The enzyme concentration in the glycerol phase did not affect the composition of the outlet product. Teflon membrane gave a somewhat higher conversion than the polypropylene membrane. The conversion increased with a decrease in flow rate, approaching the one at chemical equilibrium. The conversion was highly restricted at lower water concentrations. At water concentrations higher than 4%, free fatty acid was produced. The half life of the enzyme during the continuous reaction was about 3 weeks.

1 Introduction

In the preceding article, some characteristics of fat glycerolysis by lipase were elucidated¹⁾. It was found out that the water concentration was most influential not only for the reaction rate but also for the composition at the chemical equilibrium. The glycerolysis reaction was carried out batchwise as an emulsion system in a small agitated vessel using free enzyme. This reaction system was convenient to get a number of data concerning the effects of various factors on the fat glycerolysis. However, it has following disadvantages as an industrial scale system.

- 1) Batch operation has much less volumetric productivity than continuous one.
- 2) Emulsion system needs stirring at high speed. Furthermore, after the reaction has finished, the separation of emulsified oily products needs powerful centrifugation and/or solvent extraction.

- 3) The reaction system of free lipase plus emulsified substrate is uneconomical because it is impossible to reuse the enzyme.

Recently the authors have developed a bioreactor system which is suitable for the bioconversion of fat and fatty acid derivatives. The bioreactor utilizes a microporous hydrophobic membrane, either in flat or in hollow fiber module. With the membrane bioreactor, the authors studied continuous hydrolysis of fat^{2)~5)} and continuous synthesis of glycerides from fatty acid and glycerol^{6),7)}.

Since it is possible to overcome most of the aforementioned disadvantages inherent in the reaction system of emulsion plus free enzyme, the authors have attempted continuous glycerolysis of fat by lipase in the microporous hydrophobic membrane bioreactor. Experimental data concerning some factors governing the reaction will be reported in this paper.

2 Materials and Methods

2.1 Lipase and Chemicals

Pseudomonas fluorescens lipase, Lipase P, supplied by Amano Pharmaceutical Co., Ltd., Nagoya, was used throughout the experiments. Its hydrolytic activity, as determined by the

* Bioreactor for Enzymic Reaction of Fat and Fatty Acid Derivatives, Part VIII***.

** To whom all correspondence should be addressed.

*** Part I-VII, see Ref. 1)~7).

olive oil emulsion method described previously was about 29 units/mg.

Olive oil having a saponification value of 190, purchased from Wako Pure Chemical Industries Co., Ltd., Osaka, was used without dilution by any solvent throughout the experiments. In the long term continuous glycerolysis reaction the olive oil dehydrated by molecular sieve particles (Type 4A XH-5, Union Showa Co., Ltd., Tokyo) was used. Glycerol of 99% purity from Nippon Oil and Fats Co., Ltd., Amagasaki Works, Hyogo Prefecture, was used.

2.2 Configuration of the Membrane Bioreactor

The microporous hydrophobic membrane bioreactor used was the same one unit of plate type dialyzer as used in the previous study²⁾. Two kinds of microporous hydrophobic flat membrane were used, one made of polypropylene and the other made of poly(tetrafluoroethylene) (Teflon). The membrane made of polypropylene (Juragurd 2500, Polyplastic Co., Ltd., Tokyo) was 25 μm thick and its void fraction was 55%. The micropores of the polypropylene film were elliptically shaped pores with maximum pore diameter of 0.4 μm . The membrane made of poly(tetrafluoroethylene) (Japan Gore-Tex Inc., Tokyo) was 15 μm thick and its void fraction was 70% and the maximum pore diameter was 0.4 μm .

The setup of the microporous hydrophobic membrane bioreactor was the same as used in the previous work⁶⁾. The glycerol-water-lipase solution was passed through the narrow compartment between two sheets of membrane, while olive oil was passed on the opposite side of the membrane in the grooves of the plates.

Fig.-1 shows the neighborhood of the membrane where micropores are drawn very schematically. Owing to the hydrophobic nature of the membrane, olive oil penetrated readily the

Fig.-1 The neighborhood of the membrane.

Fig.-2 Process flow diagram of the continuous glycerolysis reaction system.

micropores while glycerol-water-lipase solution could not. However, because of the high viscosity of the glycerol-water-lipase solution, the pressure of this solution was a little greater than that of olive oil, which did not allow olive oil to pass through the membrane. Thus, the olive oil came in contact with glycerol-water-lipase solution as shown in Fig.-1. The glycerolysis reaction took place at the glycerol-side surface of the membrane. The product diffused back into the bulk flow of the olive oil phase.

A process flow diagram of the reaction system is shown in Fig.-2. Olive oil was fed at a definite flow rate into the bioreactor with two reciprocal (plunger) pumps (Micro pump, Model SP-100, Sibata Chemical Apparatus Manufacturing Co., Ltd., Tokyo). Each pump fed olive oil to each fat compartment with the same flow rate. The product formed was obtained from the outlet in a single phase. That is, the product was never mixed with the glycerol-water-lipase solution and was collected in a fraction collector. The glycerol-water-lipase solution in a beaker which was placed in a desiccator was fed at the flow rate of 7.2 ml·h⁻¹ with a peristaltic pump (Minipuls 2, Gilson France SA, Villiers Le Bel, France), and the solution from the outlet was recycled back into the beaker. In the desiccator were placed silica gel particles around the beaker to prevent moisture absorption into the glycerol-water-lipase solution. The membrane bioreactor was placed in a thermostated water bath (40°C).

2.3 Analytical Procedures

The composition of the product was deter-

mined with a TLC/FID analyzer¹⁾. Thirty to 50 mg of the output sample was diluted with 1.0 ml of chloroform before being spotted on a quartz rod. The recorded chromatograms were analyzed according to the previous paper¹⁾.

Water concentration in the glycerol-water-lipase solution was analyzed occasionally with a Carl-Fischer moisture meter¹⁾.

3 Results and Discussion

3.1 Typical Time Course

Fig.-3 shows a typical time course of the continuous glycerolysis of olive oil by *Pseudo-monas fluorescens* lipase. After 9 h transient state from the start of the continuous operation, a steady state was attained. The length of transient state depended on the flow rate of the oil: the higher the flow rate, the shorter the transient state. During the continuous operation, the water concentration in the glycerol phase increased a little, probably because the water dissolved in the olive oil transferred to the glycerol phase through the microporous membrane in the bioreactor. The water concentration in the olive oil was 0.10%, which was higher than the equilibrium value in the olive oil phase corresponding to 3.0% of water in the phase of the glycerol-water-lipase solution¹⁾. Therefore, water transferred from the oil phase to the glycerol phase to approach the thermodynamical distribution. It should be noted here that water is neither liberated nor consumed during the glycerolysis reaction.

3.2 Effect of Enzyme Concentration in

The enzyme concentration was 190 units/g-glycerol, the water concentration in the glycerol phase was 2.8~2.9% and the flow rate of olive oil was 2.5 ml·h⁻¹.

Fig.-3 A typical time course of the continuous glycerolysis reaction.

The water concentration was 2.8~3.2% and the flow rate of olive oil was 2.5 ml·h⁻¹.

Fig.-4 Effect of enzyme concentration in the glycerol solution on the composition of the outlet.

Glycerol

Enzyme concentration in the glycerol phase (in the range of 100~600 units/g-glycerol) did not affect the composition of the outlet product as shown in Fig.-4. It is quite conceivable that the conversion will decrease when the enzyme concentration is lower than the values shown in Fig.-4. In order to know whether the molecular diffusion of the oily compounds in the micropores limited the overall glycerolysis reaction, an experiment was carried out using a thinner membrane made of Teflon. The results shown in Table-1 indicates that the thinner membrane improved the conversion slightly. Thus, the molecular diffusion affected a little the overall reaction rate. However, further detailed studies with respect to both diffusion and enzyme-adsorption phenomena are needed to know the rate-limiting step. Differences in pore size, void fraction, thickness and material of the two kinds of membrane must be taken into consideration.

3.3 Effect of Flow Rate on Conversion

Table-1 Comparison of microporous polypropylene and poly(tetrafluoroethylene) membranes.

Component	Polypropylene (%)	poly(tetrafluoroethylene) (%)
TG	57	49
Total DG	36	41
Total MG	8	10
FFA	0	0

The water concentration was 2.8%, the enzyme concentration was 190 units/g-glycerol and the flow rate of olive oil was 2.5 ml·h⁻¹.

and Composition of Outlet

The effect of the flow rate of olive oil on the conversion and composition was investigated. The results are shown in Fig.-5. The conversion increased with decrease in the flow rates because of longer residence times of fat in the bioreactor. The values of the conversion and composition at the lowest flow rate were close to the ones at the chemical equilibrium which was attained in the batch reaction¹⁾.

3.4 Effect of Water Concentration on Conversion and Composition of Outlet

The effect of the water concentration in the glycerol-water-lipase solution on the conversion and composition of the outlet was investigated by varying the water concentration in the ranges indicated in Fig.-6. Alike in the batch reac-

The enzyme concentration was 190 units/g-glycerol and the water concentration in the glycerol phase was 2.8~3.0%.

Fig.-5 Effect of the flow rate of olive oil on the conversion and composition of the outlet product.

The enzyme concentration was 190 units/g-glycerol and the flow rate of olive oil was 2.5 ml·h⁻¹.

Fig.-6 Effect of the water concentration in the glycerol phase on the conversion and composition of the outlet product.

The enzyme and water concentrations in the glycerol phase were 190 units/g-glycerol and 3.2~4.1%, respectively. The flow rate of olive oil was 2.5 ml·h⁻¹.

Fig.-7 Time course of the composition in the long term continuous operation.

tions¹⁾ the conversion was highly restricted at lower water concentrations, probably because some amount of water was required for the enzyme to exhibit its glycerolysis activity. At water concentration higher than 4%, free fatty acid (FFA) was produced because the hydrolysis reaction became significant.

Fig.-6 suggests that the water concentration should be kept at 4% strictly to obtain the products without FFA and yet to achieve the highest conversion.

3.5 Operational Stability of Lipase

A long term continuous reaction was carried out to know the operational stability of the lipase. The result shown in Fig.-7 indicates that the half-life of the enzyme during the continuous reaction was about 3 weeks. Although the conversion decreased gradually in this experiment because of the unvaried feed rate of fat, it is possible to keep the conversion constant if one decreases the feed rate of fat gradually with time, adjusting it to the loss of the enzyme activity. To obtain more prolonged high conversion, experimentation at lower temperature or with more thermostable lipase may be preferable.

(Received March 6, 1986)

References

- 1) T. Yamane, M.M. Hoq, S. Itoh, and S. Shimizu, *J. Jpn. Oil Chem. Soc. (Yukagaku)*, **35**, 625 (1986), the preceeding article.
- 2) M.M. Hoq, T. Yamane, S. Shimizu, T. Funada, and S. Ishida, *J. Am. Oil Chem. Soc.*,

- 62, 1016 (1985).
- 3) M.M. Hoq, M. Koike, T. Yamane, and S. Shimizu, *Agric. Biol. Chem.*, **49**, 3171 (1985).
 - 4) M.M. Hoq, T. Yamane, and S. Shimizu, *Enz. Microb. Technol.*, **8**, 237 (1986).
 - 5) T. Yamane, M.M. Hoq, and S. Shimizu, *Yuekagaku*, **35**, 10 (1986).
 - 6) M.M. Hoq, T. Yamane, S. Shimizu, T. Funada, and S. Ishida, *J. Am. Oil Chem. Soc.*, **61**, 776 (1984).
 - 7) M.M. Hoq, T. Tagami, T. Yamane, and S. Shimizu, *Agric. Biol. Chem.*, **49**, 335 (1985).

微孔性疎水性薄膜型バイオリアクター
を用いたリパーゼによる油脂の連続
グリセロリシス*

山根恒夫・モハメド モザメル ホック・
伊藤純代・清水祥一

名古屋大学農学部食品工業化学科生物反応工学講座
(名古屋市千種区不老町1)

Pseudomonas fluorescens 産生のリパーゼによるオリブ油の連続グリセロリシス反応を、著者らが以前開発した微孔性疎水性薄膜型バイオリアクターを用いて、40°C で実施した。平膜として、ポリプロピレン膜とポリ四フッ化エチレン（テフロン）膜を調べた。オリブ油を平膜の一方の側に供給し、生成物は出口より他相を含まない単相状態で得られた。一方、少量の水分と酵素を含むグリセリンを平膜の反対側に供給し、流出液はグリセリン貯槽へ戻し再循環した。9~20 h の遷移状態の後、定常状態が得られた。グリセリン中の酵素濃度を変えても、生成物中の組成は変化しなかった。テフロン膜はポリプロピレン膜より少し高い反応率を与えた。オリブ油の流量を減少させると反応率は増大し、化学平衡に近づいた。低水分では反応率はきわめて低かった。4%以上の水分では、遊離脂肪酸の生成が見られた。酵素の半減期は約3週間であった。

* 油脂関連化合物の酵素的変換のためのバイオリアクター
(第8報)