



## Regular article

# Kinetic studies on the transesterification of sunflower oil with 1-butanol catalyzed by *Rhizomucor miehei* lipase in a biphasic aqueous-organic system



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## ABSTRACT

The kinetics of sunflower oil transesterification with 1-butanol using a homogeneous lipase (*Rhizomucor miehei*) in an aqueous-organic biphasic system were studied in a stirred batch reactor set-up. An initial screening study was performed to optimize relevant process conditions (enzyme concentration, stirring speed, 1-butanol to oil ratio). A kinetic model was developed based on concentration time profiles. Important reaction parameters were the oil concentration in *n*-hexane, the enzyme concentration, the 1-butanol to oil molar ratio; the organic to an aqueous solvent volume ratio, the temperature, the pH setting using a phosphate buffer and the agitation rate. The experimental data were modelled using the Ping Pong Bi Bi mechanism with non-competitive inhibition by 1-butanol and a term for irreversible enzyme deactivation during reaction. Agreement between model and experiments was good ( $R^2 = 0.99$ , average error 4.2%).

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## 1. Introduction

Biodiesel is commercially produced by a transesterification reaction of vegetable oil with an alcohol, in most cases methanol, in the presence of a catalyst. Alkaline catalysts, such as sodium hydroxide, are commonly used due to their low costs and high reaction rates. However, such catalysts also have several drawbacks. For instance, the alkaline catalyst has to be removed from the product, alkaline waste water requires treatment, and free fatty acids and water interfere with the reaction. An alternative for basic catalysts is the use of enzymes. Enzymes like triacylglycerol lipase (EC 3.1.1.3) effectively catalyze the transesterification of a triglyceride in either aqueous or non-aqueous systems. The advantages of enzyme catalysis include a lower reaction temperature, ease of glycerol work-up, and the possibility to process oils with a high free fatty acid and water content [1,2].

Enzymatic conversions of vegetable oils or acids derived thereof may be carried out in a single phase, using a co-solvent or a biphasic liquid-liquid system. Both free, soluble enzymes as well as immo-

bilized enzymes may be applied. Well known examples are lipases from *Candida rugosa* and *Rhizomucor miehei* [2]. In the research reported here, a biphasic liquid-liquid approach is used using a free enzyme. Advantages of the use of free enzymes instead of immobilized enzymes are the absence of possible intraparticle mass transfer limitations that may limit the overall reaction rate [3] and a lower price [4]. A disadvantage for practical applications of free enzymes in terms of reutilization, as compared to immobilized enzymes, is the more elaborate recovery of the enzyme. Another possible disadvantage of free enzymes is a lower thermostability, both at storage and operating conditions [5]. Generally a higher reaction performance can be achieved with immobilized enzymes in non-aqueous media [6]. Despite this, under anhydrous conditions the free lipase of *Rhizomucor miehei* is stable and has a high esterification activity as compared to other free lipases [7]. The biphasic aqueous-organic system for enzymatic transesterification of triglycerides offers several advantages when compared to a single phase system. The presence of water during the transesterification reaction is known to increase the enzyme activity and to prevent hydrophobic substrate and/or product inhibition [8–10]. However, the use of water may lead to incomplete conversions due to equilibrium constraints and the formation of fatty acids. By using a biphasic concept, equilibrium constraint may be partly overcome

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### Nomenclature

BuOH	Butanol
C	Concentration ( $\text{gr L}^{-1}$ )
DG	Diglyceride
FABE	Fatty acid butyl ester
FFA	Free fatty acid
Gly	Glycerol
GlyOH	Glycerol hydroxyl group
k	Kinetic reaction constant ( $\text{mol L}_{\text{aq}} \text{L}_{\text{org}}^{-1} \text{g}^{-1} \text{s}^{-1}$ )
$k_i$	Kinetic deactivation constant ( $\text{mol L}_{\text{aq}} \text{L}_{\text{org}}^{-1} \text{g}^{-1} \text{s}^{-1}$ )
$K_i$	Inhibition constant ( $\text{mol L}^{-1}$ )
$K_m$	Michaelis Menten constant ( $\text{mol L}^{-1}$ )
MG	Monoglyceride
n	Number of moles (mol)
P	Partition coefficient
$R^2$	Coefficient of determination
RML	<i>Rhizomucor miehei</i> lipase
$r_p$	Formation rate of fatty acid butyl ester (FABE) ( $\text{mol L}_{\text{org}}^{-1} \text{min}^{-1}$ )
t	Time (minute)
V	Volume (L)
$V_{\text{max}}$	Maximum enzyme velocity ( $\text{mol L}_{\text{org}}^{-1} \text{s}^{-1}$ )

### Subscripts

A	1-butanol
Ac	Acyl
aq	Aqueous phase
DG	Diglyceride
eff(t)	Effective at t
enz	Enzyme
FABE	Fatty acid butyl ester
MG	Monoglyceride
org	Organic phase
S	Oil
TG	Triglyceride
0	Initial

and the rate of hydrolysis may be reduced. In addition, the use of a biphasic system also make glycerol and enzyme recovery from the reaction mixture easier and increases the possibility of reusing the enzyme [10,11].

Kinetics studies on the transesterification of vegetable oils with methanol using enzymes have been reported [12–14]. Most of the studies involve the use of an immobilized lipase. In some cases, an additional organic solvent is added and for these systems also a number of kinetic studies are available [15–17]. Al-Zuhair et al. studied vegetable oil methanolysis catalyzed by *R. miehei* lipase in *n*-hexane as the organic solvent. The results were modelled using a Ping Pong bisubstrate mechanism with competitive inhibition of methanol and oil [17]. Confidence factors of this model were not reported.

A limited number of (kinetic) studies on the transesterification of vegetable oils catalyzed by enzymes in oil-water biphasic systems have been reported (Table 1). The reaction is assumed to involve two-steps, viz hydrolysis of the triglyceride to free fatty acids (FFA) followed by a subsequent esterification of the FFAs with the alcohol. Competitive inhibition of oil and alcohol were observed [8,18,19]. However, kinetic modelling to obtain rate equations for the individual reactions was performed in one case only (Table 1). To the authors best knowledge, studies on the enzymatic transesterification of vegetable oils using 1-butanol in a biphasic aqueous-organic system have not been reported so far.

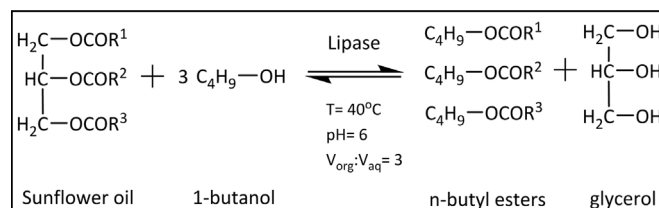


Fig. 1. Sunflower oil transesterification with 1-butanol.

Of relevance for this study are related enzyme catalyzed esterification reactions of fatty acids (oleic acid) and short chain acids with alcohols in biphasic systems. We have recently reported a kinetic model for the esterification of oleic acid 1-butanol using *R. miehei* lipase in a biphasic *n*-heptane-water system. The data were modelled using a Ping Pong mechanism with alcohol inhibition. The model included a constant partition coefficient of 1-butanol over the two phases. Parameter estimations were performed with high accuracy [20].

In this study we report a kinetic study on the sunflower oil transesterification with 1-butanol using a free lipase (*Rhizomucor miehei*) in aqueous-organic biphasic system given fatty acid butyl esters (FABE) and glycerol (Fig. 1). 1-Butanol is an interesting chemical for transesterification of vegetable oils because it can be obtained in a sustainable way via fermentation of biomass. This way it can also be made available in remote areas in developing countries. From a chemical point of view, the use of 1-butanol can be beneficial because of its greater solubility in the organic phase thereby facilitating the transesterification reaction that is assumed to take place in the organic phase.

In the first exploratory phase, the effect of substrate, product and enzyme concentration on the initial rate was determined. In the second stage, a kinetic study was performed for the reaction at variable oil intakes with all other parameters set at optimum values as determined during the exploratory studies. The experimental data were modelled using a Ping-Pong Bi-Bi mechanism.

## 2. Materials and methods

### 2.1. Materials

Commercial sunflower oil produced by Vandermoortele BV, Belgium was used as the substrate. Ethanol (absolute) and acetic acid (100%) were obtained from EMSURE®, 1-butanol (99%) and ethyl oleates (98%) were from Sigma-Aldrich. The *Rhizomucor miehei* lipase in solution ( $\geq 20,000$  Unit/g) was obtained from Sigma-Aldrich. *n*-Hexane (analytical reagent) was obtained from Lab-Scan. *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (98.5%), pentadecane (99%), and buffer compounds ( $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ , 99% and  $\text{NaH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$ , 98%) were obtained from Sigma-Aldrich. For GC-calibration analytical standards (palmitic, linoleic, oleic and stearic acid,  $\geq 99\%$ ) were obtained from Sigma-Aldrich, as well as ethyl palmitate ( $\geq 99\%$ ), ethyl linoleate ( $\geq 99\%$ ), ethyl oleate (98%), ethyl stearate ( $\geq 99\%$  for capillary GC), 1-oleoyl-glycerol ( $\geq 99\%$ ), 1,3-diolein ( $\geq 99\%$  for GC) and triolein.

### 2.2. Methods

#### 2.2.1. Experimental set up

Experiments were performed in a 300 mL batch reactor made of glass. The reactor was equipped with a heating jacket connected to a temperature controlled water bath. Stirring was provided by a twin agitator (Fig. 2). The reactor was pre-heated to  $40^\circ\text{C}$  before adding the substrates. Oil, alcohol and organic solvent were added

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