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Modelling of fish oil enzymatic transesterification for ω-3 fatty acids enrichment in membrane reactors

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Introduction

Process Intensification (PI) is one of the promising routes for several industrial sectors towards a clean, sustainable and efficient future [1]. Membrane Reactors (MRs), which are integrated devices coupling a chemical reaction with a separation through a selective membrane, are an excellent example of PI. MRs are becoming industrial reality, mainly in the hydrogen sector, and their broad applicability allows investigations of new applications. One of their applications studied in the EU-funded project MACBETH is, in the sector of biotechnology, the process to concentrate ω-3 fatty acids (EPA and DHA) starting from raw fish oil [2]. The coupling of the kinetic and of the membrane separation section requires the target acids to remain attached to the glycerol backbone, while other acids must be selectively detached. In this way, the membrane is able to separate the Ethyl Esters (EEs), depleted in target acids, and to retain the glycerides, enriched in EPA and DHA. To develop this process, it is required the presence of a lipase able to selectively detach from the glycerol backbone the acids present in fish oil, except for the targets ω-3. A lipase from Candida Antarctica (CAL-A) has been used to allow the selective reaction between oil and ethanol. In this work, two mathematical models are proposed to reproduce the experimental data of fish oil transesterification.

Experimental and Modelling

Enzyme is immobilized on beads. Batch experiments were run for 23 hours, with different amount of beads and with different ethanol concentrations. The fish oil is composed by many fatty acids, and it is assumed that they are all in the form of triglycerides. Two models are proposed: the first one, based on the work of Torres et al. [3], assumes that all acids are related to a specific glyceride (e.g. triglyceride with three EPA acids attached); the second model considers that all the acids in the oil can be randomly attached to the glycerol backbone. In both models, it is assumed that the position of the acid in the glyceride has no effect in the reaction. Reactions considered and kinetic model equations are reported in Table 1.

Table 1. Description of the two models used: on the left, model taken from literature. On the right, new model.

	Standard Model	New Random Model
Features	Each acid j is related to a specific ester (TG, DG, MG or EE). Reaction rates equivalently refer to the acid or the ester	Acids are randomly distributed. Reaction rates refer to the total amount of each acid in each ester form
Reactions	Triglyceride (TG) + Ethanol ↔ Diglyceride + Ethyl Ester (R.1) Diglyceride (DG) + Ethanol ↔ Monoglyceride + Ethyl Ester (R.2) Monoglyceride (MG) + Ethanol ↔ glycerol + Ethyl Ester (R.3)	
Reaction Rates	$rr_{i,R.1} = \theta_{i,f} \cdot C_{i,TG} \cdot C_{EtOH} - \theta_{i,b} \cdot C_{i,DG} \cdot C_{i,EE}$ $rr_{i,R.2} = \theta_{i,f} \cdot C_{i,DG} \cdot C_{EtOH} - \theta_{i,b} \cdot C_{i,MG} \cdot C_{i,EE}$ $rr_{i,R.3} = \theta_{i,f} \cdot C_{i,MG} \cdot C_{EtOH} - \theta_{i,b} \cdot C_{glycerol} \cdot C_{i,EE}$	
Model	$\frac{dC_{i,y}}{dt} = beads_{load} \cdot \sum_{j=R.1}^{R.3} \nu_{i,y,j} \cdot rr_{i,j}$ $i = acid$ $y = ester$ $j = reaction$	$\frac{dC_z}{dt} = beads_{load} \cdot \sum_{j=R.1}^{R.3} \nu_{z,j} \cdot \sum_{i=1}^{N} P_{i,z_y} \cdot rr_{i,j}$ Where $P_{i,z_y} = \frac{C_{i,z}}{\sum_z C_{i,z_y}}$ $i = acid$ $y = ester$ $j = reaction$ $z = component$

Results and discussion

Due to the high number of compounds, the different acids in the model were grouped in order to reduce the optimization variables. Few acids are then fitted, while the conversion of the other acids is calculated starting from the fitted values, since they show a similar conversion trend.

The enzyme shows a good selectivity in experiments, being then able to detach all the undesired acids and to maintain the target EPA and DHA attached to the glycerol backbone. Both versions of the model correctly fit the experimental data on the produced ethyl esters. Fitting of the standard model is shown in Fig. 1.

Reactions rates of the forward and of the backward reactions are proportional to the amount of enzyme, while they show an exponential trend as a function of the ethanol volumetric fraction in the mixture. The total amount of the different esters (TG, DG, MG, EEs) computed by the two models are compared, but they could not be experimentally verified due to the different response of the high number of compounds.

The different results of the models show that the assumption that all acids are attached to a specific glyceride, assumed so far in literature, can lead to miscalculations on the total amount of TG, DG and MGs, and particularly the amount of target ω-3 in the TG form can be highly overestimated. An example of the comparison between models prediction in terms of esters trends is shown in Fig. 2.

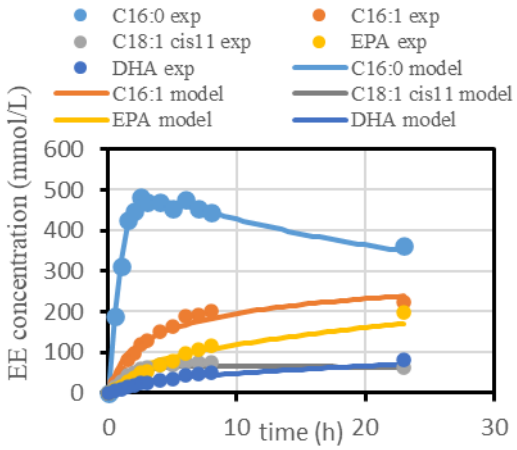

Fig. 1. Concentration of the EEs of the five acids fitted by the model over time. Results are obtained at 9% volume of ethanol in the oil-ethanol mixture and with 2 g of beads.

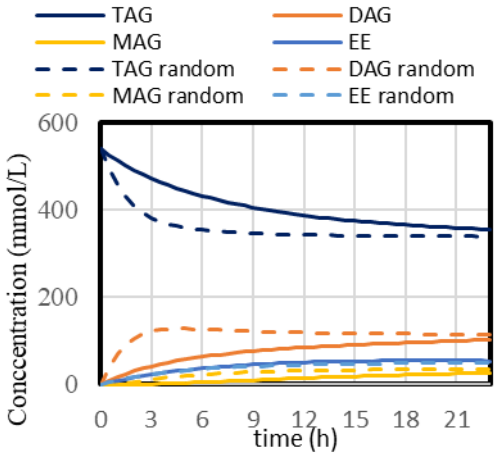

Fig. 2. Difference in concentration of EPA in each ester form (EPA as TG, DG, MG and EE) estimated with the standard and the random model.

Conclusions

In the selective ethanolysis of fatty acids from fish oil, CAL-A enzyme shows a good ability in avoiding the detachment of EPA and DHA. In this work, starting from a model proposed in literature, the conversion of the acids to ethyl esters are reproduced and expressions for the reaction rates at different ethanol concentrations have been outlined. Moreover, a new version of the model is proposed, considering a random distribution of the fatty acids. This showed that the previous model could lead to an overestimation of the presence of target acids as TG, while their presence in DG and MG was underestimated. This work put the basis for a more detailed description of the process, which is necessary for the coupling of the membrane system for the oil concentration.

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