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A combined sorption and kinetic model for multiphasic ethyl esterification of fatty acids from soybean soapstock acid oil catalyzed by a fermented solid with lipase activity in a solvent-free system

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ABSTRACT

A low-cost enzymatic catalyst for biodiesel production can be produced by growing a lipase-producing organism in solid-state fermentation and then drying the solids after the fermentation. Recently, we cultivated *Burkholderia cepacia* on a mixture of sugar cane bagasse and sunflower seed meal and used the dried fermented solid to catalyze the esterification of fatty acids with ethanol in a solvent-free system (Soares et al., Fuel (2015) 159, 364–372). During the reaction, up to 30% of the reaction medium sorbed onto the dried fermented solid, with the sorbed medium having a composition different from that of the bulk phase. In the current work, we develop a combined sorption-kinetic model to describe the reaction kinetics in this system. The sorption of the medium components onto the fermented solid follows a multicomponent Langmuir isotherm, while the kinetic equation is expressed in terms of the composition of the sorbed phase. With a single set of parameters, the model adjusted well to the experimental results obtained by Soares et al. for three different ethanol to fatty acid molar ratios (1:1, 1.5:1 and 3:1), not only for the overall conversion profile, but also for the compositions of the bulk phase and the sorbed phase. The model has potential to be used to guide the scale-up of our system.

1. Introduction

There has been a recent surge of interest in using lipases to catalyze the production of biodiesel, as a "green" alternative to the traditional alkali-catalyzed route, with some large-scale enzymatic biodiesel plants having already been established [1–3]. However, although this enzymatic route yields a higher quality product and cleaner byproducts, thereby simplifying downstream processing [4–7], its widespread commercial implementation is prevented by the high cost of producing and immobilizing the lipases.

Over the last decade, we have been investigating a strategy for reducing the costs of lipase production: lipases can be produced by solid-state fermentation and then the fermented solid can be dried and used directly as the catalyst in either transesterification or esterification reactions [8–12]. This strategy avoids processing steps that are necessary when lipases are produced by submerged fermentation, namely the recovery of the lipases from the fermentation broth and their subsequent immobilization on a suitable support material. These advantages have prompted other researchers to use fermented solids for biodiesel production in transesterification [13,14] and esterification reactions [15].

The costs of lipase-catalyzed biodiesel production can be reduced further by using solvent-free systems. These systems not only avoid the need for recovery and recycling of the solvent, but also enable the use of smaller reaction vessels, compared to situations in which the substrates are dissolved in solvents such as *n*-heptane [8,11], *n*-hexane [16,17] or *tert*-butanol [14]. Recently, we described the first use of fermented solid to catalyze the solvent-free esterification of fatty acids with ethanol, with the fermented solid being produced by growing the bacterium *Burkholderia cepacia* on a mixture of sugarcane bagasse and sunflower seed meal [10].

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In a subsequent study, we showed that up to 30% of the total mass of the reaction medium sorbed onto the fermented solid during the process and that this sorbed phase had a composition that was quite different from that of the bulk phase [12]. Since this sorbed phase represents the immediate environment of the lipases held within the fermented solid, these results have implications for the modeling of the enzyme reactor. In fact, current models of the kinetics of lipase-catalyzed reactions in solvent-free media that describe the separation of the bulk medium into organic and aqueous phases do not recognize the existence of a sorbed phase with a unique composition [18,19]. Both Foresti et al. [18], who modeled the esterification of oleic acid with ethanol by a lipase immobilized on chitosan, and Krause and Fieg [19], who modeled the hydrolysis of methyl-octanoate by lipase B of *Candida antarctica* immobilized on poly-(methyl methacrylate) beads, expressed the kinetics of the immobilized lipase in terms of the composition of the organic phase that would be predicted for a liquid-liquid equilibrium based on the global reaction medium in the absence of specific sorption phenomena. These models are not appropriate for describing reactions catalyzed by a fermented solid that contains lipases; for this, a modeling approach that takes the sorption step into account is necessary.

In a related system, namely the hydrolysis of triolein by *Candida rugosa* lipase in an emulsion, Hermansyah et al. [20] expressed the substrate concentration at the oil-water interface as being related to the bulk concentration. In the current work, we adapted this idea, developing a combined sorption-kinetic model capable of describing the reaction profiles in our system. We did this by using a multicomponent Langmuir isotherm to describe the sorption of the medium components on the fermented solid and an equation for lipase kinetics that expresses the reaction rate as depending on the composition of the sorbed phase.

2. Mathematical model

2.1. Description of the experimental system

The system used by Soares et al. [10,12] consists of a reservoir containing a reaction medium that is pumped in a closed-loop system through a glass column packed with an air-dried fermented solid that contains lipases (Fig. 1, upper part). Soares et al. [12] produced these fermented solids by growing *Burkholderia cepacia* on a mixture of sugarcane bagasse and sunflower seed meal (50% each by mass, on a dry basis). These substrates had been milled and sieved to obtain particles ranging between 0.85 and 2.36 mm. The solid-state fermentation was done in 1000-mL Erlenmeyer flasks, each containing 40 g of milled dry substrate, moistened to 75% moisture (w/w, wet basis). with phosphate buffer solution (0.1 mol L^{-1}, pH 7.0). After inoculation, they were incubated at 29 °C for 72 h. After incubation, the fermented solids were dried to a moisture content of less than 10% (w/w on a wet basis) by blowing dry air at 25 °C through them and then stored at 4 °C before being used in the esterification reactions [12]. These solids had an esterification activity of 5.8 U per gram of dry fermented solid. This activity was measured using 70 mmol L^{-1} of fatty acid and 210 mmol L^{-1} of ethanol, with 1 U corresponding to the production of 1 μmol of ethyl ester per minute, under the assay conditions of [10].

The column had an internal diameter of 2.7 cm and a height of 21 cm and was maintained at 45 °C by a water jacket [10,12]. It contained 13.3 g of fermented solids (i.e. 12.0 g of bone dry fermented solids).

The reservoir (9 cm in height, with an internal diameter of 4.4 cm) was hermetically sealed [12]. It was loaded with ethanol and with 100 g of fatty acids from soybean soapstock acid oil. These substrates were mixed as received, there was no attempt to change

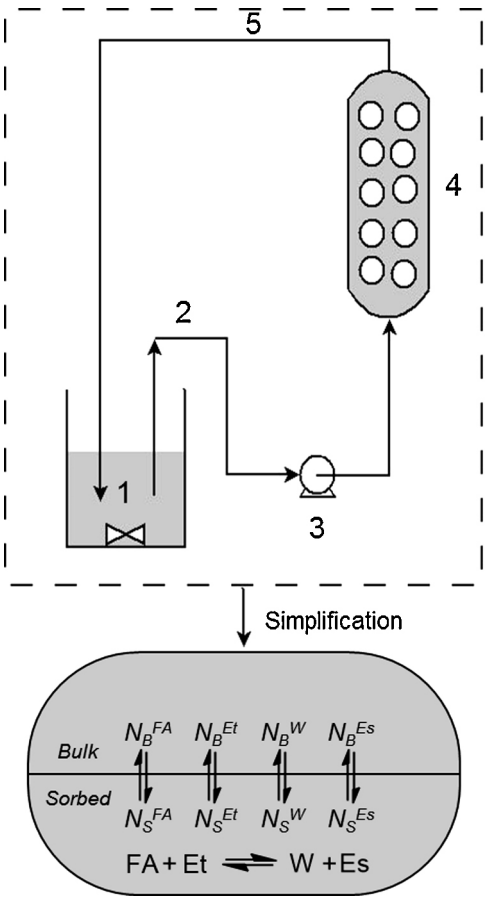

Fig. 1. The esterification system and how it was represented in the model. *N* denotes the number of moles of a component, the superscripts FA, Et, W and Es represent fatty acids, ethanol, water and ester, respectively, and the subscripts B and S represent the bulk phase and sorbed phase, respectively. The esterification reaction occurring in the reactor is also shown. Key: (1) reservoir; (2) feed of reaction mixture to the reactor; (3) peristaltic pump; (4) packed-bed reactor containing fermented solids; (5) removal of reaction mixture from the reactor.

Table 1
Initial global amounts of reaction components in the three experiments that Soares et al. [12] undertook with different initial molar ratios of ethanol to fatty acid[a].

Nominal initial molar ratio of ethanol to fatty acid	Time (h)	N_G^{FA}(mol)	N_G^{Et}(mol)	N_G^{W}(mol)	N_G^{Es}(mol)
1:1	0	0.360	0.365	0.026	0.000
1.5:1	0	0.362	0.491	0.035	0.005
3:1	0	0.361	0.981	0.054	0.012

[a] These global values were not reported directly in Soares et al. [12], rather, data were reported for the bulk reaction medium and sorbed phase. These global data represent the sum of these two phases.

the dissociation state of the fatty acid. Soares et al. [12] undertook three experiments at different initial molar ratios of ethanol to fatty acids, 1:1, 1.5:1 and 3:1, referred to as MR1:1, MR1.5:1 and MR3:1, respectively. Table 1 shows the initial amounts of fatty acids and ethanol in each of these experiments. The reservoir was stirred magnetically at 200 rpm, with reaction medium being pumped into the bottom of the column at a flow rate of 5 mL min^{-1}. The reaction medium leaving the top of the column drained back into the reservoir (Fig. 1, upper part).

For each molar ratio (i.e. MR1:1, MR1.5:1 and MR3:1), Soares et al. [12] performed six esterification reactions in which the reaction conditions were identical, but the harvesting times were different (0, 6, 12, 24, 36 and 48 h). At the harvesting time, the bulk