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Chemometric analysis of triglycerides hydrolysis in an enzyme membrane reactor

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ABSTRACT

The work concerns hydrolysis of oil in a reactor with an ultrafiltration enzyme membrane. A phenomenological model of the reactor performance and its mathematical representation have earlier been elaborated according to a network thermodynamics approach by Schnakenberg.

We try to explain when and why the process in the membrane reactor exhibits a typical consecutive kinetics, i.e., mono- and diglycerides are produced together with fatty acids and glycerol or only the latter two products are formed.

The detailed discussion on the reactor performance at different levels of selected parameters is based on chemometric simulation procedure. The parameters were: the load density of lipase immobilised in the membrane, the rate constant of uncompetitive inhibition of the lipase catalyst, and the rate of convection streams of the circulating oil and aqueous phases.

Planning of the simulation experiments has been done by the full factorial design technique while the cluster analysis has been used to discuss the resulted features of the reactor performances. This was possible after selection of the linkage distances between the particular clusters of simulated experiments, represented by "cumulative" points defined as the functions of all the product time courses in the simulated processes versus the normalised time.

1. Introduction

A catalytic membrane reactor may be an alternative for numerous useful processes [1,2], e.g., hydrolysis of triglycerides upon a catalytic action of lipase immobilised in a membrane [3–6]. The membrane fulfils a triple function: a border between organic and aqueous phases, carrier of the lipase catalyst, and phase enabling separation of the reaction products. The immobilisation of lipase can be accomplished by many methods with a broad range of load densities [3]. The reactor yield depends on its design, mode of reactant transport (diffusion and hydrodynamic flows), temperature, reactant concentrations, as well as its concentration, activity, and possible inhibition of the enzyme.

The performance model of such a membrane reactor, involving triglyceride hydrolysis catalysed by lipase that was chemically immobilised in a polymer membrane and circulating phases of olive oil and aqueous buffer solution, was already presented [7]. The performance was described using the bond graph network thermodynamic model, represented by a set of 33 differential kinetic equations [3]. All needed kinetic rate constants of the relevant processes were estimated in terms of a good agreement with the experimentally estimated time courses of the main products (glycerol and fatty acids) and the calculated time courses of other reactants. It was found that there were three parameters that controlled the reactor performance: (a) the concentration of lipase immobilised in the membrane, (b) rate constant of uncompetitive inhibition of lipase, and (c) rate of convection of the liquid phases.

The aim of the work was to establish factors influencing a course and final products of the consecutive process of triglycerides hydrolysis. Particularly, it was interesting to find conditions enabling formation of mono- and diglycerides in the enzyme reactor, usually considered as a source of fatty acids and glycerol. The goal was reached by the chemometric methods: the full factorial design of the parameters of simulated processes and the cluster analysis of the results [8]. The calculations were performed in the double precision mode, using a NOBIS computer program [9].

2. Theoretical modelling

2.1. Reactor design and assumed mechanism of the chemical process

A laboratory two-phase reactor contained: potassium phosphate buffer as a stripping phase for produced glycerol and the

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Table 1
Values of the parameters considered for 3^3 design.

	Value 1	Value 2	Value 3
Enzyme cocentration C_E [mol cm^{-3}]	5.04×10^{-7}	3.18×10^{-5}	6.03×10^{-5}
Inhibition constant k_{inh} [cm^3 mol^{-1} s^{-1}]	0	50	100
Rate of convection V [cm^3 s^{-1}]	0.10	2.55	5.00

feeding oil phase simultaneously collecting produced glycerides and fatty acids. The phases were separated by a membrane carrying chemically bonded lipase. It constituted a discontinuity border and the place of the chemical and diffusional processes. Both liquid phases were constantly circulated.

The overall hydrolysis was assumed as a sum of reversible steps of consecutive hydrolysis processes catalysed by relevant active complexes of lipase as well as by complexes of the lipase molecules partly inhibited by fatty acids. Transport of each reactant was considered as a combination of diffusion and convection.

2.2. Full factorial design of reactor performance simulations

According to our earlier experiments [7] three parameters that control the reactor performance were selected, namely the concentration of lipase immobilised in the membrane, rate constant of uncompetitive inhibition of lipase, and rate of convection streams of the circulating oil and aqueous phases.

The reactor performance reflects a typical response of a multiparameter experiment. Thus, a method that makes it possible to check the influence of combinations of the selected parameters had to be used. For that purpose, a full factorial design that enables to combine all possible levels of the parameters giving S^m permutations was chosen, where S represents the number of the levels of a selected parameter and m, the number of the parameters. Here, three levels of the above mentioned three parameters were taken into consideration (Table 1).

The remaining parameters assumed as being constant were used at the levels given in a previous work [7]. These are: rate constants of all steps of hydrolysis, thickness of diffusion layers, volumes of the cells and reservoirs, membrane working area, and mass transfer coefficients of all the reactants.

3. Results and discussion

Performances of the simulated processes were discussed firstly on the basis of the cluster analysis [8]. Linkage distances were calculated as the Euclidean distances in multi-dimentional coordinate system between points defined as "cumulative" functions of all the product time courses in the particular simulation processes versus the normalised time. This approach was inspired by Mazerski et al. [10]. The resulted dendrogram is presented in Fig. 1.

As seen, two distinct groups of simulations (1–18 and 19–27) can be distinguished. This distinction is fully correlated to plots of a ratio of the concentrations of produced glycerol and fatty acids versus the normalised time of the reactor performance. The respective bundles of curves (I and V) are shown in Fig. 2.

For the bundle V (the representatives of simulations 1–18), the concentration ratio reaches its maximum value of ca. 0.33 nearly from the beginning. This can be considered as evidence that the chemical steps of the process are quick enough in comparison to transport limitations and practically sole fatty acids and glycerol accumulate in the circulating liquid phases. The particular curves of this bundle represent the processes taking place when the membrane contains the highest concentration of lipase, regardless of the rates of convection and with very small influence of the level of the inhibition constant.

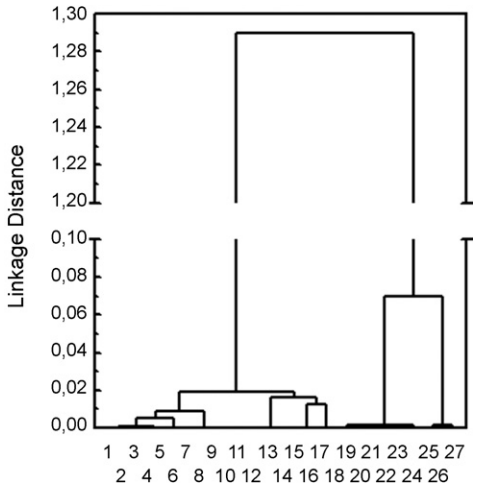

Fig. 1. Dendrogram of the results obtained from simulations given by the 3^3 design.

The other group of the dendrogram (bundle I) is constituted by simulations of the processes with the lowest lipase concentration (Fig. 2).

It has to be added that some subgroups can be recognised in Fig. 1, which represent processes influenced by lipase uncompetitive inhibition and running without such inhibition, both with meaningless influence of rates of convection.

The above results demonstrate that the rates of convection can be eliminated from further analysis and it is reasonable to make additional simulations for two parameters only, each on three levels of values, aimed at getting more data in a broaden range of the meaningful parameters (C_E up to 5.04×10^{-8} mol cm^{-3} and $k_{inh.}$ up to 2.5×10^4 cm^3 mol^{-1} s^{-1}). Thus, a new series of simulations was carried out, according to the three-level two-parameter design 3^2. The results of the simulations are also presented in Fig. 2 as the bundles of curves marked as II–IV. These bundles, together with bundle I, represent processes influenced by the lipase uncompetitive inhibition and running without such inhibition, both with meaningless influence of the rates of convection.

The difference between the time courses of the product concentrations for simulations with the highest and lowest lipase concentrations is very clear (Fig. 3a and b).

In the process presented in Fig. 3a, practically only glycerol and fatty acids are formed while the curves presented in Fig. 3b resem-

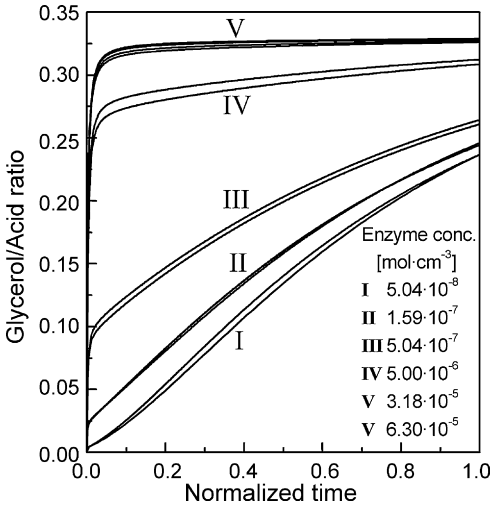

Fig. 2. Glycerol/acid concentration ratio vs. normalised time.

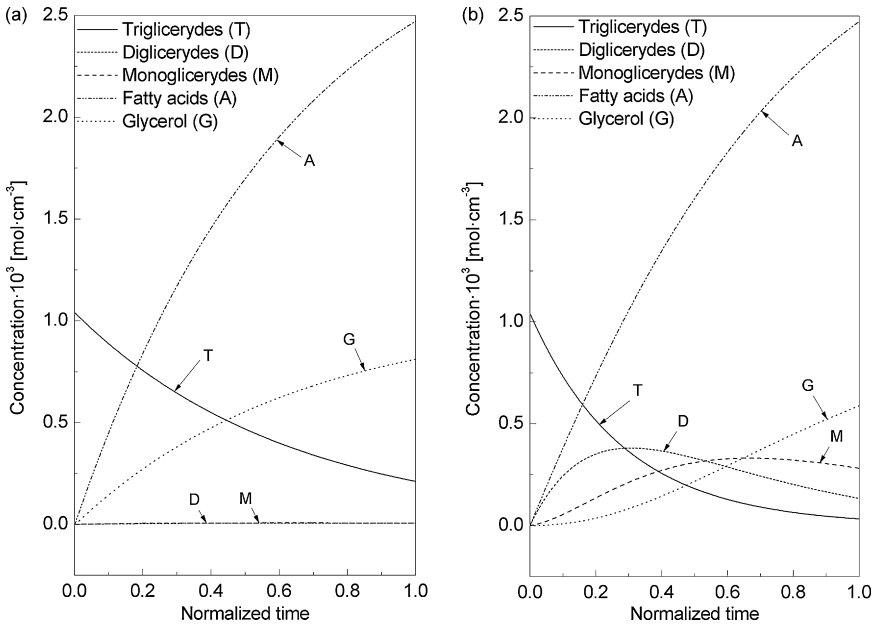

Fig. 3. (a) Reactant curves concerning the process running under a high enzyme concentration (6.3×10^{-5} mol cm^{-3}). (b) Reactant curves concerning the process running under a low enzyme concentration (5.04×10^{-8} mol cm^{-3}).

ble typical time courses for a consecutive chemical process. The maxima of the concentrations of mono- and diglycerides are distinctly separated (Fig. 3b).

Thus, it was useful to create a new dendrogram, covering all the simulations taken from the 3^2 design and some interesting representatives of the 3^3 design. The results are shown in Fig. 4.

According to the linkage distance on a level marked by a dotted line 1, the simulation processes form, as stated before, five distinct groups (I–V). The groups comprise simulations differing in the concentration of the lipase catalyst.

On the second level (line 2), the more subtle differences can be noticed. The subgroups represent simulations running at different levels of the enzyme inhibition rate constant. This results from the distances between the simulations 1 and 2 from 3, 4 and 5 from 6, etc. Moreover, the influence is more clear in the processes running with a membrane containing the lowest lipase concentrations.

The above classification and a consecutive character of the chemical processes (visualised in Fig. 3b) allow one to gain still more advanced characteristics of the reactor performance. It is based on dependences of the maximum concentrations of mono- and diglycerides on the normalised time of the simulation processes (Fig. 5).

Dotted oblique curves mark out the first order groups representing simulations with different enzyme concentrations from the lowest (I) to the highest group (V). The consecutive character of the chemical processes becomes more and more evident with diminishing concentration, which is well reflected by the diverging curves 1 and 2 that link simulations belonging to the particular primary groups.

Convergence of the curves in the simulations belonging to group V is fully in line with the already given results, as the calculated concentrations of mono- and diglycerides are on a very low level, beyond the range of the experimental detection. Thus, one can conclude that the chemical steps of hydrolysis are fully completed within the membrane and only the final products: glycerol and fatty acids penetrate into external liquid reservoirs. This conclusion agrees well with many experimental results and theoretical data [6], especially those by Pronk et al. [5] reporting that the only

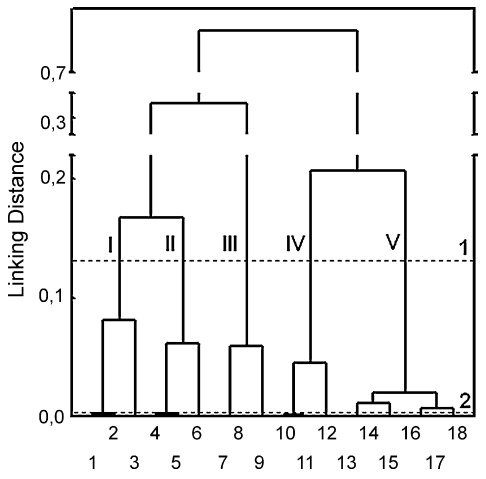

Fig. 4. Dendrogram of the simulations chosen for final analysis.

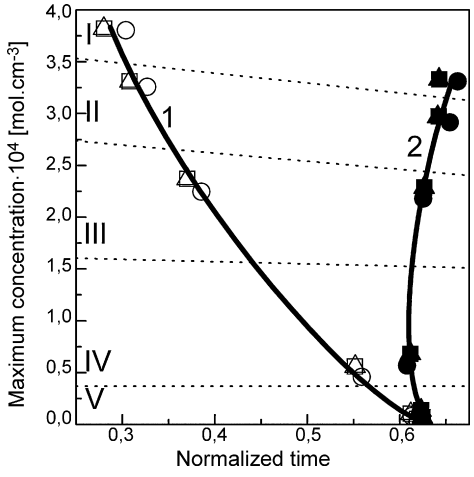

Fig. 5. Maximum concentrations of mono- and diglycerides vs. normalised time. △,○,□—diglycerides and ▲,●,■ —monoglycerides at $k_{inh.}$=25,000, 250, and 0 cm^3 mol^{-1} s^{-1}, respectively.

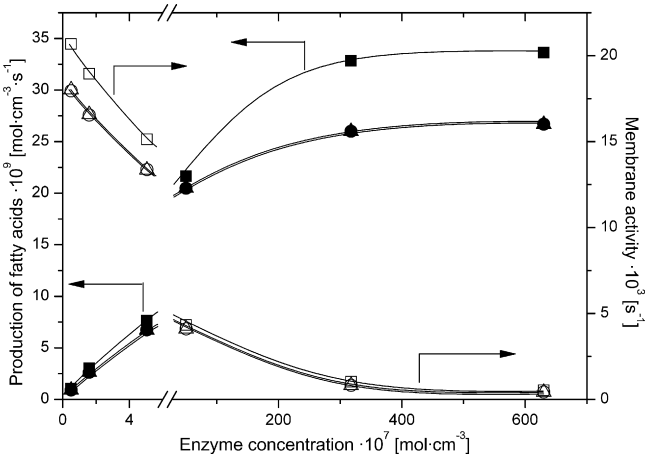

Fig. 6. Relationship between fatty acid production and membrane activity versus enzyme concentration. Symbols □,■ represent processes without inhibition while ○,● and △,▲, processes with uncompetitive catalyst inhibition with the rate constants of 250 and 25,000 $cm^3\ mol^{-1}\ s^{-1}$, respectively.

final products of the reactor are fatty acids and glycerol at the concentration ratio of 3:1. However, under certain conditions [7], the concentration of glycerol in the receiver of the enzyme membrane reactor may be very small or even not detectable while some amounts of mono- and diglycerides are being observed in addition to fatty acids [11].

In the simulation processes belonging to other groups (I–IV), the slowly forming intermediate products (mono- and diglycerides) leave the membrane and accumulate in the external reservoirs, because of the lower concentration of lipase.

It is worthwhile to mention that the large difference between the normalised times at the maximum concentrations of the produced mono- and diglycerides can be exploited to produce these compounds in the reactor adjusted to the proper mode of operation. Such possibility is evident especially when using the membrane with a low lipase concentration. In each simulation group, an influence of lipase inhibition results in distant positions of points representing processes without inhibition (marked as ○ and ● in Fig. 5) while the points for processes with two levels of the inhibition constants overlap. Evidently, the processes with the inhibition are significantly slower. This can be concluded when considering Fig. 6, in which the dependences of fatty acid production versus lipase concentration (i.e., its load density in the membrane) are shown.

As seen, positions of points representing processes with inhibition, regardless of the lipase concentration, are lower. Moreover, there is an optimum concentration of the lipase catalyst ($\sim 3 \times 10^{-5}$ mol cm^{-3}) and using its excess is pointless.

It has to be realised that processes, in which membranes with a low lipase concentration are utilised, are slow and a rate of formation of intermediates and final products is also low. However, accumulation of mono- and diglycerides is observed (Fig. 3b) under such conditions only.

4. Conclusions

The full factorial design technique and cluster analysis can successfully be used to recognise a multiparameter process of oil hydrolysis performed in the enzyme membrane reactor. Valuable conclusions on the reactor performance can be drawn based on simulations of the behaviour of an appropriate model of the reactor while applying parameters predetermined by the model. This can be recommended as a more general approach to design performances of similar reactors.

A key for a necessary linkage distance analysis between the simulations is the definition of points representing each simulated process. They have to be defined as cumulative functions derived from the time courses of concentrations of all products in the external reservoirs of the reactor.

The consecutive character of chemical steps of oil hydrolysis is observed in processes with lower concentrations of the lipase catalyst immobilised in the membrane. Reactors exhibiting such a feature could be used to obtain intermediate products, i.e., mono- and diglycerides. At a relatively high enzyme concentration and constant levels of other parameters, only final products, i.e., glycerol and fatty acids, can be formed.

There is an optimum concentration of the enzyme catalyst. At higher concentrations, the effectiveness of the enzyme in the process decreases.

In the reactors with a high concentration of the lipase catalyst, the lipase inhibition has small or meaningless influence on their performances. The convection rates do not qualitatively change features of the reactor performance in the considered range of variation.

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