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Process intensification of catalytic liquid-liquid solid processes: Continuous biodiesel production using an immobilized lipase in a centrifugal contactor separator

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HIGHLIGHTS

- Centrifugal separator contactors have been used for L-L-S reactions.
- The concept was demonstrated for continuous biodiesel synthesis using an immobilized enzyme.
- Further improvements were possible in a cascade of a CSTR and CCCS.
- Average biodiesel yield at steady state conditions was 86%-mol for a 9 h run.

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ABSTRACT

Biodiesel or fatty acid methyl ester (FAME) synthesis from sunflower oil and methanol using an immobilized lipase, an example of a liquid-liquid solid reaction, was studied in batch and various continuous reactor set-ups including the use of a centrifugal contactor separator (CCCS). The latter is an example of a highly intensified device, integrating liquid-liquid reactions and subsequent phase separations. An exploratory study in batch was performed to optimize enzyme and buffer concentrations. Close to quantitative biodiesel yields were obtained at 30 °C when using 20% (w/w) of enzyme after a batch time of about 250 min. Subsequent continuous biodiesel synthesis was performed in a stirred tank reactor (CSTR) and a CCCS device. In the latter case, the immobilized enzyme was present in the annular, outer zone of the device. Average biodiesel yields in the CSTR and CCCS were similar (72%-mol respectively) when using a weight hourly space velocity (WHSV) of 3.3 and 3.03 h^{-1} respectively, at 30 °C. Cascade experiments were performed in a CSTR followed by a CCCS with the immobilized enzyme present in both reactors. The cascade was run for 9 h without any operation issues and an average FAME yield of 85%-mol was obtained. The advantage of the use of the cascade compared to a single CSTR is an improved yield combined with an efficient separation of the biodiesel layer and the glycerol. The biodiesel yield was about constant during the run, indicating that enzyme deactivation was negligible. The performance of the various reactor configurations were modelled successfully using standard balances for continuous reactors in combination with a kinetic model derived from the batch experiments.

1. Introduction

Biodiesel has increased in popularity the last decade and is an attractive alternative for diesel fuel due to its renewable character and its ability to be used in existing engines without substantial modifications [1,2]. The worldwide production of biodiesel has grown from 2.4 billion litres per year in 2004 to 29.7 billion litres per year in 2014 [3]. Production volumes are expected to further increase in the future, giving biodiesel a bright future.

The main technology to produce biodiesel is the transesterification of triglycerides with alcohols (in most cases methanol) catalyzed by either chemo- or bio catalysts (Fig. 1). Chemo catalysts like inorganic bases are commonly used due to their low cost and high reaction rates [4]. Biocatalysts have recently attracted a lot of attention as they have shown to perform better than base

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Nomenclature

[*Acyl*]	concentration of acyl groups, mol m^{-3}
CCCS	continuous contactor centrifugal separator
CSTR	continuous stirred tank reactor
[*FAME*]	concentration of FAME, mol m^{-3}
K_M	the Michaelis Menten constant, kinetic parameter in Eq. (11), mol m^{-3}
k_r	reaction rate constant in the enzyme kinetic rate Eq. (11), mol kg^{-1} s^{-1}
MW	molecular weight, kg.mol^{-1}
[*MeOH*]	concentration of methanol, mol m^{-3}
r_{enz}	rate of the enzyme catalyzed reaction, mol m^{-3} s^{-1}
t	time, s
V	volume, m^3
w^0	initial mass, kg
w_{enz}	mass concentration of enzyme, kg m^{-3}
Y	FAME yield, %-mol

Greek symbols

ρ	density, kg m^{-3}
τ	average residence time ($=V/\phi_v$), s
φ_v	volumetric flow rate m^3 s^{-1}

Subscripts

0	initial
aq	aqueous phase
cccs	continuous contactor centrifugal separator
cstr	continuous stirred tank reactor
enz	enzyme
FAME	fatty acid methyl ester
liq	liquid
MeOH	methanol
oil	sunflower oil
tot	total

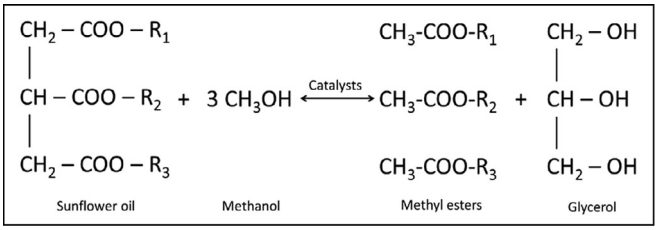

Fig. 1. Sunflower oil transesterification with methanol.

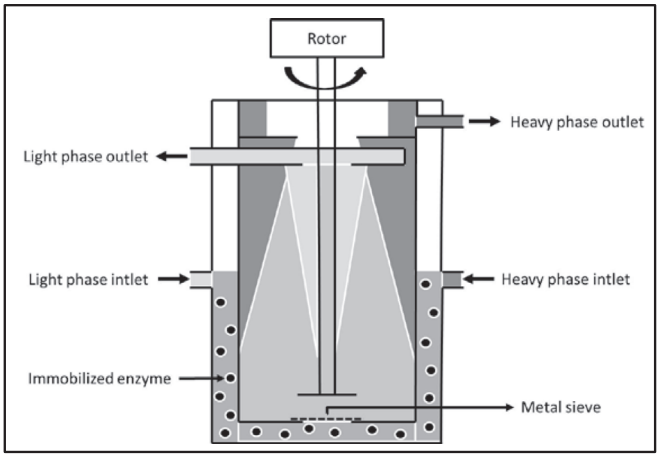

Fig. 2. Schematic representation of the continuous centrifugal contactor separator (CCCS) used in this study. Reproduced from [20].

catalysts like i) a better compatibility with oils and fats contaminated with free fatty acids (like used cooking oils), ii) soap formation is avoided, iii) a less complicated biodiesel work-up, and iv) a lower energy input [1,4].

Current commercial biodiesel processes are still typically performed in batch or fed-batch reactors. However, there is an incentive to use continuous production configurations as they allow for better product consistency and reduced downtimes [1,5–7]. Possible reactors for biodiesel production using immobilized catalysts are stirred tank and packed bed reactors [5,6,8–10]. Easy of operation and less complications regarding product-catalyst separation favour the use of packed-bed reactors [10–12]. With the use of immobilized enzymes, the recovery of the lipase is not an issue. On the other hand, the recovery of the lipase when using the free enzymes is difficult and costly while at the same time running the biodiesel synthesis with free enzymes and without recovery of the lipase would be excessively expensive. Biodiesel synthesis is an example of a biphasic liquid-liquid reaction. At the start of the reaction, the oil/fat and methanol are not fully miscible and form a liquid-liquid system. At the end of the reaction, biodiesel and glycerol again form two separate liquid phases. During reaction, intense mixing is required between the two phases to eliminate possible mass transfer limitations that will reduce the overall rate of the reactions. In addition, the product layers need to be separated after reaction by using a settler. Recently, we have shown that biodiesel synthesis may be carried out very efficiently in a continuous mode when using a continuous centrifugal contactor separator (CCCS). It is a device that integrates both intense mixing of two immiscible liquids and subsequent separation (Fig. 2) [13,14]. Originally, the CCCS-type of devices was used for cleaning-up of nuclear waste [15]. Subsequently, the CCCS was also applied in oil-water separation with oil spillages, liquid-liquid extraction [13] and reactive extraction [16–18].

The CCCS 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 suitable for a two-phase liquid-liquid catalytic reaction. The mixture is then transferred inside the centrifuge through a hole in the bottom of the rotor, where the two phases are separated by centrifugal forces whilst moving upwards, after which they leave the device through separate exits making use of an ingenious weir system [19]. As such, the device is an interesting example of process-intensification, acting both as a mixer-settler for biphasic liquid-liquid systems.

Recently, we have reported [19,20] the successful application of such a CCCS device for biphasic liquid-liquid reactions including the transesterification of sunflower oil with methanol using an alkaline catalyst and the esterification of oleic acid with 1-butanol using a liquid lipase formulation as the catalyst. In subsequent studies, the use was expanded to ethyl ester synthesis from

Jatropha oil [21], and synthesis and refining of methyl esters from sunflower oil using a cascade of two CCCS devices [22]. However, enzymatic transesterifications in a CCCS device using immobilized enzymes have not been reported to date. This will require modifications of the device to allow the containment of solids in the CCCS device.

In this study we have explored the use of the CCCS device for L-L-S reactions by investigation of the fatty acid methyl ester (FAME) synthesis from sunflower oil and methanol using an immobilized enzyme. In the first part of this study, exploratory experiments with the immobilized enzyme in batch were performed to optimize process conditions (among others catalyst, water content, and temperature). The batch experiments were modelled to obtain relevant kinetic parameters for the enzyme to be used for subsequent reactor modelling. Furthermore, experiments were performed in continuous reactors, viz. a continuous stirred tank reactor (CSTR) and a CCCS device and the results were modelled. Finally, the use of a cascade of a CSTR and CCCS device was investigated to simultaneously obtain higher biodiesel yields and to separate the product (biodiesel-glycerol) layers.

2. Materials and methods

2.1. Materials

Commercial sunflower oil was obtained from Vandermoortele BV, Belgium. Chloroform-d_1 (99.8 atom% D), methanol (99%), and buffer compounds ($Na_2HPO_4 \cdot 2H_2O$, 99% and $NaH_2PO_4 \cdot 7H_2O$, 98%) were obtained from Sigma-Aldrich. The phosphate buffer (50 mM, pH 6) was prepared by adding 6.80 g of $Na_2HPO_4 \cdot 2H_2O$ and 1.72 g of $NaH_2PO_4 \cdot 7H_2O$ to 1 L of water. TransZyme A, an immobilized enzyme, was kindly supplied by Transbiodiesel, Ltd., Israel. It is a modified lipase that is immobilized on hard shell beads. TransZyme A was chosen here because it is methanol-resistant and because it is known to maintain its activity for over 300 cycles [23].

2.2. Methods

2.2.1. Exploratory experiments in a batch reactor

A set of experiments was conducted to determine the effect of immobilized enzyme concentrations on the FAME yield in a batch reactor. Experiments were performed in a glass reactor (300 mL) surrounded by a heating jacket connected to a temperature controlled water bath and equipped with an overhead stirrer (Fig. 3). For all experiments, a stirring speed of 800 rpm and a temperature of 30 °C were used. The reactor was filled with sunflower oil (83 g), immobilized enzyme (5, 10, and 20 g), and an aqueous phosphate buffer (5 g, pH 6). The mixture was stirred for 10 min and subsequently, the reaction was started by adding the methanol (17 g) to the mixture. Each experiment was run for 6 h and samples were taken at predetermined time intervals. The samples were allowed to settle for 1 h, the organic top layer was separated from the bottom aqueous layer and the top layer was analysed.

Another set of experiments were conducted to study the effect of the buffer and water intake on catalyst performance. Experiments were performed in a glass reactor (50 mL) placed in a water bath on a temperature controlled hot-plate and stirred using a magnetic stirring bar. For all experiments, a stirring speed of 400 rpm and a temperature of 30 °C were used. The reactor was loaded with sunflower oil (4.15 g) and methanol (0.85 g). The effect of the amount of aqueous phosphate buffer solution of pH 6.0 (0.25, 0.5, 0.75, and 1 g) and distilled water (0.25 and 0.5 g) was investigated. The immobilized lipase (1 g) was used to catalyse the reaction. Each experiment was run for 90 min. After reaction, a sample was taken from the top liquid layer for analysis.

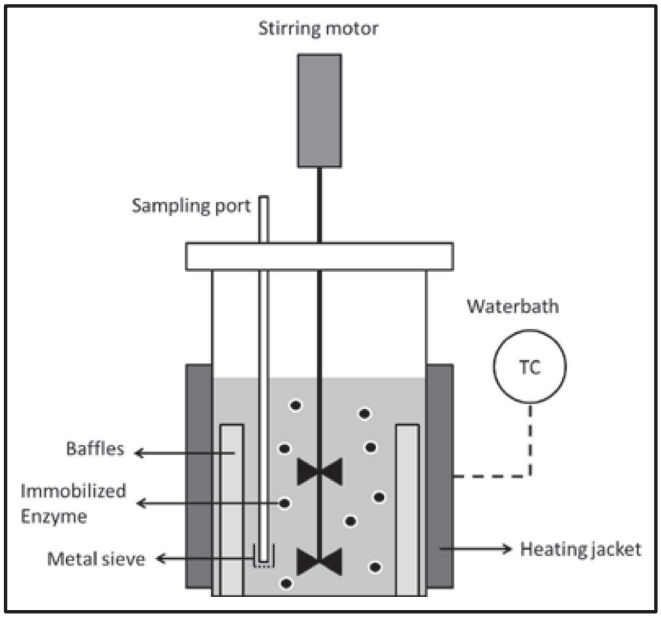

Fig. 3. Schematic representation of the batch reactor.

2.2.2. Biodiesel synthesis using immobilized enzyme in continuous reactor configurations

Experiments were carried out in three reactor configurations: i) a continuously stirred tank reactor (CSTR), ii) a continuous centrifugal contactor separator (CCCS), and iii) a cascade of a CSTR and a CCCS. An overview of experimental conditions is presented in Table 1. The weight hourly space velocity (WHSV) was set at 3.3 h^{-1} for the CSTR and 3.03 h^{-1} for the CCCS.

Table 1
Conditions for the experiments in batch, CSTR, CCCS, and a cascade with a CSTR and a CCCS.

	Value
Batch	
T (°C)	30
Stirring speed (rpm)	800
Oil intake (g)	83
Methanol intake (g)	17
Buffer intake pH = 6 (g)	5
Enzyme intake (g)	5–20
CSTR	
T (°C)	30
Stirring speed (rpm)	800
Oil feed rate (mL min^{-1})	1.8
Combined methanol-buffer feed rate (mL min^{-1})	0.6
Amount of methanol in methanol/-buffer feed (wt%)	60
Enzyme intake (g)	40
Liquid volume in reactor (mL)	222
WHSV (based on total feed flow rate), h^{-1}	3.3
CCCS	
T (°C)	30
Stirring speed (rpm)	1800
CCCS alone experiments	
Oil feed rate (mL min^{-1})	1.8
Combined methanol-buffer feed rate (mL min^{-1})	0.6
Cascade experiments (mL min^{-1})	2.4
Amount of methanol in methanol/buffer feed (wt%)	60
Enzyme intake (g)	44
Liquid volume in reactor (mL)	250
WHSV (based on total feed flow rate), h^{-1}	3.03

2.2.2.1. Experiments in a stirred tank reactor. Continuous experiments were performed in a glass reactor (300 mL) surrounded by a heating jacket connected to a temperature controlled water bath and equipped with an overhead stirrer (Fig. 4). For all experiments, a stirring speed of 800 rpm and a temperature of 30 °C were used. The reactor was filled with substrate (150 g oil, 30 g methanol, total 222 mL) and buffer solution (20 g). Subsequently 40 g of the immobilized lipase was added and the suspension was stirred for 90 min prior to the start of the reaction in the continuous mode. After this initial batch phase, the plant oil feed pump (1.8 mL min^{-1}) and the aqueous buffer/methanol feed (60 wt% methanol) pump with a flow rate of 0.6 mL.min^{-1} were started. Peristaltic pumps (Verderlab, Verder UK Ltd.) were used to feed the reactants to the reactor. The level in the reactor was maintained at a constant liquid volume by continuous removal of reactor content using a peristaltic pump (Verderlab, Verder UK Ltd.) in the outlet. The reactor outlet tube positioned in the reactor was covered by a metal filter (0.5 mm mesh size) (Fig. 4) to keep the immobilized enzyme in the reactor. The runtime was set at 270 min and a WHSV of 3.3 h^{-1} was applied. Samples were taken periodically from the top layer of the outlet stream and analysed for FAME content.

2.2.2.2. Experiments in a continuous CCCS device. Experiments were performed in a CCCS reactor (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. A metal filter (0.5 mm mesh size) was placed covering the hole at the bottom of the inner rotor to prevent the immobilized enzyme entering the separation zone (see Fig. 2). Every experiment was performed with a weir diameter of 24.13 mm, a stirring speed of 1800 rpm, and a reaction temperature of 30 °C. The CCCS device was initially filled with a mixture of substrate and buffer (150 g oil, 30 g methanol, 20 g buffer solution, pH 6) and 44 g of the immobilized lipase. The reactor content was stirred for 90 min in a batch mode. Subsequently, sunflower oil feeding was started through the light phase inlet with a flow rate of 1.8 mL min^{-1}, while a mixture of methanol and buffer (60 wt% methanol) was fed through the heavy phase inlet with a flow rate of 0.6 mL min^{-1}. Both streams were fed using a peristaltic pump (Verderlab, Verder UK Ltd.). The runtime was 360 min and a WHSV of 3.03 h^{-1} was applied. Samples were taken periodically from the light phase (biodiesel) outlet and analysed.

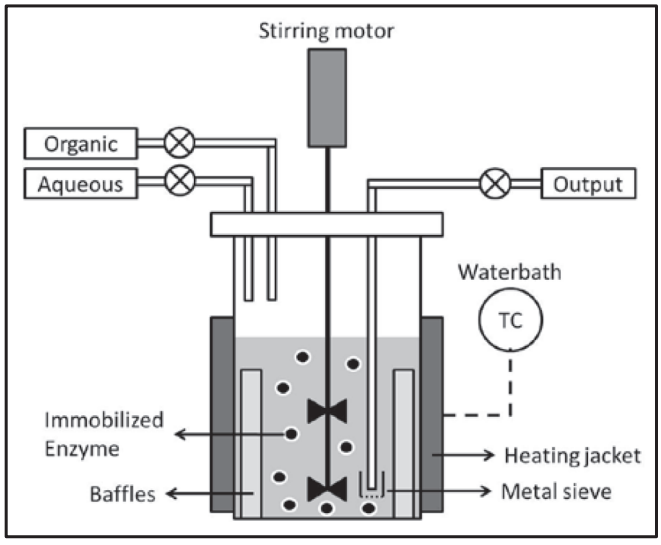

Fig. 4. Schematic representation of the continuous stirred tank reactor (CSTR).

2.2.2.3. Experiments in a continuous cascade consisting of a CSTR followed by a CCCS device. In the final stage of experimentation, the enzymatic biodiesel synthesis using the immobilized lipase was performed in a cascade of two reactors consisting of a CSTR (Fig. 4) followed by a CCCS device (Fig. 2). A schematic representation of the experimental set-up including relevant process data is given in Fig. 5. Both reactors were filled each with 222 mL of produced FAME from previous experiments and stirred for 15 min. An amount of 40 and 44 g of immobilized enzyme was added to the CSTR and CCCS, respectively. Reaction was started by turning on all pumps simultaneously and the reaction was kept running for nine hours. Samples from two sampling points (Fig. 5) were taken every hour and analysed for FAME content. Experimental details are given in Table 1.

2.3. Analytical methods

The FAME yield was determined using ^{1}H-NMR (300 MHz NMR, Varian Inc.). For this purpose, 50 μL of the biodiesel layer was mixed with $CDCl_3$ (700 μL) and measured.

2.4. Definitions

Throughout this manuscript, the substrate is defined as the sum of the amounts or flow rates of sunflower oil and methanol. The enzyme loading for the batch experiments is given in wt% on substrate intake.

The Weight Hourly Space Velocity (WHSV) is defined as the mass flow rate to the reactor (combined oil and methanol-buffer solution) per hour per unit weight of catalyst in the reactor (Eq. (1)).

$$WHSV = \frac{\phi_v \rho_{liq,average}}{w_{enz}} \tag{1}$$

The residence time in the CSTR is defined as the liquid volume of the reactor ($V_{L,total}$) divided by the total volumetric flow rate entering the reactor ($\varnothing_{V,total}$), see Eq. (2) for details.

$$\tau_{cstr} = \frac{V_{liq.tot}}{\phi_v} \tag{2}$$

The residence time in the CCCS is defined similarly. Here, the actual $V_{liq,tot}$ was determined experimentally by measuring the liquid volume left in the CCCS after reaction. For this purpose, the inlet and outlet valves were closed and the liquid was drained from a valve in the bottom of the CCCS, collected and measured using a volumetric cylinder.

The FAME yield, *Y*, expressed as %-mol, was determined by ^{1}H-NMR (*vide infra*) and is calculated from the intensities of methyl ester group of FAME (δ 4.1 ppm) and the intensity of the methyl end groups of the fatty acid chain of FAME (δ 0.89 ppm) (Eq. (3)).

$$Y = \frac{methyl\,ester\,peak\,area}{methyl\,end\,group\,peak\,area} \cdot 100\% \tag{3}$$

The volumetric production rate of FAME is defined as the amount of FAME produced per volume liquid per time (kg m^{-3} - min^{-1}) using Eq. (4).

$$Volumetric\,production\,rate = \frac{3\phi_{v,oil}\rho_{oil}Y}{V_{liq,tot}} \frac{MW_{FAME}}{MW_{oil}} \tag{4}$$

For calculations of the volumetric production rates in the individual CSTR and CCCS experiments, the total liquid volume in the respective reactors was used. For reactions in the cascade the sum of the total liquid volumes of the CSTR and the CCCS device was used.

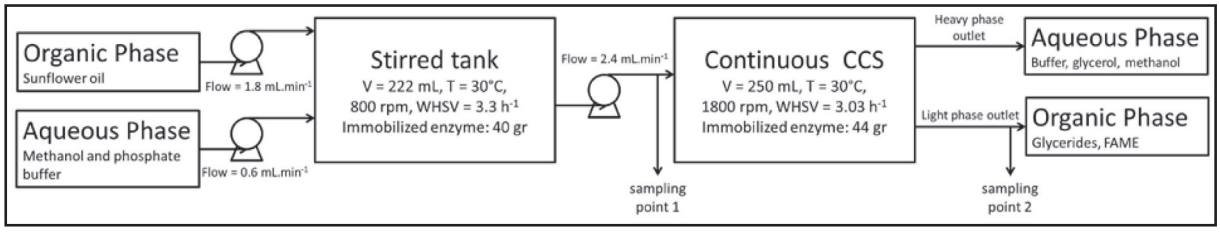

Fig. 5. Schematic representation of the cascade consisting of a CSTR and CCCS.

2.5. Kinetic modelling

The kinetic modelling activities were performed using Matlab where the reaction rate constants of the batch experiments were optimized using the lsqnonlin routine. For the modelling of the batch experiments, the following auxiliary equations were used:

$$V_{liq.tot} = \frac{w^0_{oil}}{\rho_{oil}} + \frac{w^0_{MeOH}}{\rho_{MeOH}} + \frac{w^0_{aq}}{\rho_{aq}} \quad (5)$$

$$[Acyl]_0 = \frac{3w^0_{oil}}{MW_{oil}V_{liq.tot}} \quad (6)$$

$$[MeOH]_0 = \frac{w^0_{MeOH}}{MW_{MeOH}V_{liq.tot}} \quad (7)$$

$$[FAME] = \frac{Y}{100} \cdot [Acyl]_0 \quad (8)$$

$$[Acyl] = [Acyl]_0 - [FAME] \quad (9)$$

$$[MeOH] = [MeOH]_0 - [FAME] \quad (10)$$

3. Results and discussion

3.1. Biodiesel synthesis using the immobilized enzyme in a batch set-up

Exploratory experiments to study the effect of the immobilized enzyme concentration on the FAME yield were performed in a batch reactor at 30 °C using 83 g of sunflower oil, 17 g of methanol and 5 g of an aqueous phosphate buffer of pH 6. As such, the reactions were carried out in the presence of a small amount of an aqueous phase, which is known to be beneficial for the activity of the lipase [24]. Three enzyme concentrations were used (5, 10, and 20% w/w) and the results are given in Fig. 6. At least duplicate experiments were performed and the results are the average of the experiments. The difference between the FAME yields for the various experiments was small (<5% at most), indicative for good reproducibility of the reactions.

The results show that the FAME yield (Fig. 6) at a fixed batch time increases with higher enzyme concentrations. Close to quantitative FAME yields were obtained when using 20 wt% of enzyme after about 250 min. The activity of the enzyme was compared with other transesterifications catalyzed by immobilized enzymes in batch set-ups (Table 2). Comparison though is cumbersome as enzyme intakes and batch times differ considerably. However, it is clear that activity of TransZyme A is good and FAME yields > 95% are attainable with this enzyme.

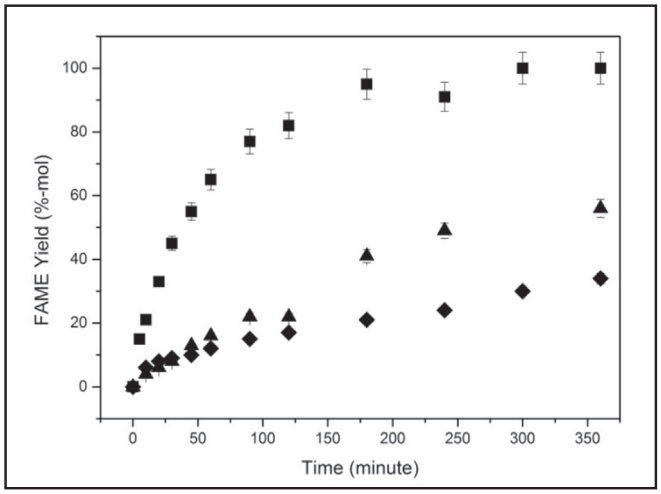

Fig. 6. FAME production using 5% (◆), 10% (▲), and 20% (■) of immobilized lipase in a batch reactor. Error bars represent deviation from triplicate data. Conditions are given in Table 1.

3.2. Kinetic modelling for batch experiments

The experiments with biodiesel synthesis with immobilized enzyme in the batch set-up were performed with 5, 10 and 20 g of enzyme. The initial amounts of sunflower oil, methanol and aqueous phosphate buffer were the same in all experiments, see Table 1. Initially, the kinetics was modelled using a typical Michealis-Menten kinetic expression [31] as given in Eq. (11), because it is versatile and the best-known two-parameter model for enzyme kinetics.

$$r_{enz} = \frac{k_r w_{enz}[Acyl]}{K_M + [Acyl]} \quad (11)$$

However, it was found that $K_M \gg [Acyl]_0$ and thus a simple first order model was used (Eq. (12)). This first order model was found adequate to describe the concentration profiles, as illustrated by the yield curves shown in Fig. 7.

$$r_{enz} = \frac{d[FAME]}{dt} = k_r \cdot w_{enz}[Acyl] \quad (12)$$

where w_{enz} denotes the mass concentration of enzyme (kg m^{-3}) and $[Acyl]$ denotes the concentration (mol m^{-3}) of acyl groups in the solution that are not converted to FAME yet. The value for k_r was $(30 \pm 3) \times 10^{-6}$ m^3 kg^{-1}_{enz} s^{-1} at 30 °C for the experiments with 5% and 10% enzyme. With the run using a higher amount of enzyme (20%) a significantly higher value of the rate constant was obtained. A possible cause is the difference in enzyme to water ratio for the three reactions, which is known to have an important effect on enzyme kinetics. In the next Section 3.3 the influence of the amount of water in biodiesel synthesis is discussed in detail. In the reactor

Table 2
Representative examples of batch studies on biodiesel synthesis using immobilized enzymes.

Enzyme type	Substrates	Reaction condition	Yield (%)	Ref.
T. lanuginosus lipase	Canola oil Methanol	Enzyme loading: 0.02% w/w oil Reaction time: 24 h	90	[25]
T. lanuginosus lipase	Sunflower oil Methanol	Enzyme loading: 1% w/w oil Reaction time: 24 h	97	[26]
Lipozyme TL IM combined with Novozyme 435	Rapeseed oil Methanol	Enzyme loading: Lipozyme: 3% w/w oil Novozyme 1% w/w oil Reaction time: 12 h	95	[27]
P. cepacia lipase	Jatropha oil Ethanol	Enzyme loading: 10% w/w oil Reaction time: 8 h	98	[28]
C. rugosa lipase	Soybean oil Methanol	Enzyme loading: 60% w/w oil Reaction time: 60 h	87	[29]
Lipozyme TL IM	Palm oil Oleyl alcohol	Enzyme loading: 24.7% w/w oil Reaction time: 3 h	79.5	[30]
TransZyme A	Sunflower oil Methanol	Enzyme loading: 23% w/w oil Reaction time: 6 h	>95	This Study

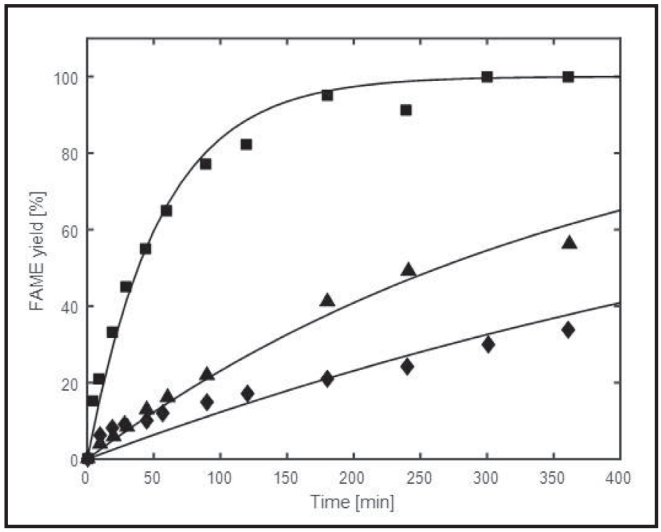

Fig. 7. Experimental and modelling results for FAME synthesis using 5% (◆), 10% (▲), and 20% (■) immobilized enzyme in a batch reactor. Symbols: measured; lines: calculated according to Eq. (12). Conditions are given in Table 1.

modelling section, a value for k_r of 30×10^{-6} m^3 kg enzyme^{-1} s^{-1} is used, which is the value obtained with 5% and 10% enzyme.

3.3. Effect of buffer and water concentration on biodiesel yield in a batch set-up

It is well known that the amount of water on pure plant oil intake is an important process variable for biodiesel synthesis using immobilized enzymes. It is speculated that water is needed to sustain the enzyme activity in organic media [24], though an excess leads to reduced reaction rates. An overview of available data on optimum amounts of water required to obtain highest biodiesel yield is given in Table 3. A study by Shah et al. [28] showed that highest product yield (98% w/w, 8 h batch time) for the ethanolysis of Jatropha oil using an immobilized *Pseudomonas cepacia* lipase was obtained in the presence of 5% (w/w) water. Lu et al. [32] performed the methanolysis reactions of triglycerides with an immobilized *Candida* lipase in the presence of various amounts of water. In this particular case, 20% (w/w on triglyceride) of water was shown to give the best results (approx. 80% yield after 12 h). Babaki et al. [33] reported that higher amounts of water (30%) are required to give highest yields (approx. 90% after 50 h) for canola oil methanolysis using an immobilized *Thermomyces lanuginosus* lipase. Thus, it can be concluded that the optimum amount of water is, amongst others, a function of the type of immobilized enzyme.

Table 3
Optimum water concentrations for biodiesel synthesis using various types of immobilized enzymes.

Lipase type	Optimum water concentration (% w/w oil)	Ref.
Pseudomonas cepacia	5	[28]
Candida sp. 99–125	10–20	[32,34]
Thermomyces lanuginosus	20 – 30	[33]
TransZyme A	12	This study

In addition, it has also been shown that the use of a buffer is advantageous. The optimum pH for immobilized enzymes depends on the immobilization procedure and the support [35,36]. Several studies showed that the optimum pH for the free enzyme and the immobilized enzyme differs considerable. Shaw et al. [37] reported that the optimum pH for *Candida rugosa* lipase shifts from 7.5 for the free enzyme to 8.5 after immobilizing the enzyme on PVC, Sepharose, chitin, and agarose. A shift to higher pH values was also observed for *C. rugosa* lipase (from 8 to 8.5) [35] after immobilization on chitin. However, also shifts to lower pH values have been reported, for instance from 7 to 6.5 after immobilizing the free enzyme on Celite [38]. In this study, a pH value of 6 was used.

The effect of buffer concentration on the biodiesel yield using the immobilized lipase was investigated by performing batch experiments at 30 °C. In the set-up, an amount of 5 g of substrate (4.15 g sunflower oil and 0.85 g methanol) was used; see experimental section for further details regarding intakes. Separate experiments were performed for water only (0.25 and 0.5 g) and an aqueous phosphate buffer with a pH of 6 (0.25, 0.5, 0.75, and 1 g). All experiments were done in duplicate and the results are given in Fig. 8.

A strong effect of the aqueous phosphate buffer concentration, on the biodiesel yield was observed. At low concentrations, the yield increases with concentration, reaches a maximum and then is lowered again when working at higher buffer concentrations. Highest yields of 70% were obtained at a buffer concentration of 10 wt% on substrate (oil plus methanol). This optimum buffer content is in the range of optimum water contents reported in the literature for other immobilized enzymes (Table 3). The observed maximum in Fig. 7 is likely the results of two competing processes. At low water concentrations, the addition of a small amount of

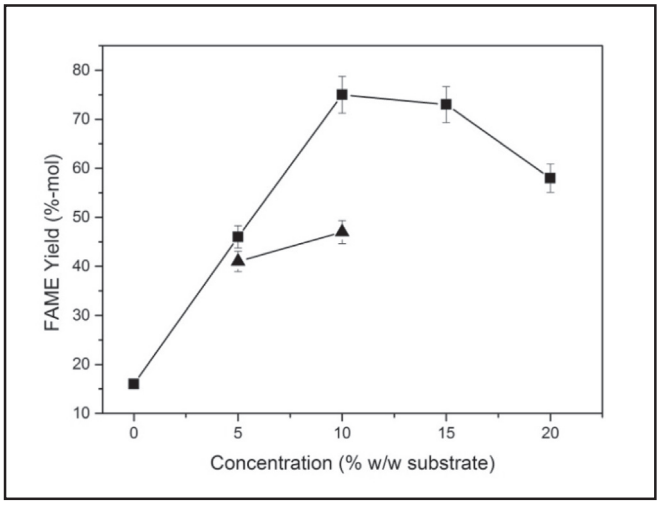

Fig. 8. Effect of the phosphate buffer (pH 6) (■) and water (▲) concentrations on FAME yield in a batch reactor. Error bars represent deviation from duplicate data, lines for illustrative purpose only.

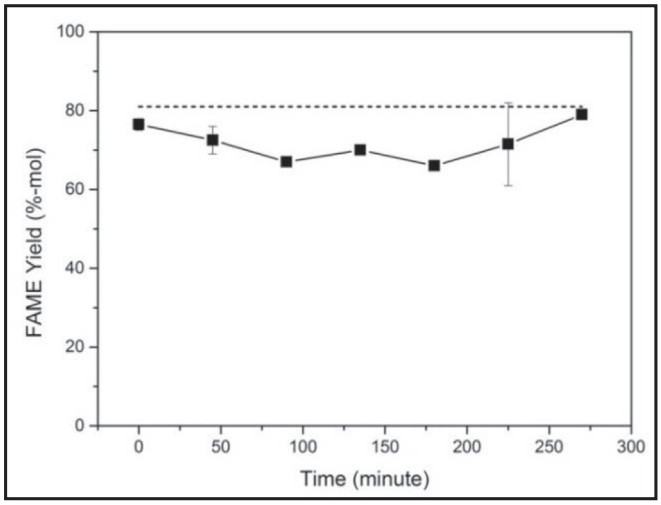

Fig. 9. FAME yield versus runtime in a CSTR using the immobilized enzyme. Experimental conditions are given in Table 1. Error bars represent deviation from duplicate experiments. Dotted line: calculated according to Eq. (14).

water to the oil phase will lead to an increase in activity due to a larger coverage of the immobilized enzyme with water molecules, which is beneficial for the enzyme activity [24,39]. On the other hand, the use of excessive water has a negative influence on the biodiesel yield, among others due to the formation of larger amounts of FFA's [24]. A large excess of water is also expected to be adsorbed by the enzyme support and result in more than full coverage of the enzyme, preventing organic substrate to access the enzyme [39].

The data given in Fig. 8 also clearly indicate that the use of a buffer leads to improved biodiesel yields compared to water alone, in agreement with literature data (*vide supra*). This is particularly evident for water concentrations of 10 wt% on substrate. As such, the subsequent experiments in the continuous set-ups were performed using a buffer solution.

3.4. Biodiesel synthesis using immobilized enzyme in a continuous CSTR and CCCS reactor

3.4.1. Experiments in the CSTR

The experiments in the CSTR (Fig. 4) were carried out at 30 °C, a stirring speed of 800 rpm and a WHSV of 3.3 h^{-1}, see Table 1 for additional details. An experiment was started in a batch mode for 1.5 h to reduce the time to reach steady state operation in the set-up. The runtime was set at t = 0 h when the feed pumps were started. The results for two duplicate experiments are provided in Fig. 9.

The average FAME yield at steady state conditions versus the run time is about constant and is on average 73%-mol with a standard deviation of 5% (Fig. 9). It is evident that excessive catalyst deactivation does not occur during the runtime of the reaction in the CSTR.

The kinetic rate equation obtained from the batch experiments was used to model the experiments with the continuous CSTR. The component balance for FAME in the CSTR reads

$$V_{cstr}\frac{d[FAME]_{cstr}}{dt} = k_r \cdot w_{enz,cstr}[Acyl]_{cstr} V_{cstr} - \varphi_v[FAME]_{cstr} = 0 \quad (13)$$

This balance can be rewritten to obtain directly the yield of FAME for the CSTR:

$$Y_{cstr} = 100\frac{k_r \cdot w_{enz,cstr} V_{cstr}}{\varphi_v + k_r \cdot w_{enz,cstr} V_{cstr}} \quad (14)$$

From Eq. (14) the yield of FAME with the CSTR was calculated as 81%, see Fig. 9. In the calculation, the rate constant as obtained from the batch experiments was corrected for the water content. Here, the water content was approximately 10%, while the batch kinetic experiments were performed with 5% water. According to the results with varying amounts of water the rate increases with 65% as the amount of water increases from 5% to 10%, see Fig. 8. Consequently, in the calculations here, the rate constant was increased with 65% to compensate for the difference in water content.

3.4.2. Experiments in the CCCS

Another set of continuous experiments was performed in a continuous centrifugal contactor separator (CCCS) reactor. The experiments were carried out using a phosphate buffer of pH 6 at 30 °C, a stirring speed of 1800 rpm and a WHSV of 3.03 h^{-1} (Table 1). The immobilized catalyst was added to the annular, outer zone of the device (Fig. 2). A perforated plate was placed in the hole in the centrifuge to prevent the immobilized enzyme from entering the separation zone. An experiment was started by filling the CCCS device with a reaction mixture obtained by combining a number of reaction products from batch experiments. This procedure was followed to reduce the time to reach steady state in the device. The experiment was performed in duplicate, see Fig. 10 for details.

The CCCS device was operated for a runtime of 400 min without operational issues. In addition, phase separation at the exit was excellent and visually the biodiesel produced showed only a slight haze without the formation of a separate water phase upon standing. The FAME yield increased with the runtime for the first 180 min and then levels off to a more or less constant value for the remaining runtime. The initial increase is likely due to the start-up procedure. The average FAME yield in the steady state phase was 72%-mol, which is very close for that in the CSTR. The standard deviation for the average steady state yield is about 6%.

The FAME yield versus runtime does not show a clear decrease (actually shows a slight increasing trend), indicating that enzyme stability is good. As such, the enzyme matrix is able to withstand shear stresses in the annular zone of the CCCS device, a known deactivation pathway for immobilized enzymes [40,41].

The kinetic model obtained from the batch experiments, was also used to model the experiments of the CCCS. A similar approach as for the CSTR (*vide supra*) for the continuously operated CCCS

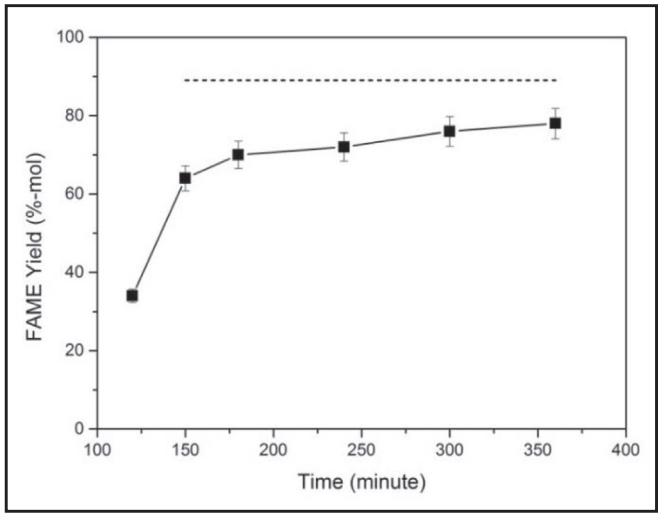

Fig. 10. FAME yield versus runtime in a CCCS using the immobilized enzyme. Experimental conditions are given in Table1. Error bars represent deviation from duplicate data. Dotted line: calculated according to Eq. (16).

reactor results in the following equations for the FAME component balance and the FAME yield

$$V_{cccs}\frac{d[FAME]_{cccs}}{dt} = k_r \cdot w_{enz,cccs}[Acyl]_{cccs}\, V_{cccs} - \varphi_v[FAME]_{cccs} = 0 \quad (15)$$

$$Y_{cccs} = 100\frac{k_r \cdot w_{enz,cccs}V_{cccs}}{\varphi_v + k_r \cdot w_{enz,cccs}V_{cccs}} \quad (16)$$

Again the reaction rate constant k_r as obtained from the batch experiments was corrected for the amount of aqueous buffer present in the reaction mixture before application in the calculation. When using this approach, a FAME yield of 89% is obtained, see Fig. 10. In this case the calculated FAME yield is consequently higher than the experimental points. The reason for this discrepancy is not completely clear, but part of the explanation may be that the CCCS somehow has not reached a steady state within the timeframe shown in Fig. 10, as witnessed by the increasing values of the yield vs. time.

3.4.3. Process metrics for the continuous experiments

To compare our experiments with other studies, the volumetric biodiesel production rate for both continuous reactor configurations was determined using Eq. (4). The volumetric production rate of CSTR was 0.55 kg m^{-3} min^{-1}, compared to 0.46 kg m^{-3} min^{-1} for the CCCS. This difference, even though FAME yields of both reactors are similar, is due to the slightly higher liquid volume in the CCCS (265 mL compared to 222 mL for the CSTR) and as such the residence time in the CCCS (τ_{CCCS}) is higher than in the CSTR (τ_{CSTR}). This in turn decreases volumetric production rate of CCCS. In previous work from our group [42], we reported on the continuous transesterification of sunflower oil with butanol using 150 g. L_{aq}^{-1} of a liquid *Rhizomucor miehei* lipase formulation in a CSTR. A volumetric production rate of 0.25 kg product m^{-3} min^{-1} was obtained when using a 90 min residence time, and 0.38 kg m^{-3} - min^{-1} for a residence time of 60 min. As such, the immobilized enzyme shows higher volumetric production rates than for a typical free enzyme, with the additional advantage that the development of (expensive) catalyst recycling strategies is not required. The volumetric production rates reported here in the CCCS for the immobilized enzyme are by far lower than for the methanolysis of sunflower oil using conventional base catalysts. For instance, we reported [20] volumetric production rates of up to 61 kg m^{-3} - min^{-1} when using NaOH as the catalyst. However, these differences are in line with the far higher reactivity of base catalysts compared to enzymes.

3.5. Biodiesel synthesis using immobilized enzyme in a cascade of a CSTR and a CCCS reactor

Biodiesel syntheses using the immobilized lipase in the CSTR and CCCS separately showed average FAME yields in the steady state of about 72%-mol (Figs. 9 and 10). To further enhance the biodiesel yield, a cascade of a CSTR and CCCS in series was explored. A number of experiments were performed using this configuration with conditions as given in Table 3 and Fig. 5. Samples were taken periodically from the CSTR outlet (sampling point 1) and from the outlet biodiesel phase of the CCCS (sampling point 2). The cascade was run for a total of 9 h.

As with the separate CCCS experiments, visual observations showed that the separation of both liquid outlet phases of the CCCS was good, indicative for good performance of the separation part of the CCCS device. Analyses show that steady state is reached in both reactors after about 3 h (Fig. 11). The time to reach steady state in the CSTR is by far longer than found for individual CSTR experiments (Fig. 9), likely due to the application of different start-up procedures (see experimental section). The average FAME yield in the cascade was 85.7%-mol, and the highest value was 91%, observed at the end of the runtime. As such, the addition of the CCCS to a CSTR leads to an improved yield and allows for the efficient separation of the biodiesel and glycerol layer after reaction.

The experimental data for the cascade were also modelled using the batch kinetic expression as input (Eq. (12)). The FAME yield of the CSTR is again given by Eqs. (13) and (14), resulting in a FAME yield of 81% for the CSTR. The FAME balance for the CCCS has to be extended compared to Eq. (15) with a contribution for the FAME yield that is already achieved in the CSTR:

$$V_{cccs}\frac{d[F]_{cccs}}{dt} = k_r \cdot w_{enz,cccs}[A]_{cccs}\, V_{cccs} - \varphi_v[F]_{cccs} + \varphi_v[F]_{cccs}^{in} = 0 \quad (17)$$

where $[F]_{cccs}^{in}$ is associated with the FAME concentration that leaves the CSTR, $[F]_{cccs}^{in} = [F]_{cstr}$. Eq. (17) can be rewritten to obtain an

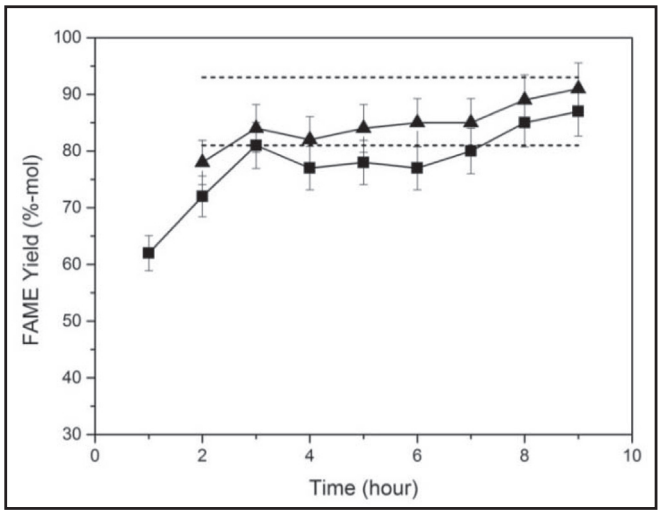

Fig. 11. FAME yield versus runtime in a cascade of a CSTR (■) and CCCS (▲) using an immobilized lipase. Experimental conditions are given in Table 1. Error bars represent deviation from duplicate data. Symbols: measured data; solid lines to guide the eye; dotted lines: calculated results from Eqs. (14) (lower line) and (18) (upper line).

expression for the FAME yield in the exit of the CCCS that is now in a cascade with the CSTR:

$$Y_{cccs} = 100\frac{k_r \cdot w_{enz,cccs} V_{cccs} + \varphi_v Y_{cstr}}{k_r \cdot w_{enz} V_{cccs} + \varphi_v} \quad (18)$$

For the cascade a FAME yield of 93% is calculated. The modelling results are compared to the measured data in Fig. 11. Especially after prolonged operation of the system, i.e. at higher time values, the agreement between calculated and experimental yield values is remarkably good.

The volumetric production rate of the cascade system was calculated based on Eq. (4) and using the average FAME yield in the steady state (3–9 h runtime). The volumetric production rate was 0.30 kg m^{-3} min^{-1}, which is higher than found in our previous study on biodiesel production using a liquid lipase formulation (*Rhizomucor miehei)* in a similar cascade set-up (0.23 kg m^{-3} - min^{-1}) [42].

4. Conclusions

We have shown the proof of concept for the use of an immobilized enzyme for biodiesel synthesis in a CCCS device and an average steady state yield of 72%-mol was obtained when using a weight hourly space velocity (WHSV) of 3.03 h^{-1}at 30 °C. Higher yields were obtained in a cascade consisting of a CSTR and a CCCS device in series. Here, under optimized conditions, the average FAME yield at steady state conditions was 86%-mol. The biodiesel yield is about constant during a runtime of 9 h, indicating that deactivation of the immobilized enzyme by for instance shear stresses in the device does not play a major role. The FAME yields were modelled using a kinetic expression obtained from batch experiments and the mass balances for the two reactors and agreement between experiments and model was satisfactorily. The volumetric production rate in the cascade was higher than for biodiesel synthesis using a free *Rhizomucormiehei* lipase in a similar cascade of reactors; though lower than for conventional biodiesel synthesis using alkaline catalysts. To the best of our knowledge, this is the first example of a L-L-S reaction carried out in the CCCS device and as such is an absolute novelty of this paper. It opens new avenues for the use of the CCCS devices for various challenging chemical transformations, for instance for liquid-liquid processes using heterogeneous catalysts, possibly also in combinations with a gas phase substrate. These studies are in progress and will be reported in due course.

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