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Process Intensification of Enzymatic Fatty Acid Butyl Ester Synthesis Using a Continuous Centrifugal Contactor Separator

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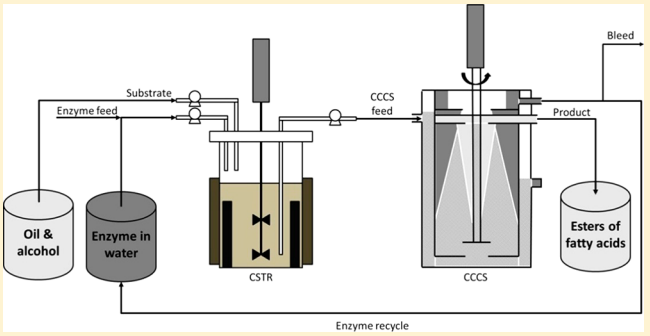
ABSTRACT: Fatty acid butyl esters were synthesized from sunflower oil with 1-butanol using a homogeneous *Rhizomucor miehei* lipase in a biphasic organic (triglyceride, 1-butanol, hexane)− water (with enzyme) system in a continuous setup consisting of a cascade of a stirred tank reactor and a continuous centrifugal contactor separator (CCCS), the latter being used for integrated reaction and liquid−liquid separation. A fatty acid butyl ester yield up to 93% was obtained in the cascade when operated in a once-through mode. The cascade was run for 8 h without operational issues. Enzyme recycling was studied by reintroduction of the water phase from the CCCS outlet to the stirred tank reactor. Product yield decreased over time to an average of 50% of the initial value, likely due to accumulation of 1-butanol in water phase, loss of enzyme due to agglomeration, and the formation of a separate enzyme layer.



1. INTRODUCTION

Biodiesel has become an important biofuel in recent years. It is renewable and can be used in existing compression ignition engines without substantial modifications.[1,2] The global annual production of biodiesel has increased dramatically in the past decade: from 2.4 billion liters in 2004 to 29.7 billion liters in 2014.[3] The biodiesel demand is expected to increase even more in the years ahead.

Biodiesel is composed of methyl esters of long chain fatty acids and is produced from vegetable or animal oils and fats.[4] Biodiesel production is typically performed using a base-catalyzed transesterification of the oil/fat with an alcohol.[4−6] Methanol is the most frequently used alcohol due to its low cost and the availability of globally accepted product specifications for methylesters.[5,7] However, recent studies have shown that esters from longer chain alcohols such as propanol and butanol may have some advantages compared to esters from methanol or ethanol.[7] For instance, the cetane number of biodiesel, a prime fuel quality indicator in diesel engines, increases when using higher alcohols instead of methanol.[7]

Increasingly, enzymes are being used for the transesterification of triglycerides as they have certain advantages over basic catalysts. These include a better compatibility with feeds with high free fatty acid (FFA) contents, no possibility for soap formation, simplified product workup, and a lower energy input.[1,4] However, higher costs and lower reaction rates limit their commercial use. One of the methods to allow for the enzymes to be recycled and to reduce costs is the use of immobilized enzymes. However, these are in general more expensive than the free enzymes, and diffusion limitation of reactants/products in the immobilized enzyme matrix may reduce the overall rate of individual reactions considerably.

In this study we have explored the use of a free enzyme in a biphasic aqueous−organic system (Figure 1). A biphasic approach allows for an efficient recycling of the enzyme after the reaction is complete by a simple separation of the organic product phase and the water phase with the free enzyme. The advantages compared to the use of immobilized enzymes are a potential reduction of the production costs and the absence of intraparticle diffusion limitations that may have a negative effect on product formation rates. In addition, the presence of water during the transesterification reaction is known to increase enzyme activity and to prevent hydrophobic substrate and/or product inhibition.[8−10] However, reactions in water only may lead to incomplete conversions due to equilibrium constraints and the formation of fatty acids. By using a biphasic aqueous−organic system, equilibrium constraint may be partly overcome and the rate of hydrolysis may be reduced.[10,11]

Biodiesel synthesis using homogeneous enzymes in biphasic aqueous−organic systems has been studied and modeled;[8,11−14]

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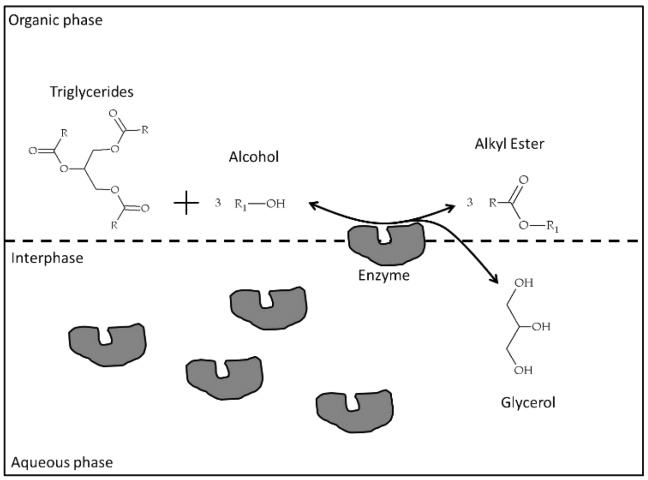

Figure 1. Simplified schematic representation of an enzymatic transesterification in an aqueous−organic biphasic system.

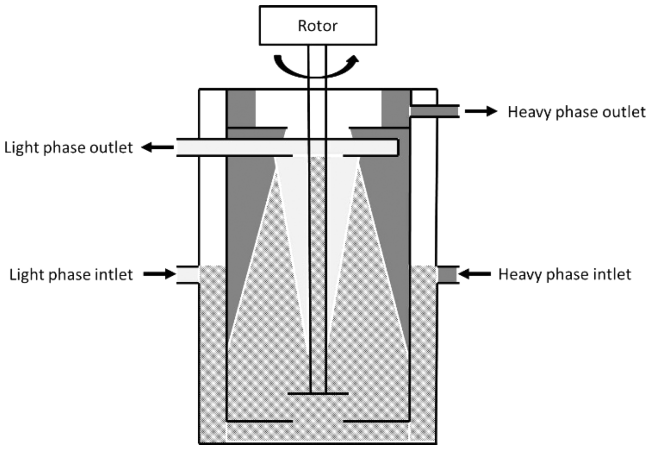

Figure 2. Continuous centrifugal contactor separator (CCCS). Reprinted with permission from ref 22. Copyright 2008 ACS Publications.

however, most of the studies were performed in a batch reactor setup. A fed-batch approach has been reported recently with stepwise alcohol addition to minimize alcohol inhibition of the enzyme.[15−17] Studies on biodiesel synthesis and related fatty acid esters using free enzymes in continuous units are limited. Price et al.[17−20] in collaboration with Novozymes performed studies at various process scales including in continuous stirred tank reactors using free enzymes for biodiesel synthesis. A kinetic model based on the Ping Pong Bi−Bi model was developed for biodiesel synthesis from rapeseed oil and methanol using a liquid formulation of the *Thermomyces lanuginosus* lipase (Callera Trans L).[18] In further studies, the model was used to compare the performance between fed batch reactors and continuous stirred tank reactors (CSTRs). The model predicted that a cascade of 5 CSTRs is required (with a combined residence time of 30 h) to reach a final biodiesel concentration within 2% of the 95.6 mass % achieved in a fed-batch operation for 24 h.[19] Finally, an experimental scale-up study using both fed batch and CSTRs for biodiesel synthesis using liquid *T. lanuginosus* lipase (NS-40116) was performed successfully.[20] These studies clearly show interest in the use of liquid enzyme formulations for biodiesel synthesis.

A continuous centrifugal contactor separator (CCCS) is a device that integrates both the intense mixing of two immiscible liquids and the subsequent separation (Figure 2).[21,22] The device basically consists of a hollow rotor positioned in a larger vessel. The two immiscible liquid phases are introduced in the annular zone between the outside of the rotor and the inside of the outer housing. Here, an efficient and fast mixing between the two phases occurs, which is advantageous for a two-phase liquid−liquid catalytic reaction to eliminate mass transfer limitations. The mixture is then transferred inside the centrifuge through a hole in the bottom plate, where the two phases are separated by centrifugal forces while moving upward, after which they leave the device through separate exits making use of an ingenious Weir system.[23] As such, the device is an interesting example of process intensification, acting both as a mixer and as a settler for biphasic liquid−liquid systems.

We have reported on the use of the CCCS device for esterification of fatty acids and the transesterifications of plant oils with alcohols.[23−26] For example, Kraai et al.[23] used the CCCS device for the transesterification of sunflower oil with methanol using an alkaline catalyst. Excellent biodiesel yields and volumetric production rates were obtained. Base-catalyzed biodiesel synthesis was further studied in the CCCS device, including optimization studies,[24] as well as ethyl ester synthesis from Jatropha oil and ethanol,[25] and synthesis and refining of methyl esters from sunflower oil using a cascade of two CCCS devices.[26] In addition to chemocatalysis, the esterification of a long chain fatty acid with 1-butanol in a biphasic organic−water system using an enzyme was also reported by us.[23]

However, enzyme-catalyzed transesterification reactions of plant oils with alcohols have not been reported using the CCCS, and this is the focus of the research reported in this manuscript. Here, we provide the proof of concept for the synthesis of butyl esters from sunflower oil and 1-butanol in a cascade of a continuous stirred tank reactor and a CCCS device using a homogeneous *Rhizomucor miehei* lipase. In this concept, the majority of the reaction is performed in the stirred tank reactor, whereas the CCCS device acts as a second reactor as well as an efficient liquid−liquid separator. The concept is of particular interest as it allows for recycling of the enzyme in the aqueous phase, potentially making such processes economically more viable (Figure 3).

In the first exploratory phase, the effects of the enzyme concentration and residence time in a continuous stirred tank reactor on the ester yield were determined. The results were modeled using a dynamic reactor model incorporating enzyme kinetics as recently determined by our group.[27] In the second part, a cascade concept using the continuous reactor and the CCCS in series was explored and the possibility for enzyme recycling was investigated. The activity of the enzyme was determined for an experiment using a 12 h runtime, and the cascade runs were modeled using first-principle engineering models.

2. MATERIALS AND METHODS

2.1. Materials. Commercial sunflower oil produced by Vandermoortele BV, Belgium, was used as the substrate. 1-Butanol (99%) and *R. miehei* lipase in solution (≥20 000 Unit.g^{-1}) were obtained from Sigma-Aldrich. *n*-Hexane (analytical reagent) was obtained from Lab-Scan. Chloroform-*d* (99.8 atom % D) was obtained from Sigma-Aldrich.

2.2. Methods. *2.2.1. Enzymatic Biodiesel Production in the Continuous Stirred Tank Reactor.* Experiments were performed in a glass reactor (300 mL) surrounded by a heating

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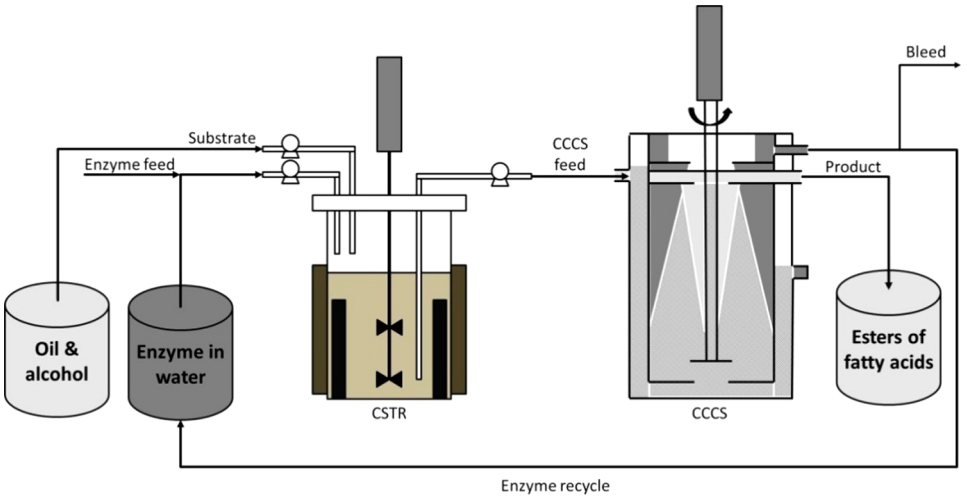

Figure 3. Scheme of the concept of biodiesel synthesis in a cascade of a continuous stirred tank reactor and a CCCS device with catalyst recycle.

jacket connected to a temperature-controlled water bath and equipped with a stirring device containing two turbines (Figure 4). For all experiments, a stirring speed of 800 rpm and a

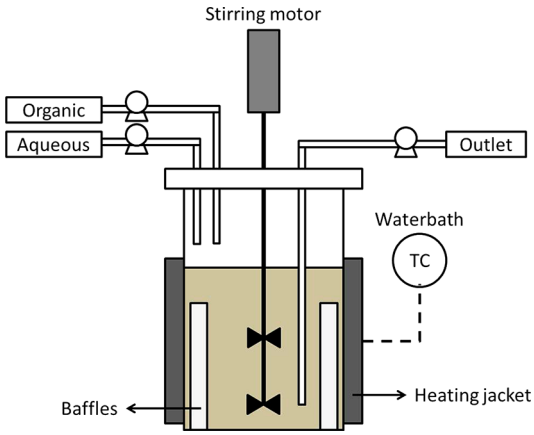

Figure 4. Schematic representation of the continuous stirred tank reactor.

temperature of 40 °C were used. The two liquid inlet streams (water and organic phase) were fed to the reactor using peristaltic pumps (Verderlab, Verder UK Ltd.). The level in the reactor was maintained at 200 mL total liquid volume by continuous removal of reactor content using a peristaltic pump (Verderlab, Verder UK Ltd.).

Three different residence times were applied (30, 60, and 90 min). The experiments were initiated by an initial batch reaction involving filling the reactor with hexane (150 mL) containing 40 $g \cdot L^{-1}$ sunflower oil and 15 $g \cdot L^{-1}$ 1-butanol (molar ratio oil to 1-butanol of 4.5) and an aqueous phase (50 mL) containing the enzyme (20−250 $g \cdot L^{-1}$). The reactor was heated to 40 °C under stirring, and the reaction was allowed to proceed for a time equal to the predetermined residence time. The continuous experiment was started by starting the feed and outlet pumps. For the experiment at a residence time of 60 min, the feed rates of the feed pumps were set at 2.5 $mL \cdot min^{-1}$ for the organic phase and 0.83 $mL \cdot min^{-1}$ for the aqueous phase (Table 1). The exit feed pump was set at 3.33 $mL \cdot min^{-1}$ to maintain a constant liquid volume in the reactor (200 mL). The feed rate was adjusted for experiments at other residence times.

Table 1. Experimental Conditions for the Experiments in the Continuous Stirred Tank Reactor and a Cascade with a Stirred Tank Reactor and a CCCS

variable	value	range
stirred tank reactor		
T (°C)	40	
stirring speed (rpm)	800	
τ (min) in stirred tank reactor experiments		30−90
τ (min) for cascade experiments	60	
organic feed rate ($mL \cdot min^{-1}$)[a]	2.5	
oil feed rate ($mL \cdot min^{-1}$)	0.109	
1-butanol feed rate ($mL \cdot min^{-1}$)	0.046	
aqueous feed rate ($mL \cdot min^{-1}$)[a]	0.83	
enzyme feed rate ($mL \cdot min^{-1}$)	0.104	
enzyme concentration ($g \cdot L^{-1}$)		20−250
liquid volume in reactor (mL)	200	
CCCS device		
T (°C)	40	
stirring speed (rpm)	1800	
Weir size (mm)	27.94	
liquid feed ($mL \cdot min^{-1}$)	3.33	
τ (min)	69	

[a]for experiments with a residence time of 60 min in the stirred tank reactor.

The runtime of each experiment was at least equal to three times the residence time. An overview of the experimental conditions is given in Table 1. Samples were taken from the outlet stream during the experiment every 15 min within the first hour and every 30 min afterward. The phases were separated using a separation funnel. The hexane and 1-butanol in the organic phase were removed using a rotary evaporator (60 °C, 300 mbar), and the remaining product was analyzed using ^1H NMR.

2.2.2. Enzymatic Biodiesel Production in a Cascade of a Continuous Stirred Tank Reactor and a CCCS. Experiments were performed in a cascade of two reactors consisting of a continuous stirred tank reactor as described above (Figure 4) and a subsequent CCCS device (Figure 2). The CCCS used in this study was a CINC V02 (350 mL geometric volume) equipped with a heating/cooling jacket connected to a temperature-controlled water bath and a high-mix bottom plate. The heating jacket encased the complete CCCS device so

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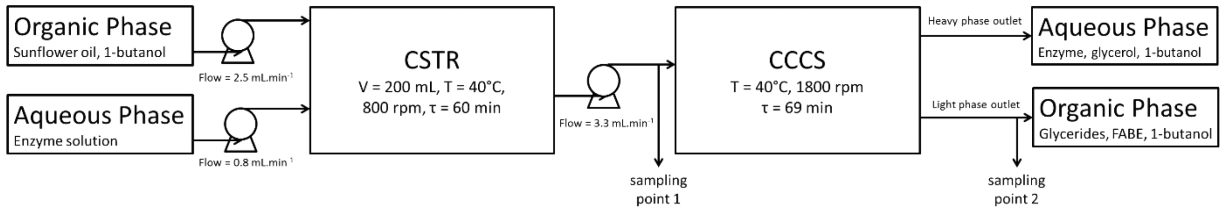

Figure 5. Schematic representation of the cascade with a stirred tank and CCCS in series.

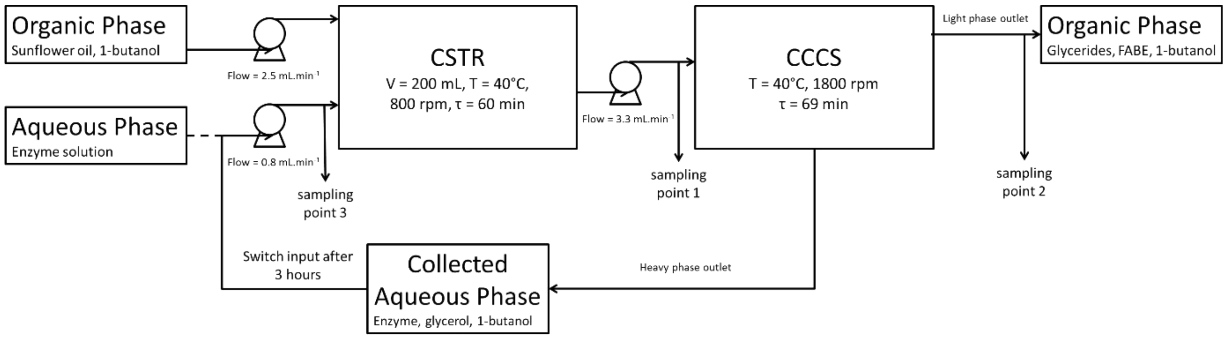

Figure 6. Schematic representation of the continuous cascade consisting of a stirred tank reactor and CCCS device with enzyme recycle.

that the annular, centrifugal, and outlet zones could all be temperature controlled by applying a high water flow at the desired temperature through the heating jacket. The two liquid inlet streams (water and organic phase) were fed to the continuous stirred tank reactor using peristaltic pumps (Verderlab, Verder UK Ltd.), while a peristaltic pump (Verderlab, Verder UK Ltd.) was used to remove the liquids from the stirred tank vessel. A schematic representation of the experimental setup is given in Figure 5.

In the first stage of experimentation, optimization of the Weir size, of particular relevance to obtain a good separation of the organic and aqueous phase in the outlet of the CCCS, was performed. For this purpose, experiments using three different Weir sizes that were available (23.5, 26.04, and 27.94 mm) were carried out. A representative feed stream consisting of a representative reaction product obtained from experiments carried out in the stirred tank reactor (vide supra) was fed into the CCCS device at a flow rate of 3.3 mL/min. The CCCS temperature was maintained at 40 °C, and stirring speed was set at 1800 rpm. The output of each liquid phase was collected, and the amount was measured using a volumetric cylinder. The separation performance for a particular Weir was determined by measuring the amounts of the two phases in each of the outlet streams.

The cascade experiments were performed by initially filling the stirred tank reactor with hexane (225 mL) containing 40 g·L^{-1} sunflower oil and 15 g·L^{-1} 1-butanol (oil to 1-butanol molar ratio of 4.5) and an aqueous phase (75 mL) containing 150 g·L^{-1} enzyme and stirred at 800 rpm and 40 °C for 1 h. After 1 h, 100 mL of this biphasic mixture was transferred to the CCCS. The actual experiment was then started by switching on the feed pumps to the stirred tank reactor and the intermediate feed pump to the CCCS. The runtime for each experiment was 8 h (τ_{CSTR} = 60 min; τ_{CCCS} = 69 min). The aqueous feed consisted of the enzyme solution in water. The organic feed consisted of the oil and butanol solution in hexane. The volumetric flow rates of the pumps were as follows: organic input, 2.5 mL·min^{-1}; aqueous input, 0.8 mL·min^{-1}; continuous reactor output/CCCS input, 3.3 mL·min^{-1}. The heavy and light phase outlets of the CCCS were collected and measured using a volumetric cylinder. The quality of the phase separation in the separation part of the CCCS device was determined visually and quantitatively by placing the outlet streams in volumetric cylinder for 1 h and then measuring the volumes of both liquid phases.

Samples of the mixed phase from the stirred tank reactor (Figure 5, sampling point 1) and the organic outlet phase of the CCCS (Figure 5, sampling point 2) were taken every hour and analyzed using ^{1}H NMR after removal of the solvents. An overview of experimental conditions is given in Table 1.

2.2.3. Experiments in the Cascade of a Continuous Stirred Tank Reactor and a CCCS with Enzyme Recycle. Enzyme recycle in the continuous stirred tank, CCCS, set up was done using the set up and conditions mentioned above. The system was run with fresh enzyme in aqueous phase for 3 h, based on experience, before using aqueous phase collected from CCCS heavy phase output as input of stirred tank reactor (Figure 6). Samples of mixed phase from stirred tank reactor (Figure 6, sampling point 1) and organic phase from CCCS (Figure 6, sampling point 2) were drawn every hour and analyzed using ^{1}H NMR after solvent removal. A sample of the aqueous phase was taken from sampling point 3 (Figure 6) every 2 h and analyzed for enzyme activity and protein, glycerol, and 1-butanol content. An overview of experimental conditions for the recycle experiments is given in Table 2.

2.3. Analytical Methods. The FABE yield was determined using ^{1}H NMR. Samples of 1 mL were taken from the CSTR and CCCS outlet streams. Absolute acetic acid (0.1 mL) was added to the samples from the CSTR outlet to inactivate the enzyme, and subsequently, the organic and aqueous layers were separated. The samples from the CCCS outlet were already phase separated and were analyzed as such. Hexane and 1-butanol were removed from the organic layer of all CSTR and CCCS samples using a rotary evaporator (60 °C, 300 mbar). A 50 μL amount of the hexane-and-1-butanol-free sample was mixed with 700 μL of $CDCl_3$ in an NMR tube. The mixture was analyzed using a 300 MHz NMR (Varian Inc.). The FABE yield was determined by comparing the intensity of quartet signal of

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Table 2. Experimental Conditions for the Experiments in a Continuous Stirred Tank Reactor and a Cascade with a Stirred Tank Reactor and a CCCS Including Enzyme Recycle

variable	value
stirred tank reactor (CSTR)	
T (°C)	40
stirring speed (rpm)	800
T_{CSTR} (min)	60
organic feed rate (mL·min^{-1})	2.5
oil feed rate (mL·min^{-1})	0.109
1-butanol feed rate (mL·min^{-1})	0.046
aqueous feed rate (mL·min^{-1})	0.83
enzyme feed rate (mL·min^{-1})	0.104
enzyme concentration (g·L^{-1})	150
liquid volume in the reactor (mL)	200
CCCS device	
T (°C)	40
stirring speed (rpm)	1800
Weir size (mm)	27.94
liquid feed rate (mL·min^{-1})	3.33
T_{CCCS} (min)	69

the CH_2 group of the ester end group (δ 4.1 ppm) with respect to the signal intensity of the methyl end group of fatty acids (δ 0.89 ppm).[25]

Lipase activity was determined using a method described by Kwon and Rhee,[28] while the protein concentration was determined gravimetrically using the TCA precipitation method. For this purpose, 1000 μL of the sample was added to 250 μL of TCA solution (from a stock solution consisting of 5 g of TCA diluted with 3.5 mL of distilled water) and incubated for 10 min at 4 °C. The mixture was centrifuged at 14 000 rpm for 5 min, and then the supernatant was separated for glycerol and 1-butanol concentration determination. The residue (proteins) was washed and centrifuged using cold acetone twice and then dried at 60 °C and weighted.

The glycerol and 1-butanol concentration in the recycled aqueous enzyme solution were determined using HPLC. Before injection, the sample was diluted 100 times using distilled water. The solution was injected into an HPLC (HP 1200 series) equipped with a Biorad organic column (Aminex HPX-87H, 60 °C) and UV and RI detector (HP 1260). The samples were run for 80 min with sulfuric acid (5 mM, 0.05 mL·min^{-1}) as the eluent. Glycerol and 1-butanol concentrations were determined by comparing peak intensities with a calibration curve made by using pure compounds.

2.4. Definitions. The residence time in the continuous stirred tank reactor was defined as the total liquid volume in the reactor ($V_{L,total}$) divided by the total liquid stream (organics plus water) entering the reactor per minute ($\varphi_{V,total}$); see eq 1 for details.

$$\tau_{CSTR} = \frac{V_{L,total}}{\varphi_{V,total}} \quad (1)$$

The residence time in the CCCS was defined similarly; see eq 2 for details.

$$\tau_{CCCS} = \frac{V_{L,vol}}{\varphi_{V,total}} \quad (2)$$

$V_{L,vol}$ was determined by measuring the liquid volume left in CCCS after closing the valves of the inlet and outlets of the CCCS after an experiment. The liquid was drained from a valve in the bottom of the CCCS and collected and measured using a volumetric cylinder.

The FABE yield is expressed in %-mol and determined by comparing the peak area of butyl ester group of the ester group of FABE (δ 4.1 ppm) with respect to signal intensity of the methyl end groups of the fatty acid chains (δ 0.89 ppm).

$$\text{FABE yield} = \frac{\text{butyl ester peak area}}{\text{methyl end group area}} \times 100\% \quad (3)$$

The reported FABE yield for a continuous experiment is the average FABE yield during steady state operation of the cascade.

The volumetric production rate of FABE is defined as the amount of FABE produced per volume liquid per time (kg·m^{-3}·min^{-1})

$$\text{volumetric production rate} = \frac{3\Phi_{oil} Y \left(\frac{MW_{FABE}}{MW_{oil}}\right) \rho_{oil}}{V_{L,vol}} \quad (4)$$

where Φ_{oil} = volumetric flow rate of the sunflower oil (m^3·min^{-1}), ρ_{oil} = oil density (kg·m^{-3}), Y = FABE yield (%·mol), $V_{L,vol}$ = liquid volume (m^3), MW_{FABE} = molecular weight of FABE (kg mol^{-1}), and MW_{oil} = molecular weight of oil (kg mol^{-1}).

For calculations of the volumetric production rate in the stirred tank reactor, the total liquid volume in the stirred tank reactor was taken. For reactions in the cascade, $V_{L,vol}$ is the sum of the total liquid volume in the stirred tank reactor and the CCCS device.

3. RESULTS AND DISCUSSION

3.1. Enzymatic Biodiesel Production in a Continuous Stirred Tank Reactor. Exploratory biphasic experiments with sunflower oil as a representative example of a pure plant oil and 1-butanol as the alcohol were done in a continuous stirred tank reactor (CSTR) to determine the optimum residence time and enzyme concentration. The experiments were performed at 40 °C with an organic to aqueous volume ratio of 3 and an oil to 1-butanol molar ratio of 4.5. These values were taken from optimized batch experiments performed earlier in our group.[27] An overview of experimental conditions is given in Table 1. The reactions were started up in batch mode, and at t = 0 the pumps were started. The FABE yield versus the runtime for two typical experiments at different residence times using an enzyme concentration of 20 g·L_{aq}^{-1} enzyme is given in Figure 7. At the lower residence time (30 min) an average of FABE yield of 12%-mol was obtained. Improved yields (22%-mol) were obtained by increasing the residence time to 60 min. The slight decrease in yield over time when using the 60 min residence time is likely related to the relatively short runtime. In this case, with the runtime larger than the residence time, i.e., larger than 60 min, a steady state appears to have been reached, while for a runtime shorter than the residence time the steady state may not have been reached already. This is confirmed by performing experiments at prolonged runtimes (up to 8 h, see the next section). Experiments in the CSTR were performed at least in duplicate, and the FABE yield at a certain time is the average of a duplicate or triplicate. The results as given in Figure 7 show that the reproducibility of the reactions is good.

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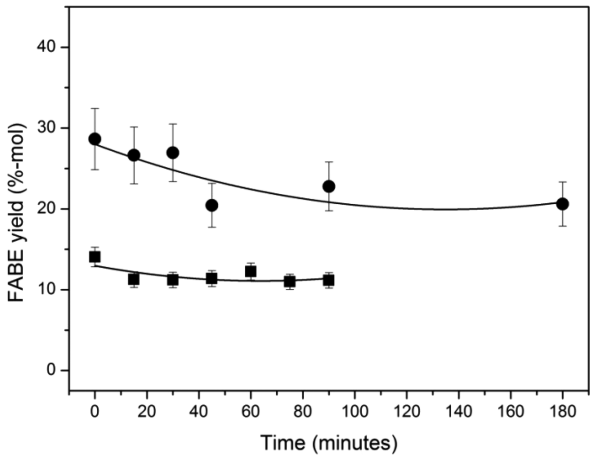

Figure 7. FABE yield versus time produced in a continuously stirred tank reactor using residence times of 30 (■) and 60 min (●) with an enzyme concentration of 20 $g{\cdot}L_{aq}^{-1}$. Experiments were done in duplicate; lines are for illustrative purpose only.

These preliminary experiments clearly show that the enzyme is capable of catalyzing the transesterification of pure plant oil with 1-butanol in a biphasic system, though optimization of process conditions will be required to improve the yields.

To further enhance the FABE yield, a number of experiments with higher enzyme concentrations and longer residence times were performed at otherwise similar conditions (Table 1), and the results are shown in Figure 8. The FABE yield is a clear

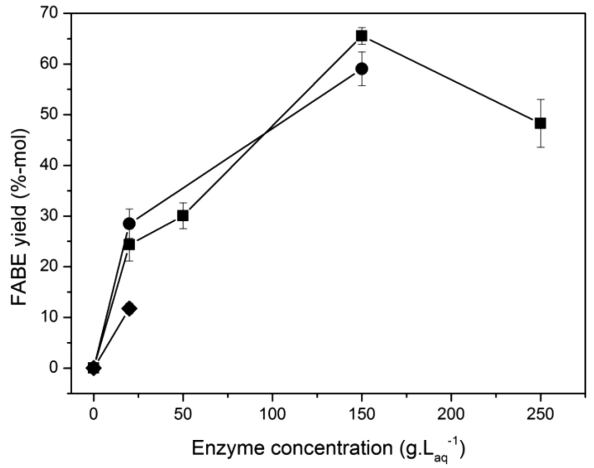

Figure 8. Average product yield versus enzyme concentration at different residence times for reactions in the CSTR: (♦) 30 (only a single point was measured), (■) 60, and (●) 90 min.

function of the enzyme concentration (Figure 8), and a clear optimum at about 150 $g{\cdot}L_{aq}^{-1}$ is visible when using a residence time of 60 min. This maximum is most likely related to the available interfacial area in relation to the amount of enzyme. The enzymatic reaction is known to be an interfacial reaction, with the enzyme residing in the water phase and the oil substrate in the organic phase (Figure 1). When considering the volumetric phase ratio used in the experiment (V_{org}/V_{aq} = 3), the biphasic system is best described as a reverse micellar system,[29] with the enzyme contained in an aqueous droplet. At an enzyme concentration of about 150 $g{\cdot}L_{aq}^{-1}$, the interface surface is likely fully covered by enzyme and a further increase does not lead to higher reaction rates.[10] The actual reduction in the activity of the enzyme using a 250 $g{\cdot}L_{aq}^{-1}$ enzyme concentration may be caused by enzyme agglomeration as well as a higher extent of enzyme inactivation by 1-butanol due to a lower effective water content in the reverse micelle.[30]

The highest average FABE yield within the experimental window was about 65.5%-mol, obtained when using a residence time of 60 min. Further optimization studies, e.g., by prolonging residence times and the use of other phase ratio's, were not performed as a FABE yield of 65.5%-mol was considered sufficient with respect to subsequent experimentation in a cascade of a stirred tank reactor and a CCCS device.

The volumetric production rate of the experiment using 150 $g{\cdot}L_{aq}^{-1}$ of enzyme calculated using eq 4 was 0.25 kg $FABE{\cdot}m^{-3}{\cdot}min^{-1}$ when using a 90 min residence time, and 0.38 $kg{\cdot}m^{-3}{\cdot}min^{-1}$ when using a residence time of 60 min. These volumetric production rates are slightly lower than those reported by Price et al.[17] for a *T. lanuginosus* lipase (Callera Trans L) in a fed batch reactor (0.47 $kg{\cdot}m^{-3}{\cdot}min^{-1}$).

3.2. CSTR Modeling. The experimental data obtained in the CSTR reactor (Figure 8) were modeled using the mass balances in combination with the known enzyme kinetics for sunflower oil transesterification with 1-butanol using the homogeneous lipase (*R. miehei*) in an aqueous−organic biphasic system (Ping Pong Bi Bi mechanism with non-competitive inhibition by 1-butanol and a term for irreversible enzyme deactivation during reaction).[27] In the modeling of the reactors, any influence of mass transfer limitations on the performance was neglected. The time scale of the experiments is of the order of tens of minutes or even hours. The mass transfer coefficient, $k_l a$, of intensively stirred reactors typically is on the order of 0.01 1/s or larger. This translates to a time scale for mass transfer of 100 s, or less, which is much smaller than that of the experiments.

3.2.1. Model Development. The continuous stirred tank experiments were operated in two stages. In a first stage the reactor was operated in batch mode during a time $0 \le t \le t_{batch}$. After this initial batch stage the operation in actual continuous mode started by switching on the organic and aqueous feed pumps and outlet pump.

The enzyme in the system is confined to the aqueous phase and is subject to deactivation according to first-order kinetics.[27]

$$(r_{enz})_{deactivation} = k_{i,enz} C_{enz,act} \tag{5}$$

where $C_{enz,act}$ denotes the active enzyme concentration in the aqueous phase. During the initial operation of the reactor in batch mode the amount of active enzyme decreases according to

$$\frac{dC_{enz,act}}{dt} = -k_{i,enz} C_{enz,act} (0 \le t \le t_{batch})$$
$$(C_{enz,act})_{t=0} = C^{0}_{enz,act} \tag{6}$$

In the second stage, where the reactor is operated in the continuous stirred tank mode, the amount of active enzyme varies in time due to the addition of active enzyme with the inflowing aqueous stream, $\phi_{v,aq,in} C^{in}_{enz,act}$, the outflow with aqueous stream, $\phi_{v,aq,out} C_{enz,act}$ and the decrease caused by deactivation $-k_{i,enz} C_{enz,act} V_{aq}$. Thus, the amount of active enzyme in the continuous stirred tank reactor is described by

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$$V_{aq}\frac{dC_{enz,act}}{dt} = \phi_{v,aq}(C^{in}_{enz,act} - C_{enz,act}) - k_{i,enz}C_{enz,act}V_{aq}(t \geq t_{batch})$$
$$(C_{enz,act})_{t=0} = C^{0}_{enz,act} \quad (7)$$

Equations 6 and 7 can be solved for the concentration of active enzyme

$$0 < t \leq t_{batch}: C_{enz,act} = C^{0}_{enz,act}\exp[-k_{i,enz}t] \quad (8)$$

$$t > t_{batch}: C_{enz,act} = C^{0}_{enz,act}\exp\left[-k_{i,enz}t_{batch} - (1 + k_{i,enz}\tau_{aq})\frac{t - t_{batch}}{\tau_{aq}}\right] + C^{in}_{enz,act}\frac{1 - \exp\left[-(1 + k_{i,enz}\tau_{aq})\frac{t - t_{batch}}{\tau_{aq}}\right]}{1 + k_{i,enz}\tau_{aq}} \quad (9)$$

The buildup of the product, acyl butyl ester, in the organic phase of the reactor can be described by the differential equations

$$V_{org}\frac{dC_{P,org}}{dt} = r_P V_{org}(0 \leq t \leq t_{batch})$$
$$V_{org}\frac{dC_{P,org}}{dt} = \phi_{v,org}(C^{in}_{P,org} - C_{P,org}) + r_P V_{org}(t \geq t_{batch})$$
$$(C_{P,org})_{t=0} = C^{0}_{P,org} = 0 \quad (10)$$

where again the two stages of batch and continuous operation of the reactor have to be taken into account. Previously, we established the production rate of acyl butyl ester by the enzymatic transesterification of triglycerides with *n*-butanol in an aqueous−organic two-phase system based on the Ping Pong mechanism as[27]

$$r_P = \frac{k_{enz}C_{enz,act}}{\left(1 + \frac{K_{m,S}}{C_{S,org}}\right)\left(1 + \frac{C_{A,org}}{K_{I,A}}\right)} \quad (11)$$

where $C_{S,org}$ denotes the acyl concentration in the organic phase and $C_{A,org}$ the butanol concentration also in the organic phase.

Similarly, we can write the balance for the amounts of acyl groups, S, in the reactor

$$V_{org}\frac{dC_{S,org}}{dt} = -r_P V_{org}(0 \leq t \leq t_{batch})$$
$$V_{org}\frac{dC_{S,org}}{dt} = \phi_{v,org}(C^{in}_{S,org} - C_{S,org}) - r_P V_{org}(t \geq t_{batch})$$
$$(C_{S,org})_{t=0} = C^{0}_{S,org} \quad (12)$$

The number of moles of butanol in the reactor, N_A, is given by

$$N_A = V_{org}C_{A,org} + V_{aq}C_{A,aq} \quad (13)$$

and its variation by

$$\frac{dN_A}{dt} = -r_P V_{org}(0 \leq t \leq t_{batch})$$
$$\frac{dN_A}{dt} = \phi_{v,org}(C^{in}_{A,org} - C_{A,org}) - \phi_{v,aq}C_{A,aq} - r_P V_{org}(t \geq t_{batch})$$
$$(N_A)_{t=0} = N^{0}_{A,org} \quad (14)$$

where the amount of acyl groups is written in terms of its concentration in the organic phase, $C_{S,org}$, and the amount of butanol as its total number of moles in the reactor, N_A, because butanol is present in both the organic and the aqueous phases.

For the conditions used here it is reasonable to assume that the concentrations of butanol in the aqueous and organic phases are always at equilibrium. Then the concentration of butanol in the organic phase, $C_{A,org}$, is related to N_A via $C_{A,org} = N_A/(V_{aq}/P + V_{org})$ and eq 14 can be rewritten explicitly in terms of $C_{A,org}$

$$\frac{dC_{A,org}}{dt} = -r_P\frac{R_V P}{1 + R_V P}(0 \leq t \leq t_{batch})$$
$$\frac{dC_{A,org}}{dt} = \frac{1}{1 + R_V P}\left[R_V P\left(\frac{C^{in}_{A,org} - C_{A,org}}{V_{org}/\phi_{v,org}} - r_P\right) - \frac{C_{A,org}}{V_{aq}/\phi_{aq}}\right](t \geq t_{batch})$$
$$(N_A)_{t=0} = N^{0}_{A,org} \quad (15)$$

where $R_V = V_{org}/V_{aq}$ denotes the volume ratio of the organic and aqueous phases.

The observable is the yield of FABE which was defined as

$$\text{yield} = \frac{C_P}{C_P + C_S} \times 100\% \quad (16)$$

3.2.2. Model Results. The parameters used for the modeling activities are provided in Table 3.

Table 3. Parameters Used in the Modeling Activities

parameters	value
experimental conditions	
V_{org}	0.15 L
V_{aq}	0.05 L
$C_{C,org}{}^{0}$	40 g·L_{org}^{-1}
kinetic parameters	
k_{enz}	0.794 × 10^{-3} mol L_{org}^{-1}·g^{-1}·L_{aq}·s^{-1}
$k_{i,enz}$	0.007 s^{-1}
$K_{m,S}$	0.335 mol L_{org}^{-1}
$K_{I,A}$	0.0715 mol L_{org}^{-1}
physical properties	
P	0.6 [(mol_A/L_{org})]/[(mol_A/L_{aq})] = ($C_{A,org}/C_{A,eq}$)equilibrium
$M_A = M_{butanol}$	74.1 g mol^{-1}
$M_S = M_{oil}$	885 g mol^{-1}

The modeled concentration profiles of the substrates and the products as well as the enzyme concentration versus the time for an experiment in the CSTR with a residence time of 90 min and an inlet enzyme concentration of 20 g·L^{-1} is given in Figure 9. Clearly, the nonstationary behavior at the startup of the reactor in batch mode is visible. Of interest is the modeled steady state concentration of the enzyme, which is about 16.5 g·L^{-1}, due to enzyme deactivation; this value is lower than the inlet enzyme concentration (20 g·L^{-1}).

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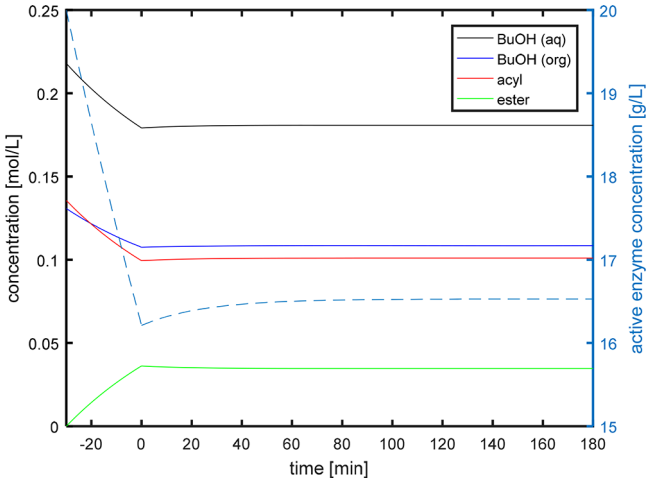

Figure 9. Modeled concentrations versus runtime for the CSTR experiments calculated with τ = 60 min and C^0_{enz} = 20 g·L^{-1}. Solid lines, reagents and products; dashed line, enzyme concentration on the right-hand axis.

The modeled FABE yields in the steady state versus the enzyme concentration for various experiments at different residence times are given in Figure 10.

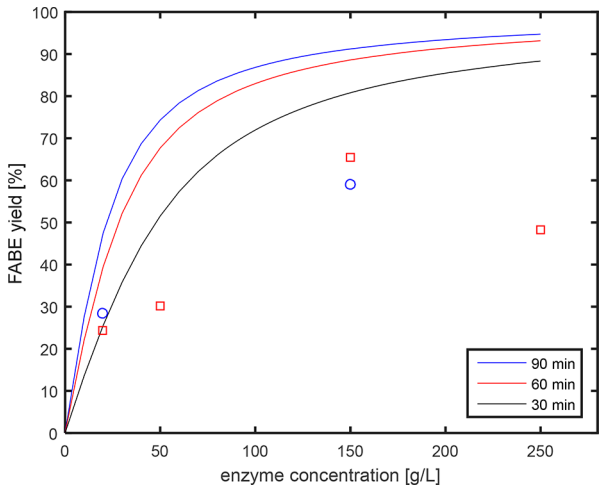

Figure 10. FABE yield vs enzyme concentration at three residence times for the CSTR experiments. Lines: calculated. Symbols: experimental data, available only for τ = 60 (red symbols) and 90 min (blue symbols), respectively.

The model lines predict that the FABE is a function of the enzyme concentration, with higher concentrations leading to higher FABE yields. In addition, it also clearly shows that, as expected, the residence time is of importance and highest FABE yields are attained at the highest residence times, However, the effect of changing the residence times from 60 to 90 min is by far less than when going from 30 to 60 min, particularly when considering intermediate enzyme concentrations (50−150 g·L^{-1}).

The experimental data points are also given in Figure 10. Clearly, the fit between model and experiments at low enzyme concentrations is reasonably good, whereas the deviation between experiment and model is considerable at the highest enzyme concentration. In addition, the model predicts a steady increase in the FABE yield versus enzyme concentration, whereas the experimental point at higher enzyme concentrations (τ of 60 min) shows a reduction. The latter may be explained by considering that the kinetic model has only been derived for enzyme concentrations below 200 g·L^{-1}.

Possible explanations for the deviation between the model and the experimental data points are related to the actual enzyme concentration in the CSTR reactor. It is well possible that the shear forces in this device are higher than for the setup used to determine the enzyme kinetics, and these are known to affect the deactivation rate of the enzyme. In addition, the formation of a small amount of a third layer between the glycerol and the ester layer was observed experimentally which may be enriched in enzymes due to agglomeration.[31,32] Both effects will lead to an effective reduction of the enzyme concentration in the CSTR, leading to reduced FABE yields. To gain some insights into the importance of these effects, the effect of higher levels of enzyme deactivation and effective removal of the enzyme to a third phase were modeled using the following equations

$$k_{i,\mathrm{enz,optimized}} = k_{i,\mathrm{enz}} F_1 \tag{16a}$$

$$C^0_{\mathrm{enz,act,optimized}} = C^0_{\mathrm{enz,act}} F_2 \tag{17}$$

The experimental point with an enzyme concentration of 250 g·L^{-1} is neglected in the optimization. However, the number of data points proved to be insufficient to obtain reliable values for the parameters F_n (n = 1,2). The result for a manual optimization is given in Figure 11. As such, it seems that the experimental data can be modeled using a 3 times higher value for the kinetic constant of enzyme deactivation and by halving the amount of enzyme due to the formation of a third layer with inactive enzyme.

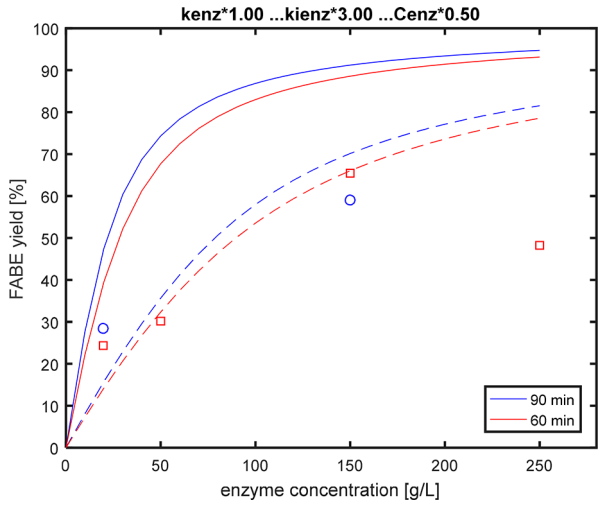

Figure 11. FABE yield vs enzyme concentration at three residence times for the CSTR experiments, see Figure 10. Dashed lines: calculated with manual optimized kinetics.

3.3. Enzymatic Biodiesel Production Using a Cascade of a Continuously Stirred Tank Reactor and a CCCS.

3.3.1. Phase Separation Optimization in the CCCS. Before performing actual enzymatic reactions in the CCCS device, the phase separation performance for the device regarding the organic and aqueous phase outlets was investigated. This is a critical performance indicator, as it is essential to have good separation between the two outlet liquid streams, meaning that

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the organic phase should not contain significant amounts of the aqueous phase and vice versa. Phase separation optimization experiments were carried out using a representative reaction product of experiments carried out in the stirred tank reactor containing glycerides, fatty acids, butyl ester and 1-butanol in hexane (organic phase), and enzyme, glycerol, and 1-butanol in water (aqueous phase). The inlet flow rate of the CCCS feed was set at 3.3 mL·min^{-1}. Separation performance is known to be a function of the size of certain rings in the top of the CCCS device (Weir size). When using a Weir size of 23.49 mm, as suggested by the supplier based on among others density differences between the two phases to be separated, separation performance was not on par. A small amount of aqueous phase was present in light phase output, while a significant amount of the organic phase was found in the heavy phase output (Table 4). Optimization of the Weir size revealed that good separation

Table 4. Separation Performance of the CCCS for various Weir Sizes

	light phase output		heavy phase output	
Weir size (mm)	organic phase (%-vol)	aqueous phase (%-vol)	organic phase (%-vol)	aqueous phase (%-vol)
23.49	93.9 ± 0.6	6.1 ± 0.6	27.1 ± 4.0	72.9 ± 4.0
26.03	93.9 ± 0.2	6.1 ± 0.2	24.3 ± 4.3	75.7 ± 4.3
27.94	98.3 ± 1.4	1.7 ± 1.4	7.1 ± 4.2	92.9 ± 4.2

performance was obtained with a size of 27.94 mm, though quantitative separation is not possible, possibly due to the presence of the enzyme at the interface, affecting surface-related properties (e.g., surface tension).

3.3.2. Experimental Studies in a Cascade of a Stirred Tank Reactor and a CCCS Device in Series. Initial experiments in the continuous stirred tank reactor gave a FABE yield of 65.5%-mol when using 150 g·L_{aq}^{-1} of enzyme and 60 min residence time (vide supra). To further enhance the conversion and to separate the two outlet streams, a CCCS device was positioned in series after the stirred tank reactor. A schematic representation of the continuous setup is given in Figure 6; relevant process parameters are given in Table 1. An experiment was started by performing a batch-type experiment in the stirred tank reactor. After a predetermined time, part of the content of the stirred tank reactor was transferred to the CCCS device. At this time the feed pumps to the stirred tank reactor and the CCCS were started, and this was actually the start of an experiment. As such, these experiments were carried out in a once-through mode without enzyme recycle. Operational issues were not encountered for the 8 h runs. Samples were taken periodically from the outlet of the stirred tank reactor and the outlet of the CCCS device (organic phase) and analyzed. The average FABE yield versus runtime for several 8 h runs at both sampling points is given in Figure 12.

The FABE yield in the outlet of the stirred tank reactor varies between 63.4%-mol and 77.9%-mol. Of interest is to see that the FABE yield in the outlet of the cascade is on average higher than 90%-mol, and values of 93.9%-mol were observed. These findings indicate that besides allowing for an efficient separation of the outlet streams the CCCS also acts as a reactor, and on average a 16.5%-mol FABE yield is obtained in the device (Figure 12). As such, we have shown the proof of principle for the continuous synthesis of FABE in a cascade of a stirred tank reactor and a CCCS device with integrated liquid−liquid separation to give FABE in yields up to 90%-mol.

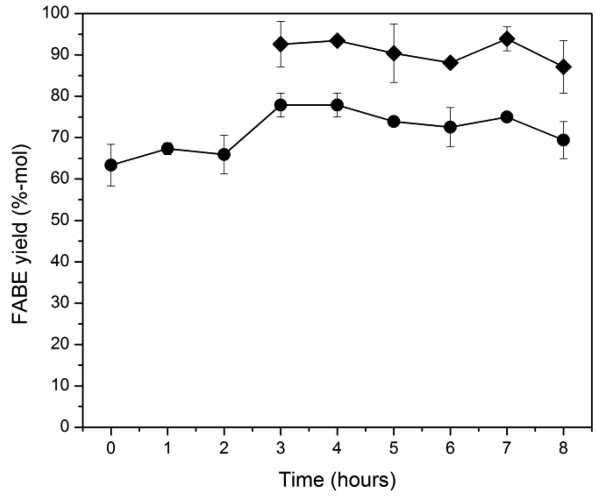

Figure 12. FABE yield for a cascade experiment using a continuous stirred tank reactor coupled with the CCCS: (●) outlet of the stirred tank reactor and (♦) outlet of the CCCS.

The volumetric production rate of FABE in the cascade was calculated using eq 4 and found to be 0.23 kg·m^{-3}·min^{-1}. This value is lower than that of the CSTR alone (0.38 kg·m^{-3}·min^{-1}, which is due to the by far higher total liquid volume of the two reactors in the cascade experiment (460 mL) compared to that of the CSTR alone (200 mL). The volumetric production rate is much lower than that reported by Kraai et al. for a base-catalyzed biodiesel synthesis reaction,[24] which was 61 kg·m^{-3}·min^{-1} using a standalone CCCS device. These differences are due to the by far lower intrinsic reaction rates of enzymes compared to base catalysts for biodiesel synthesis.[33]

3.4. Effect of Enzyme Recycle on Product Yield. Recycle of the homogeneous enzyme is of high importance to reduce the cost of enzymatic biodiesel production.[20] The concept using a biphasic liquid−liquid system with the enzyme having a preference for the water phase allows in theory for efficient enzyme recycle (Figure 3). The effect of enzyme recycle was experimentally investigated by reintroducing the aqueous phase outlet of the CCCS device back to the continuously stirred tank reactor. The experiment was started by performing a reaction for 3 h in the cascade at similar conditions as the cascade experiment discussed above (Table 2). During this period, the aqueous stream from the CCCS outlet containing the enzyme was collected. Subsequently, this enzyme containing recycle stream was reintroduced into the stirred tank reactor, and the feeding of fresh enzyme was ceased (Figure 6). After enzyme recycle was started, the experiment was run for another 9 h. Samples to determine the product yield were taken from CSTR outlet/CCCS inlet (sampling point 1, Figure 6) and from the CCCS light phase outlet (sampling point 2, Figure 6), while samples for enzyme activity analysis were taken from the aqueous phase inlet stream of CSTR (sampling point 3, Figure 6). The results are given in Figure 13. It can be seen that the product yield in the exit of the cascade decreased steadily during the recycle experiments.

To gain insight into this reduction in activity over the runtime of the experiments, the enzyme activity and the protein content of the feed inlet stream to the stirred tank reactor were measured and the results are given in Figure 14. It is clear that the protein content is reduced when starting the recycle stream (t = 3 h) to about 80% of the original value and then remains constant (except for the drop between 10 and 12 h runtime).

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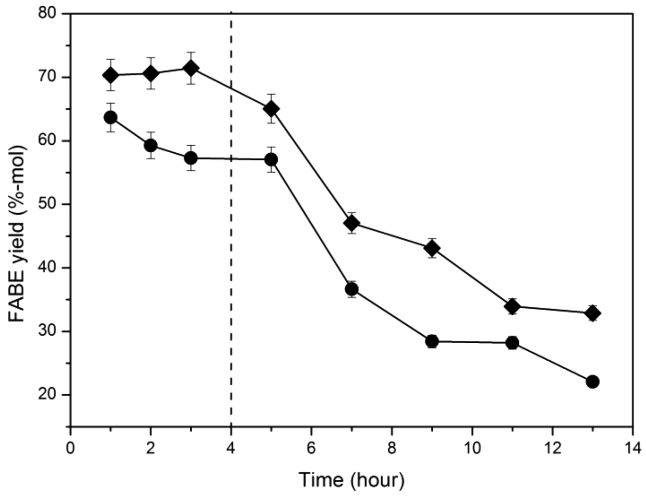

Figure 13. Effect of enzyme reuse on product yield of continuous reactor output (●) and continuous reactor coupled with CCCS output (♦). Enzyme reuse started at the fourth hour (dash line).

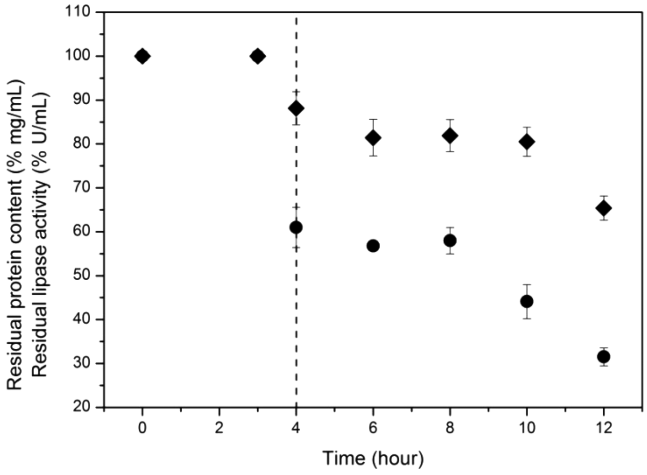

Figure 14. Residual protein content (♦) and lipase activity (●) during enzyme reusability experiment. Enzyme reuse started at the fourth hour (dash line).

The explanation for this reduction is accumulation of enzyme in an (visually observed) interphase layer between the aqueous and organic phase in the enzyme recycle collection vessel. The formation of such interphase layers enriched in enzymes has been reported for biphasic liquid−liquid systems in the literature.[31,32]

The lipase activity of the remaining enzyme in the recycle stream was also determined, and also a reduction in activity was observed when starting enzyme recycle (Figure 14). The activity was about constant between runtime 4 and 8 h but reduced sharply afterward. The initial drop in activity is partly due to enzyme loss in the interphase layer, but given the higher reduction in activity compared to protein content (Figure 14), other factors play a role as well. Possibilities are both inhibition of the enzyme by 1-butanol and irreversible enzyme deactivation. Indeed, both factors were observed in batch studies when using this particular biphasic enzyme system, and both were quantified using kinetic modeling.[27]

To determine the extent of 1-butanol inhibition, the amount of 1-butanol in the recycle stream was measured experimentally and the results are given in Figure 15. After a run time of 3 h,

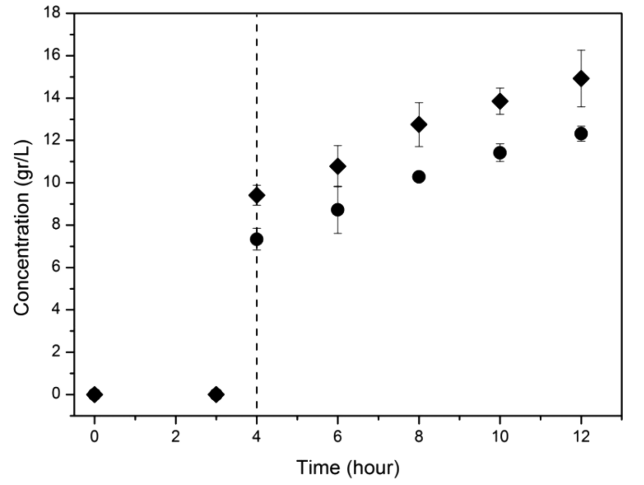

Figure 15. Glycerol (♦) and 1-butanol (●) accumulation in aqueous phase during enzyme recycle experiments. Enzyme reuse started at the fourth hour (dash line).

the amount of 1-butanol in the recycle stream increased due to the start of the recycle stream. This increase is caused by the fact that 1-butanol (i) is fed in excess to the oil to the cascade and as such is not quantitatively converted and (ii) has a certain solubility in the aqueous stream. As such, 1-butanol inhibition of the enzyme plays a role and will lead to a reduction in the FABE during the recycle experiments. In addition, the glycerol formed during reaction has a higher affinity for water than for the organic product phase and as such accumulates in the aqueous recycle stream (Figure 15). The presence of substantial amounts of glycerol may also affect the distribution of 1-butanol between the aqueous and the organic product stream. A higher affinity of 1-butanol in the aqueous phase is expected at higher glycerol concentrations, which will lead to higher levels of enzyme inhibition by 1-butanol.

The trends in Figures 13 and 14, i.e., a reduction in FABE yield, may be due to (i) accumulation of enzyme in a separate layer in the enzyme recycle vessel, (ii) 1-butanol inhibition, and (iii) possibly also some irreversible enzyme deactivation (as observed in batch studies). The residual enzyme activity as given in Figure 14 was modeled, and the results are given in the next paragraph.

3.5. Modeling of the Enzyme Activity for the Cascade Experiment with Enzyme Recycle. A model was developed for the concentration of active enzyme in the system for the experiment where the effects of enzyme recycling on the product yield were studied. The enzyme is again assumed to deactivate according to first-order kinetics as shown in eq 5. These experiments are characterized by 4 consecutive stages concerning the way the reactors are operated.

In the first stage the stirred tank reactor (STR) is filled with the two-phase reaction mixture and operated in batch mode over the time interval of $0 \le t \le t_{batch}$. The amount of active enzyme decreases according the first-order rate law

$$V_{aq,str}\frac{dC_{str}}{dt} = -k_{i,enz}C_{str}V_{aq,str}(0 \le t \le t_{batch}) \quad (18)$$

In this section the subscripts enz,act and aq are dropped from the symbol C for shortness, and all concentrations denote the concentrations of the active enzyme in the aqueous phase.

In the second stage, an amount $V_{ccs,0}$ of the reaction mixture is transferred from the STR to the continuous centrifugal

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contactor separator (CCCS). The STR is from now operated in a continuous mode with its aqueous feed stream containing fresh enzyme at a concentration of C_{str}^{in}, and its exit flow is input to the CCCS. This way the CCCS is gradually filling up while operating in a semibatch mode over the time interval of $t_{batch} \leq t \leq t_{fill}$. The concentrations follow from

$$V_{aq,str}\frac{dC_{str}}{dt} = \phi_{v,aq}(C_{str}^{in} - C_{str}) - k_{i,enz}C_{str}V_{aq,str} \quad (t_{batch} < t \leq t_{fill}) \tag{19}$$

$$\frac{dV_{aq,cccs(t)}C_{cccs}}{dt} = \phi_{v,aq}C_{str} - k_{i,enz}C_{cccs}V_{aq,cccs(t)} \quad (t_{batch} < t \leq t_{fill}) \tag{20}$$

where the volume of the aqueous phase in the CCCS increases over time according to $V_{aq,ccs(t)} = V_{aq,ccs,0} + \phi_{v,aq}(t - t_{batch})$.

In the third stage, both the STR and the CCCS are completely filled and operated in a continuous mode. The exit of the STR is input to the CCCS, and the aqueous exit of the CCCS is accumulated in a storage vessel (SV). The amounts of active enzyme in the STR, CCCS, and the SV follow from

$$V_{aq,str}\frac{dC_{str}}{dt} = \phi_{v,aq}(C_{str}^{in} - C_{str}) - k_{i,enz}C_{str}V_{aq,str} \quad (t_{fill} < t \leq t_{switch}) \tag{21}$$

$$V_{aq,cccs}\frac{dC_{cccs}}{dt} = \phi_{v,aq}(C_{str} - C_{cccs}) - k_{i,enz}C_{cccs}V_{aq,cccs} \quad (t_{fill} < t \leq t_{switch}) \tag{22}$$

$$\frac{dV_{aq,sv(t)}C_{sv}}{dt} = \phi_{v,aq}C_{cccs}(t_{fill} < t \leq t_{switch}) \tag{23}$$

where the volume of the aqueous phase in the SV increases over time according to $V_{aq,sv(t)} = \phi_{v,aq}(t - t_{fill})$.

Finally, in the fourth stage of operation, starting at $t = t_{switch}$, all 3 vessels are operated in a continuous mode where the aqueous input stream to the STR is switched from the fresh enzyme solution to the enzyme solution in the SV. This way a closed cycle of the aqueous enzyme solution is established and the system operates with a complete recirculation of enzyme without any addition of fresh enzyme as shown in Figure 6. The concentrations of active enzyme can now be calculated from

$$V_{aq,str}\frac{dC_{str}}{dt} = \phi_{v,aq}(C_{sv} - C_{str}) - k_{i,enz}C_{str}V_{aq,str}(t > t_{switch}) \tag{24}$$

$$V_{aq,cccs}\frac{dC_{cccs}}{dt} = \phi_{v,aq}(C_{str} - C_{cccs}) - k_{i,enz}C_{cccs}V_{aq,cccs}(t > t_{switch}) \tag{25}$$

$$V_{aq,sv}\frac{dC_{sv}}{dt} = \phi_{v,aq}(C_{cccs} - C_{sv})(t > t_{switch}) \tag{26}$$

The resulting eqs 18−26 describe the various stages of operation of the experiment with enzyme recycling. The equations were solved to obtain calculated values of the residual enzyme activity at sampling point 3 (see Figure 6) at the entrance of the STR. For the time interval $0 \leq t \leq t_{switch}$ this value is associated with the makeup enzyme concentration in the feed stream from the stock solution of enzyme, while for the time $t > t_{switch}$ the value is associated with the concentration of active enzyme in the SV.

Figure 16 shows a comparison of the modeling results and the measured data points, where the calculated line for $t > t_{switch}$ was obtained with a manually optimized correction factor of 0.55 for the deactivation rate constant $k_{i,enz}$ as obtained from our previous work.[27] A reasonably good fit is obtained this way, especially when taking into account the complexity of the recirculation system, including a stirred reactor, a continuous centrifugal contactor separator, and a storage vessel, over a period of 12 h.

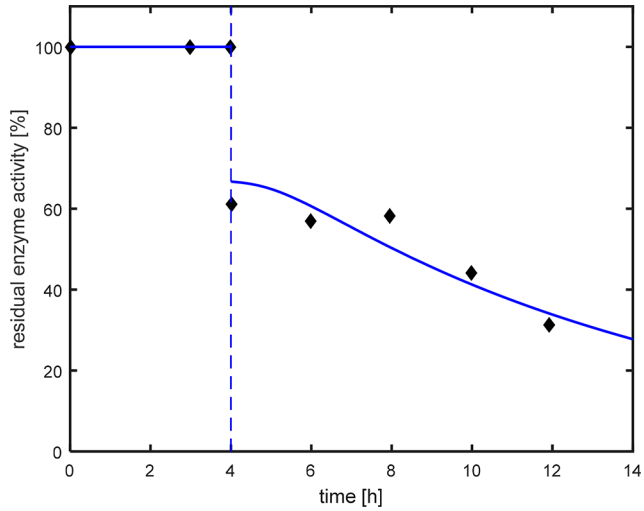

Figure 16. Residual lipase activity measured at sampling point 3 in the enzyme recycling test. Symbols, measured; solid lines, calculated from eqs 18−26; dashed line, start of enzyme recycling.

4. CONCLUSIONS

In this study, we have shown the proof of concept for the continuous production of butyl ester biodiesel from sunflower oil and 1-butanol using a free lipase in an aqueous−organic biphasic in a single CSTR as well as a cascade of a CSTR and CCCS reactor. The experimental data obtained in the CSTR reactor were successfully modeled using the mass balances in combination with the known enzyme kinetics for sunflower oil transesterification with 1-butanol using the homogeneous lipase (*R. miehei*) in an aqueous−organic biphasic system (Ping Pong Bi Bi mechanism with noncompetitive inhibition by 1-butanol and a term for irreversible enzyme deactivation during reaction). Optimization studies in the cascade lead to steady state FABE yields of up to 92% -mol for a runtime of 8 h. Operational issues were not observed for these continuous once-through experiments. Volumetric production rates were close to those reported for biodiesel synthesis in fed batch and continuous setups using a free enzyme (*T. lanuginosus* lipase). Enzyme recycling was investigated by reintroduction of the aqueous phase containing the enzyme in the feed of the cascade. FABE yield was shown to decrease over the runtime, likely due to enzyme loss by formation of an interfacial layer combined with enzyme inhibition by 1-butanol and irreversible enzyme deactivation. The enzyme activity versus runtime was measured and successfully modeled. Measures to improve the concept and to allow for a more efficient recycle strategy involve the use of a separation unit in the recycle stream to remove butanol and glycerol (bleed) and as such reduce

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enzyme inhibition by 1-butanol. These dedicated process studies are beyond the scope of this manuscript.

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Notes

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NOMENCLATURE

A,P,S	denotes the components *n*-butanol, ester, and acyl, respectively
C	concentration, mol L^{-1}
CCCS	continuous centrifugal contactor separator
$C_{enz,act}$	concentration of active enzyme in the aqueous phase, $g{\cdot}L^{-1}$
$C^{0}_{enz,act}$	initial concentration of active enzyme in the aqueous phase in the batch operation stage, $g{\cdot}L^{-1}$
$C^{in}_{enz,act}$	concentration of active enzyme in the aqueous feed in the continuous operation stage, $g{\cdot}L^{-1}$
$K_{m,S}$	Michaelis Menten constant, = 0.355 $mol{\cdot}L^{-1}$
k_{enz}	kinetic rate constant, = 0.794×10^{-3} mol $L_{org}{}^{-1}.g^{-1}{\cdot}L_{aq}{\cdot}s^{-1}$
$k_{i,enz}$	deactivation constant of the enzyme, = 0.007 s^{-1}
P	distribution coefficient of butanol, $P = (C_{A,org}/C_{A,aq})_{eq}$
R_V	phase volume ration, $R_V = V_{org}/V_{aq}$
$(r_{enz})_{deactivation}$	deactivation rate of the enzyme, $g{\cdot}L_{aq}{}^{-1}.s^{-1}$
STR	stirred tank reactor
SV	storage vessel
t_{batch}	time of the initial stage of operation in batch mode, s
V	volume, L
$V_{aq,ccs,0}$	amount of aqueous phase transferred, with the reaction mixture, from the STR to the CCCS at the time $t = t_{batch}$, L

Greek Symbols

ϕ_v	volumetric flow rate, $L{\cdot}min^{-1}$
τ	average residence time $(= V/\varphi_v)$, s

Subscripts

A,P,S	denotes the components *n*-butanol, ester, and acyl, respectively
aq	aqueous phase
cccs	continuous centrifugal contactor separator
eq	at equilibrium
org	organic phase
str	stirred tank reactor
sv	storage vessel

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