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Published in:
Process Biochemistry

Link to article, DOI:
10.1016/j.procbio.2020.06.005

Publication date:
2020

Document Version
Peer reviewed version

Esterification using a liquid lipase to remove residual free fatty acids in biodiesel

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Abstract

Lately, the price of liquid formulated lipase enzymes, usable in biodiesel production, has been significantly reduced. This enables one-time use of these enzymes for transesterification, and the process is used industrially. However, the process suffers a drawback by leaving 2-3% free fatty acids in the crude biodiesel, which reduces the profitability. This article discusses a novel enzymatic FFA esterification reaction utilizing liquid lipase B from *Candida antarctica (*CALB) along with glycerol at low water concentrations to eliminate the residual FFA. The reaction setup was found able to reduce the free fatty acid concentration to within biodiesel specifications of <0.25 wt.% FFA. Additionally, two alternative process setups are proposed, which were both found viable through a combination of experiments and simulations, and can be developed into full-scale processes. The resulting two-step enzymatic biodiesel process - transesterification followed by esterification - provides a potential process layout for the industrial production of biodiesel.

Keywords.
Biodiesel, FAME, lipase, esterification, drying process, high yield, *Candida antarctica* lipase B

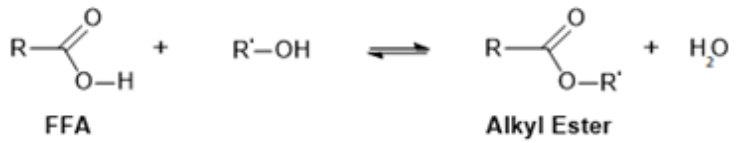
1. Introduction

One of the most valuable tools in achieving sustainable chemical production is the use of enzyme-based process technology [1,2]. An excellent example is the enzymatic production of fatty acid methyl esters (FAME), commonly termed biodiesel [3]. For biodiesel production, the advantages of using enzymes are lowered operating temperatures and pressures, better quality of glycerol byproduct, and less waste, all of which reduce operating costs. In fact, the enzymatically-catalyzed biodiesel production process has already been commercialized and is currently in use in industry [4,5]. Today, a liquid lipase formulation (marketed by Novozymes as Eversa Transform 2.0) is a widely used biocatalyst for this particular application. It catalyzes the conversion of triglycerides, as well as free fatty acids (FFAs), with low waste generation and good profitability compared to the conventional alkaline-catalyzed process [6]. A particularly attractive feature of the enzymatic process is that it allows the use of cheap, low-quality feedstocks with minimal pretreatment, unlike the alternative chemical procedure [3]. Indeed, using this technology, economically viable processing of high FFA-containing feedstocks becomes possible.

However, even though the current enzymatic process results in a high yield of FAME on triglyceride, the product still contains 2-3 wt.% FFA (regardless of the feedstock composition), since the reaction is equilibrium limited [7]. Today, FFA is removed by saponification, which is an expensive processing step and therefore should be avoided [8,9]. Tit is expensive mainly because of significant amounts of FAME lost in the saponification, handling of waste and water, as well as re-acidification for recycle of the soap stock.

It is well known that avoiding saponification is potentially achievable through enzymatic esterification of the remaining 2-3 wt.% FFA (Scheme 1). Nevertheless, this reaction is reversible, meaning that to convert all the residual FFA to alkyl ester, the equilibrium needs to be driven by using either an excess of methanol, or else by removing water during the

reaction. However, in practice only water can be removed, because an upper methanol concentration limit exists, above which the enzyme becomes unstable [10].



Scheme 1. Reversible esterification of FFA with an alcohol (typically methanol).

It is well known that lipases such as *Candida antarctica* lipase B (CALB) catalyze reactions at a liquid-liquid interface [11,12]. Furthermore, CALB shows relatively high activity and stability at very low water concentrations making it ideal for this case [13,14]. In this work we have investigated the idea of using glycerol as the main component of the reaction system to obtain a liquid-liquid interface and serve as a liquid enzyme carrier. Use of liquid enzyme has the added advantage of using a cheaper enzyme formulation as well as avoiding the need for filtration, when compared to immobilized enzyme formulations [15]. Additionally, the enzyme was found recoverable through simple decantation of the glycerol phase. One problem with using a liquid enzyme formulation is that it introduces some water with the enzyme, although this is mitigated by dry glycerol binding the water [16]. A similar study using another enzyme, *Calera Trans L* (CTL), in a liquid-liquid system quite similar to this work, also confirms the difference in FFA at equilibrium between an immobilized enzyme and liquid enzyme formulation [17].

Despite the benefit, in the scientific literature there are few reports of this particular reaction [11], especially to achieve low residual FFA concentrations. Key to this is removal of water, which we have examined both using a stirred bubble reactor (SBR) [11], and alternatively flash columns [18]. A combination of process model simulations and experimental results have been used to show a first proof-of-concept and thereby substantiate that this can be a viable novel process.

2. Process principles

2.1. Theoretical effects of stepwise and continuous water removal

Although the reaction of interest here uses a liquid formulation of CALB, the principle of operation can be illustrated by a previously published model using immobilized CALB (Novozym 435) for the esterification reaction [19]. A simulation of an esterification using this model is shown in Figure 1. Here water is removed stepwise through an arbitrary drying procedure (set at 15 minutes) to 250 ppm, after which methanol is again added to 4 % (w/w).

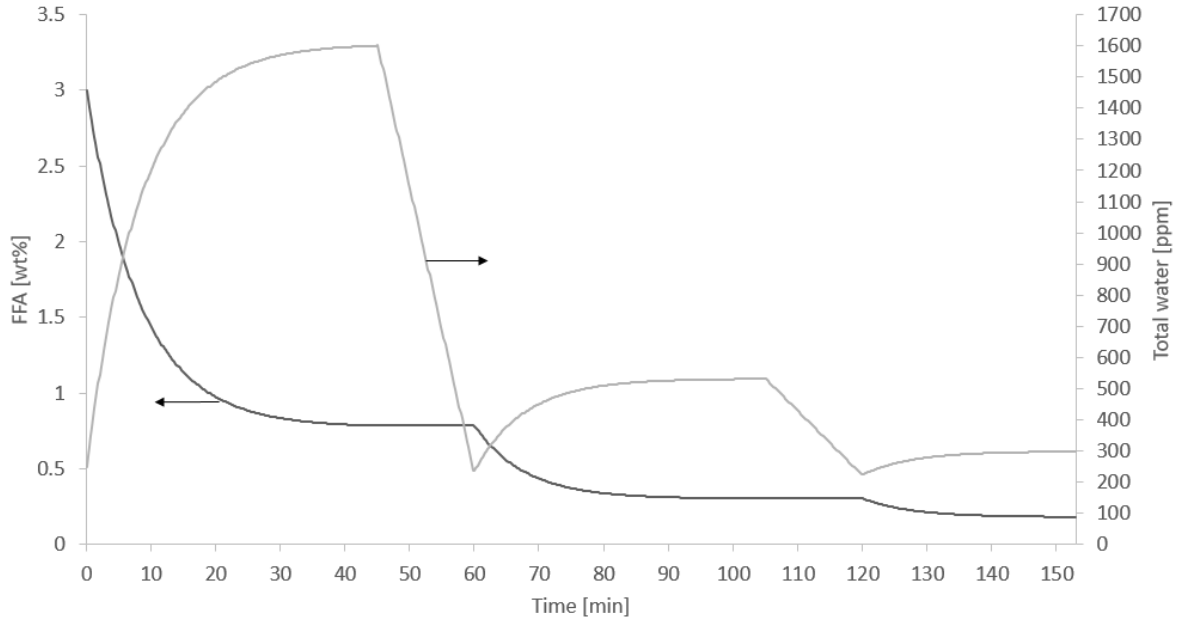


Figure 1. Model simulation of esterification using stepwise water removal. Reaction runs used 5 % (w/v) Novozym 435. After drying 4 % (w/w) methanol was added. The black line represents the simulated FFA concentration, and the grey line, the simulated water concentration [adapted from19].

According to the simulation shown in Figure 1, a stepwise repeated reaction-drying procedure for the esterification of FFA can achieve a residual FFA concentration of less than 0.25 wt.%. Indeed, it is easy to conceive of such a process using several steps of reaction and drying using flash columns [18].

An alternative to stepwise removal of water could be continuous drying, where a continuous shift in equilibrium towards complete FFA esterification is obtained. Using the same model, whilst continuously removing (an arbitrarily chosen) 0.5 % water per minute, gives the simulation shown in Figure 2. The SBR process utilizes this principle.

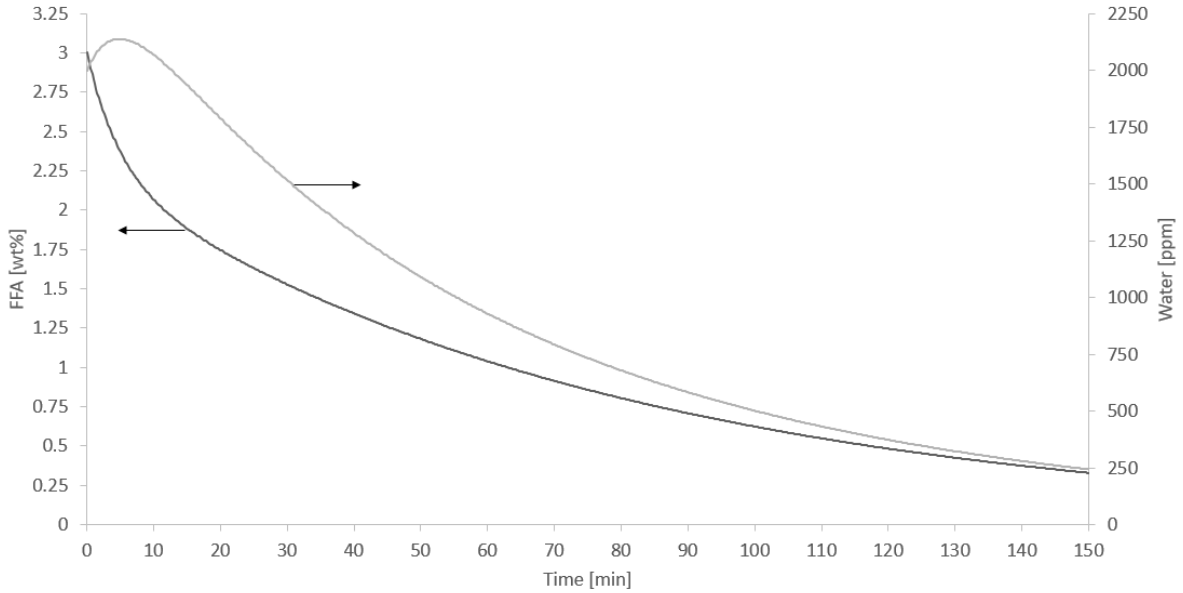


Figure 2. Continuous reaction and drying model simulation. The esterification was catalyzed by 5 % (w/v) Novozym 435. 2 % (w/w) methanol is always present. The black line represents the simulated FFA concentration, and the grey line, the simulated water concentration.

2.2. Stirred bubble reactor (SBR)

The stirred bubble reactor (SBR) system uses a methanol-rich gas, bubbling through a reaction vessel, in which the enzymatic esterification reaction is catalyzed. In the reaction vessel, methanol transfers from the methanol-rich gas bubbles to the liquid. At the same time, the reaction produces water as a by-product which is preferentially transferred into the gas phase. The resulting gas phase leaves the reaction vessel and is bubbled through a second vessel containing liquid methanol. In this vessel water is condensed and absorbed by the liquid methanol. The relatively dry methanol-rich gas in the headspace of the

methanol vessel is then circulated back to the reaction vessel. This results in a net transfer of water from the reactor to the methanol vessel. A patent on a method similar to the SBR examined here, was previously filed [20]. Figure 3 shows an overview of the process, where the first vessel has been termed the 'reactor', and the second the 'condenser'.

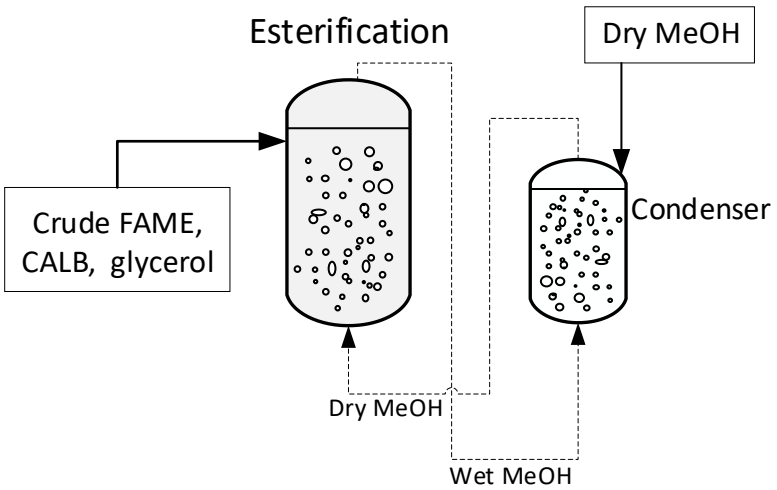


Figure 3. Overview of the stirred bubble reactor process. Left: Esterification reactor. Right: (condenser) containing initially dry methanol. The dashed lines represent a dry methanol gas stream from the headspace of the condenser to the reactor, and wet methanol gas stream from the reactor back to the condenser.

In order for the SBR process to work, two requirements must be met. First, the methanol concentration needs to be maintained at a reasonable level in the reactor at steady-state. Hence, while being high enough to favor the equilibrium towards FAME, it should not be at a concentration so high that it results in enzyme inactivation. Second, the driving force for the evaporation of water into the gas phase bubbling though the reactor should be adequate. Both are determined by the vapor-liquid equilibrium of methanol and water in the condenser and are correlated with the temperatures of the two liquids and the amount of methanol (compared to feedstock), in the condenser. This determines the driving force, and thereby the rate of mass transfer, and hence final water and methanol concentrations.

3. Materials and methods

3.1. Materials

Glycerol and methanol were of analytical grade with purity >99.5 %. Glycerol had a water concentration of around 1000 ppm, methanol had a water concentration of around 200 ppm. Oleic acid was of technical grade with purity >90 %, with the remainder being mainly other fatty acids. The enzyme used was a liquid formulation of CALB (containing 43.7 wt. % water) (NS88007, Novozymes A/S, Bagsvaerd, Denmark). FAME/Biodiesel (Acidity equivalent to ~0.2 wt. % FFA) from palm oil with added oleic acid was used as feedstock.

Esterification reactions were carried out at three different scales. 30 g scale experiments were conducted in 150 mL square flasks (DURAN). 300 g scale experiments were conducted in centrifuge tubes (Polycarbonate, 500 mL, 69 x 160, Beckman Coulter Life Sciences). Both were run in a shaking incubator (New Brunswick Scientific™ Innova™ 44 Incubator Shaker) at 250 rpm, maintained at 40°C. 450 g scale experiments using the SBR system were conducted in a 1 L temperature-controlled glass reactor with a height of 14 cm and a diameter of 10 cm, and mixed with a 4-vertical winged propeller at 500 rpm, and maintained at 40°C. The condenser comprised a cylindrical vessel initially containing 500 mL of dry methanol. It was agitated with a 4-vertical winged propeller at 500 rpm, and maintained at 25°C.

3.2. Methods

3.2.1. Reaction initiation

Prior to the reaction, mixtures of crude FAME, glycerol and methanol were preheated to 40°C. Enzyme was then added to start the reaction (t=0). Reaction progression was monitored by regularly taking samples (2 mL), which were heated to 100°C for 10 minutes followed by 2 minutes of centrifugation to separate the two liquid phases.

3.2.2. Analysis

Samples were analyzed for acidity in the FAME phase using the AOCS method and calculated as an oleic acid equivalent [21]. Methanol and glycerides were quantified using the Medium Infrared System (Bruker Tensor II) supported by the Eurofins Quality Trait Analysis (QTA) calibration service according to the AOCS specification [22]. Water content was measured using Karl Fischer titration (831 KF Coulometer with 774 Oven Sample Processor) and calculated through component mass balances.

3.2.3. Full factorial design

A full factorial design with three center points was carried out at the 30 g scale using single step reactions to equilibrium with two levels of each factor: 0.5-5 % glycerol, 2-6 % methanol and 0.3-0.8 % enzyme (w/w of crude FAME). Results were used to show the impact of glycerol, methanol and enzyme concentrations on the equilibrium FFA concentration without water removal.

3.2.4. SBR operation

The circulating gas flowrate between the vessels was 0.4 L min^{-1} (or 0.8 vvm (volume of gas per volume of reactor liquid per minute)).

Glycerol was added to a loading of 5 % (w/w crude FAME). Methanol and enzyme loadings were varied and are given in % (w/w crude FAME).

Development and simulation using an empirical mathematical model of the SBR system was implemented and conducted in MATLAB®, using thermodynamic data obtained from literature [23, 24, 25].

3.2.5. Thermal incubation study

Thermal deactivation of enzyme was measured at 30 g scale. First a mixture of biodiesel without added oleic acid, 5 % glycerol and 6 % methanol (w/w of biodiesel) was pre-heated

to the required temperature. Then 4.55 % enzyme (w/w biodiesel) was added, and the mixture incubated for 15 minutes. Following incubation, the mixture was rapidly cooled to 40°C. Then 10 % oleic acid (w/w biodiesel) was added, and the esterification rate was measured by sampling at 15 minute intervals for 1 hour while maintaining the mixture at 40°C. The excessive loading of enzyme and oleic acid were deliberately used to provide rapid rate measurements.

3.2.6. Enzyme reuse study

Centrifuge tubes were used as reactors, and the scale allowed for isolation of the glycerol phase between batches through centrifugation at 1500 rpm for 10 minutes and subsequent manual decanting. After each batch, fresh preheated FAME and methanol were added, and the subsequent esterification reaction mixture was sampled to allow measurement of the reaction rate.

The initial rates of reaction during the first 4 hours of each reaction were obtained through linear regression of the first 3-5 measurements, until the R^2 value was beneath 0.95. The equilibrium FFA concentration after 20 hours was measured for each batch.

3.2.7. Flash VLLE simulations

Flash column VLLE calculations were done computationally using the UNIFAC thermodynamic model in the steady state process simulation software Pro/II [26].

4. Results and discussion

4.1. The effect of glycerol on equilibrium

The effect of glycerol addition was studied in a full factorial experiment with three center points. Table 1 shows the linearized model obtained from the study.

Factor	Estimate	p-value
Intercept	2.686	<0.0001
Glycerol (0.5-5 [%])	-0.211	0.0016
Methanol (2-6 [%])	-0.153	0.0148
Enzyme (0.3-0.8 [%])	1.239	0.0140

Table 1. Estimated linear model parameters and their statistical significance for prediction of equilibrium FFA in wt. %. Loading ranges stated in % (w/w of FAME). Initial FFA concentration in the FAME was 2.9 wt. %, with minimum FFA measured after reaction being 1.3 wt. %. Total water concentrations range from 1700 ppm at 0.3 % (w/w of FAME) enzyme to 3600 ppm at 0.8 % enzyme. Glycerol and methanol have negligible impact on the total water concentration. Equilibrium was reached in 8-16 hours.

Comparing the effect of methanol and glycerol upon the equilibrium FFA concentration, shows the significance of glycerol. Glycerol is currently thought to impact the equilibrium through reduction in the apparent water concentration. Additionally, glycerol addition was found to be essential for the activity of the enzyme, which approached zero without glycerol present (data not shown). We attribute this to the formation of the liquid-liquid interface, upon which the enzyme acts, in the presence of glycerol. As expected, an increase in methanol loading resulted in higher conversion of FFA. For the enzyme dosage we found that the FFA content at equilibrium was higher at higher enzyme loading which we suggest is due to the additional water associated with using a higher enzyme concentration.

Whilst the presence of glycerol reduces the effect of water, it also shifts the equilibrium of the transesterification reaction towards formation of glycerides rather than FAME. We therefore

examined this and found a slight increase in glyceride levels during a single step esterification reaction (without drying) from 0.05 wt. % total glycerin in FAME to 0.25 wt. % at equilibrium in reactions run with a glycerol loading of 5 % (w/w of FAME). This is the precise glycerin limit according to biodiesel specifications (European Standard), but stems primarily from the formation of monoglycerides, which increased from 0.24 to 0.61 wt. %, and glycerol, which increased from an immeasurably low value to 0.09 wt. %. Both were measured using the QTA method. However, the glycerol is reduced in subsequent downstream processing like water washing, resulting in FAME within the biodiesel specification.

4.2. Enzyme and glycerol reuse

Having established a working liquid CALB enzyme system for esterification, we examined enzyme reusability. This is typically the primary benefit of immobilized enzymes, but our proposed liquid enzyme system has a similar advantage, since the enzyme is situated primarily at the phase interface between the two liquid phases. This enables isolation of most of the enzyme with the glycerol phase by decanting/centrifugation. Figure 4 shows the measured esterification rates on sequential batches of FAME. Rates were measured using the standard procedure in 300 g scale batches.

Some enzyme activity is lost as shown in Figure 4, but the majority is recovered. The total enzyme activity loss is around 15 %, meaning around 7.5 % per batch. In summary, the results show excellent enzyme stability in use and the potential for reuse (translating to a low enzyme addition requirement per batch, when operating at steady state industrially).

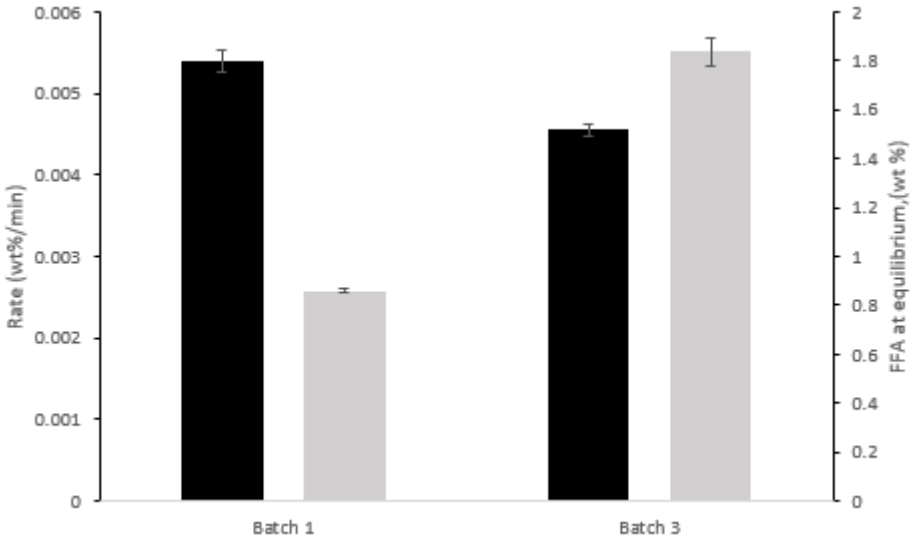


Figure 4. Initial reaction rates (black columns) and final equilibrium FFA concentrations (grey column) in 300 g scale experiments. Initial mixture contained 3.2 wt% FFA with and enzyme loading of 1 % (w/w of FAME) and 5 % added glycerol. In each batch of FAME esterified, 6 % methanol was added.

4.3. Stepwise flash/reaction process

Removal of water can be done through the well-established flashing operation, using the difference in boiling points between water and FAME. A stepwise repeated reaction – flash system would therefore allow the equilibrium to be shifted towards further conversion of FFA, as the model simulation shows in Figure 1.

The results in Figure 5 show reactions at different initial FFA and water concentrations, and the corresponding FFA conversions. The figure can be used to estimate the number of reaction-flash steps required. For example, a reaction starting with 3.6 wt. % FFA reaches equilibrium at 0.9 wt. % FFA, subsequent drying and a second reaction with fresh methanol would achieve an FFA concentration beneath 0.5 wt. %. In fact, these reactions were conducted at elevated water concentrations relative to those obtainable through biodiesel flashing industrially. At industrial scale, water would be controlled by an additional flash prior to the reaction. Furthermore, in an industrial plant, less enzyme would be added (and hence

less water) since glycerol (with enzyme) would likely be recycled. This means that in an industrial plant even lower FFA concentrations can be expected, than those predicted from Figure 5.

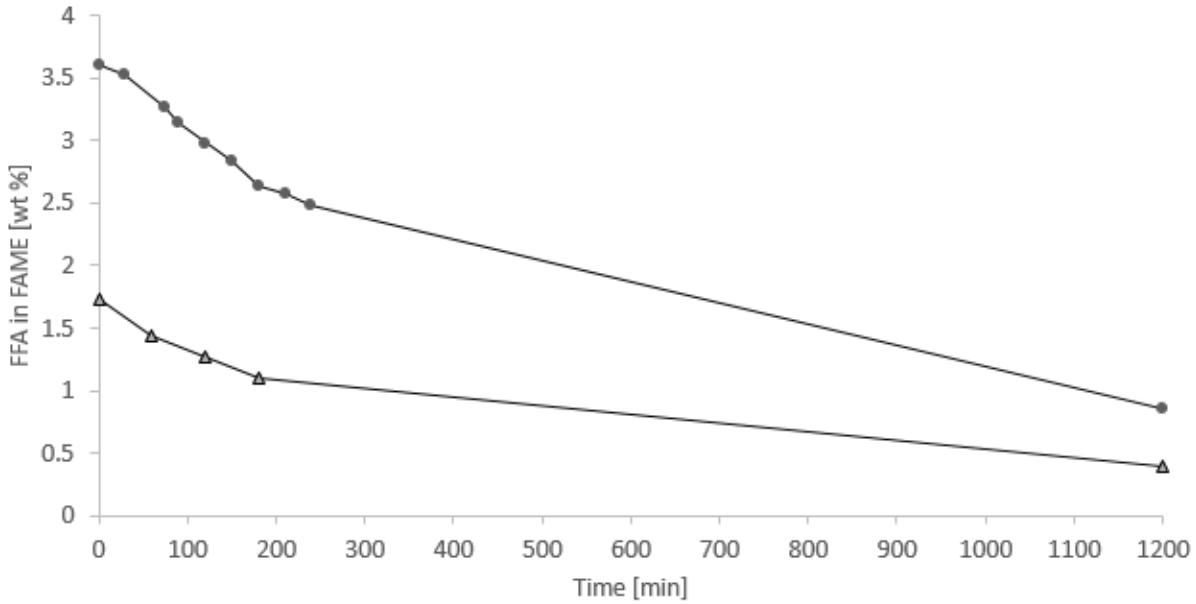


Figure 5. Reactions imitating first and second step reactions at high initial water concentrations. Circles: 1 % NS88007, 5 % glycerol, 4 % methanol, 300 g FAME, Initial water ≈ 5500 ppm. Triangles: 0.455 % NS88007, 5 % glycerol, 6 % methanol, 30 g FAME, Initial water ≈ 2000 ppm.

4.4. CALB thermal stability in flash columns

Through flash simulations, it was established that temperatures above 50°C, along with industrially feasible pressures of around 0.15 bar would be necessary to obtain effective water removal. Additionally, a maximum of 15 minutes residence time was assumed necessary in the flash columns. To obtain the maximum allowable temperature with respect to enzyme stability, a thermal incubation study was conducted. As shown in Figure 6, CALB was found thermally stable, with no activity loss measured after 15 minutes at 55°C. This set the maximum operating temperature for subsequent flash simulations.

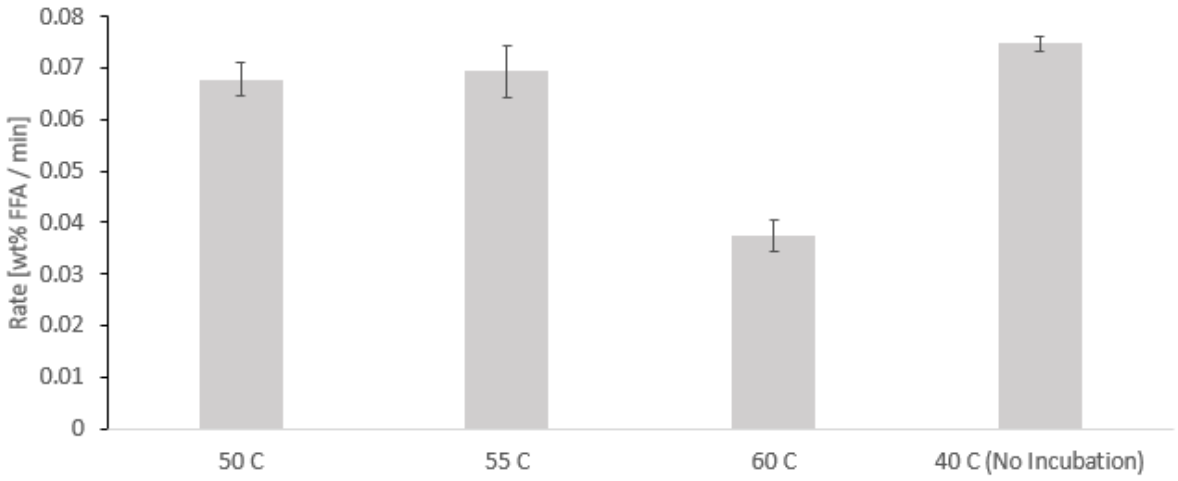


Figure 6. Thermal incubation study. 30 g scale reactions, 15 minutes incubation at indicated temperatures with 6 % methanol, 5 % glycerol, and 4.55 % NS88007 (w/w of FAME). FFA to 10 wt. % was added prior to rate measurements. Rate measured at 40°C.

4.5. Flash column simulations

Utilization of the UNIFAC thermodynamic model for simulation of phase equilibria yielded flash column simulations [26]. A simple one-step flash column was simulated operating at an inlet temperature of 55°C and a pressure of 0.15 bar in the column, with an inlet feed consisting of 32.64 % M-palmitate, 5.87 wt. % M-stearate, 41.00 wt. % M-oleate, 7.65 wt. % M-linoleate, 4.49 wt. % glycerol, 5.39 wt. % methanol, 2.70 wt. % FFA (oleic acid), and 2500 ppm water, simulating wet crude FAME (as if directly from the Eversa Transform transesterification reaction vessel) with addition of 5 % glycerol and 6 % methanol (w/w of crude FAME). This resulted in a water concentration of 963 ppm in the liquid flash column outlet, but also reduced the methanol concentration to 2.28 wt. %. More efficient water removal was obtained through a recycle loop around the flash column and heat exchanger, giving an equilibrium water concentration of 500 ppm when recycling 60 % of the liquid outlet. This means the water concentration might be reduced significantly beneath that required to obtain in specification FAME (<0.25 wt. % FFA), especially when considering the results of Figure 5, where 0.40 wt. % FFA was measured after a reaction starting from 1.73 wt. % FFA and around 2000 ppm water (calculated from mass balances).

4.6. Continuous SBR Process

In a similar manner, as the above proposed stepwise flash process, the utilization of a stirred bubble reactor has also been examined. Using an equal volume of methanol and FAME the overall mass transfer coefficient (k_La) of the system was estimated to be 0.60 h^{-1}, assuming the system would be able to remove all water present, without any reaction occurring.

Results from a proof-of-concept experiment are shown in Figure 7. As explained previously, as a result of bubbling the reaction mixture with relatively dry methanol-rich gas, the FFA concentration decreases. The methanol concentration stabilizes at a concentration of 2 wt. % while the FFA concentration decreases over time. The reaction was concluded after 7 hours when the FFA concentration fell beneath 0.5 wt. %.

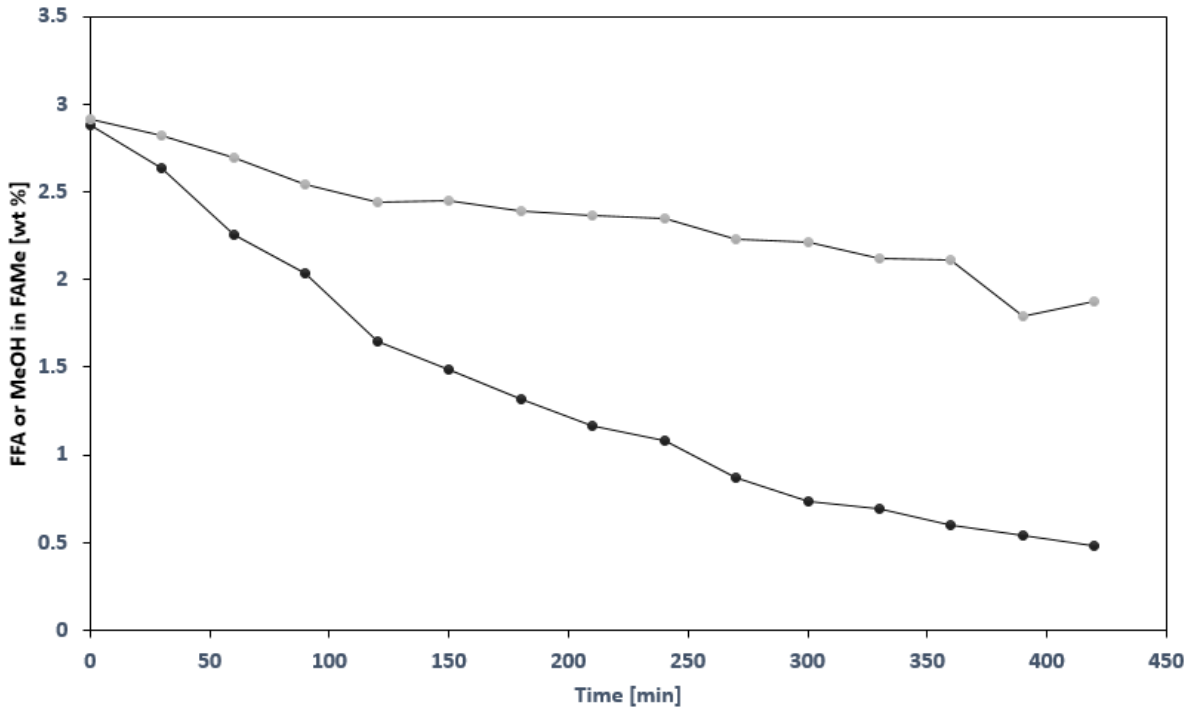


Figure 7. Simultaneous reaction and water removal using the SBR method. Light grey dots are representing methanol concentration [wt. %] and black dots are representing FFA concentration [wt. %]. Conducted using 450 g B100 Biodiesel spiked to 3 wt. % FFA oleic acid, 4 % (w/w of FAME and oleic acid) methanol, an equal volume of methanol

corresponding to 500 mL in the condensing vessel and enzyme concentration of 0.455 % (w/w of FAME and oleic acid) NS88007 with 5 % (w/w of FAME and oleic acid) of glycerol.

These results confirm that the process should be able to ensure a final FFA concentration within the specification limits. The process relies on a range of parameters, which were then examined through modelling relating mass balances, partial pressures, gas flowrates and solubility characteristics. The model allows for calculation of the concentration change of water and methanol in both the esterification reactor and the condenser, while considering the temperature dependency of the relevant partial pressures. Equation (1) shows the balance over the esterification vessel of a component i.

$$\frac{\mathrm{d}C_{R,i}(t)}{\mathrm{d}t} = \frac{Q_g \ \mathrm{M}_i \ \rho_R}{R \ V_R} \left(\frac{\pi_{C,i}}{T_C} - \frac{\pi_{R,i}}{T_R} \right) \quad \left[\frac{\mathrm{wt\%}}{\mathrm{s}} \right] \tag{1}$$

Where $C_{R,i}$ and $\pi_{j,i}$ are the mass concentration [kg/m^3] and partial pressure [Pa] of component i, T_j is the absolute temperature [K] in reactor or condenser. Q_g is the volumetric gas flowrate [kg/(m^3 s)] and M_i is the molar mass of component i [kg/mol]. Using this as the basis, in the complete system there are two equations for each component, since a similar equation for the condenser can also be applied (with opposing signs for the driving force). The partial pressure of component i is assumed to be related to the molar liquid fraction of the component. Using the modified and extended Raoult's law, the vapor pressure of a component in a mixture can be described as in equation (2):

$$\pi_i = \gamma_i x_i p_i^o \tag{2}$$

Where x_i and γ_i are the molar ratio and activity of component i, respectively, and $p^o{}_i$ is the vapor pressure of the pure component, which is calculated by the Antoine equation. The thermodynamic activity of the different components was estimated using solubility

parameters and molar volumes from the Wilson Equation for binary systems [23]. Relevant physicochemical parameters for the modelling are shown in Table A1 in the appendix.

It should be noted that no binary interaction parameters describing the interaction in a FAME-water system have been obtainable from scientific literature. Hence, water was modelled as ideal in the reactor and it was assumed that only methanol and water would be volatile, so no evaporation of FAME occurs. To simplify the model, terms for reaction and mass transfer rates were excluded, meaning the model works on an arbitrary timescale.

Simulation of the SBR system was done in MATLAB®. Simulations were run using a methanol mass fraction of 0.2 (w/w) and an initial water concentration of 3000 ppm while varying the temperature difference between tanks. This resulted in the plot shown in Figure 8.

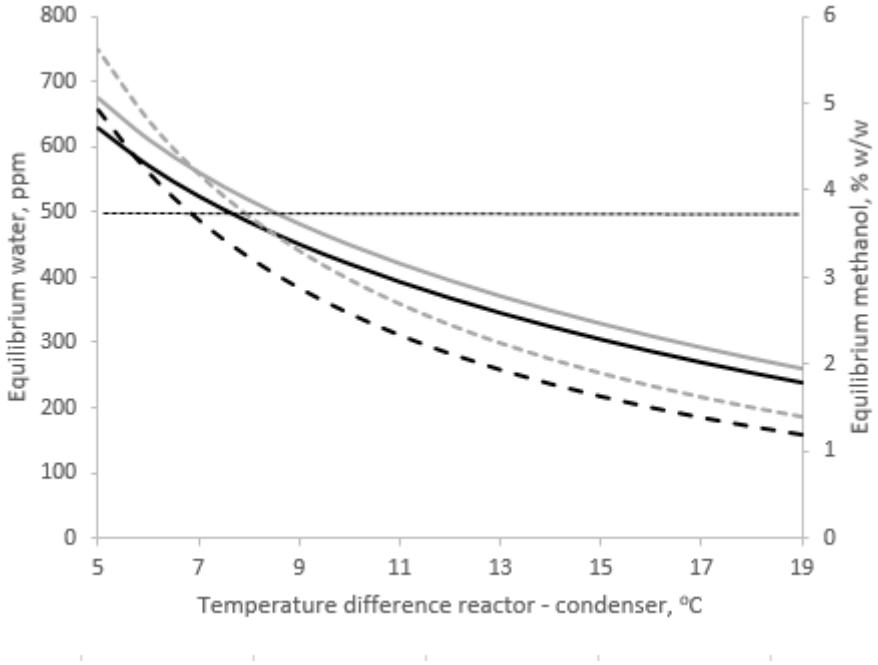


Figure 8. Simulation results on water (Solid lines) and methanol (Dashed lines) equilibria when variating the condenser temperature at either a reactor temperature of 40 or 50°C (Light-grey and black lines, respectively) at a methanol mass fraction of 0.2 (w/w) and initial water concentration of 3000 ppm.

Figure 8 shows that increasing the reactor temperature, T_R, from 40 to 50°C hardly influences the attainable equilibrium. This suggests that increasing the reactor temperature should only be done if a significant increase in enzymatic activity is observed. In contrast, variation in the condenser temperature shows a larger effect. Indeed, keeping the temperature difference small results in higher methanol and water concentrations and therefore faster reaction rates, while increasing the temperature difference reduces the water and methanol concentrations. At a temperature difference of 8°C (reactor at 40°C and condenser at 32°C) and a methanol mass fraction of 0.2 (w/w) it is estimated that the system should be able to achieve a water concentration of 500 ppm from an initial concentration of 3000 ppm. The rate of mass transfer will be governed by the volumetric gas flowrate and the driving force (difference in vapor pressure).

Potentially, operating the system in an intermittent manner i.e. drying by bubbling for a limited time, followed by reaction (throughout which water will accumulate) and subsequent drying, might be one way to achieve a high driving force and reaction rate. A high driving force could be maintained by a substantial temperature difference between the reactors, while adding methanol in between gassing stages. The addition of methanol would be required due the stripping of methanol from the reactor while at a high temperature difference (Figure 8).

5. Industrial implementation

The results presented in this study indicate the viability of enzymatic esterification to reduce the FFA concentration to that required for in-specification biodiesel. For the first time this represents a wholly enzymatic solution for the commercial and sustainable production of biodiesel. In the following section we discuss the remaining issues for industrial implementation.

5.1. Process Flowsheets

Figures 9 and 10 are the proposed layouts for flash and SBR processes, respectively. Both processes are shown here as an extension of the current Eversa Transform 2.0 transesterification, which has been documented previously and already commercialized. Following the Eversa Transform transesterification, the heavy (aqueous enzyme-rich) phase should be decanted prior to entering the subsequent esterification polishing process.

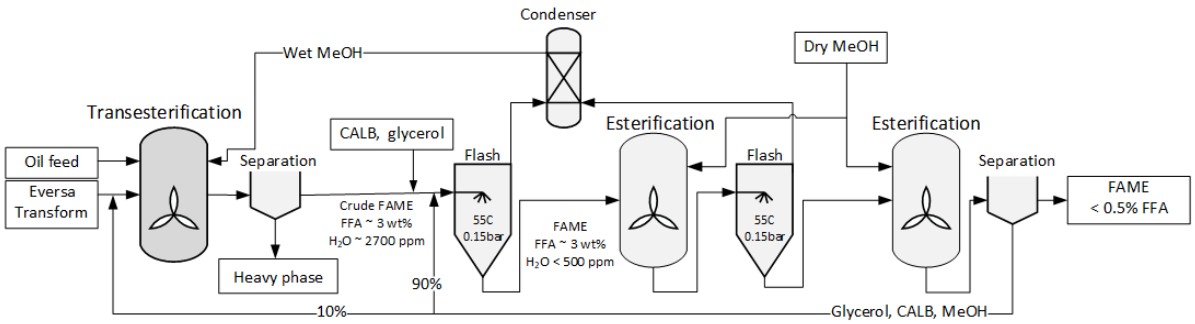


Figure 9. Stepwise esterification and flash process. Reactions are experimentally proven, while the flash columns have only been simulated until now. This process can operate both as batch or continuously using CSTRs with increased size and residence time.

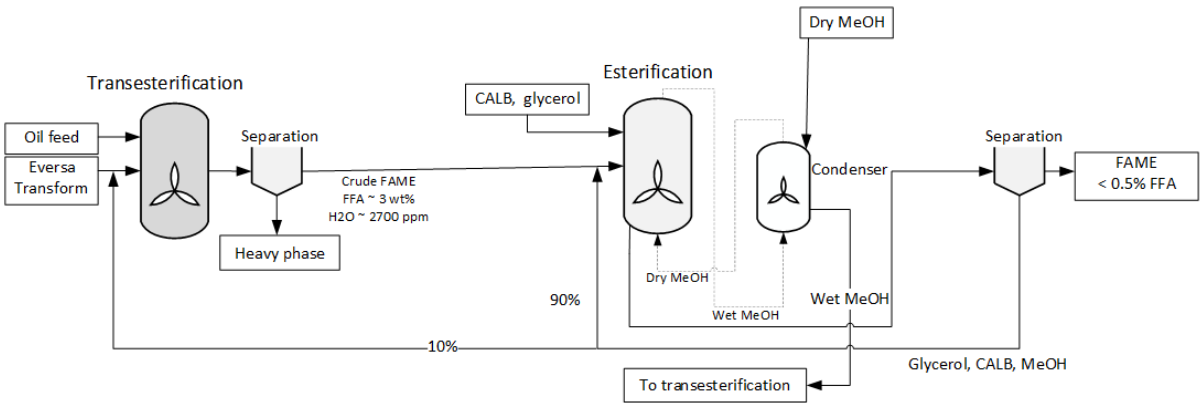


Figure 10. SBR process flowsheet. Currently only considered as a batch process.

The reaction results shown in Figure 5 (esterification with flash drying) suggest that only 2 esterification steps would be required, and with the flash column operation obtained from the UNIFAC model, a simple system for stepwise esterification and flashing could be designed as shown in Figure 9. After the Eversa Transform transesterification reaction, the water rich

components should be mixed and flashed to reduce the water concentration prior to the reaction. After the first reactor, residual FFA has been converted to FAME giving an increased water concentration. A flashing step then removes a significant amount of this water (and methanol), thereby shifting the equilibrium towards further FFA conversion in the second reactor. Finally, a decanting step is used to recycle most of the enzyme and glycerol, whilst removing a fraction through a purge in order to reduce the accumulation of undesired byproducts. Additionally, methanol rich vapor from the flash columns and the purged enzyme/glycerol phase are recyclable to the Eversa Transform reaction step. Experiments (not shown) suggest 8-16 hours of reactor residence time per stepwise reaction would be necessary depending on whether continuous or batch reactors are used and dependent upon enzyme loading. Reactor volumes would then depend on the desired production capacity and the choice of operating mode. These times are commercially realistic and give viable reactor sizes (and of similar magnitude to those currently employed in industrial enzymatic biodiesel process (27)).

The simulated results shown in Figure 8 use a batch reaction using the SBR process, and on this basis a single batch would be sufficient for reduction of the FFA to beneath 0.5 wt. %. With increased residence time and addition of dry methanol, the equilibrium might even be shifted within specification FFA concentrations. Figure 10 shows the proposed batch operating system, where the same recycling principles as in the stepwise flash system, have been used. Net reaction time will be shorter in this system, because batch operation is proposed.

5.2. Benefits from reducing the size of the caustic wash

Existing plants currently recycle soap stock stemming from the caustic wash. A significant portion of the FAME is trapped when separating the soap, thereby reducing overall productivity. The recycled soap is acidified (with sulphuric acid) to yield FFA. This also represents an added cost. Therefore, the proposed FFA esterification processes will not only

increase the productivity, but also reduce or remove the need for caustic and sulphuric acid additives, which also reduces waste.

5.3. Achieving a biodiesel product within specification

An important point is the European specification on FFA concentration of 0.25 wt. % (or 0.5 mg KOH/g FAME). The proposed SBR process has been shown to reach the specified FFA concentration, as previously described, while the flash process has not. The lowest FFA concentration reached in the flash process was around 0.40 wt. %, which was reached at elevated water concentrations relative to that obtainable industrially (implying better results would be expected industrially). Therefore, a caustic wash might be necessary to reach the specified FFA concentrations with the flash process. Additionally, the caustic wash is also sometimes used for removal of sulphur compounds and other impurities, such as glycerides, and addition of caustic might therefore still be required (although much reduced), regardless of the FFA concentration.

5.4. Reuse of enzyme

Most of the enzyme activity was found recyclable by decanting the glycerol phase. This lowers enzyme cost, the amount of water added through liquid enzyme addition, and likewise reduces the costs associated with addition of dry glycerol. The experimental results shown in Figure 4 indicated a 7.5% activity loss per batch, suggesting that a 90% recycle should be possible. Reuse is thus a key part of the proposed processes, since the economics are highly dependent on it. It should be noted that the experiments conducted in this work have been carried out at an enzyme load of 0.455 wt. % corresponding to an expected economically viable steady-state concentration, assuming five reuses.

6. Future optimization

Optimization of both processes is still possible and includes selection of temperature, pH, mixing, glycerol and methanol loadings as well as methanol addition regimes. The experiments reported here were all run at 40°C, but higher temperatures might also be feasible. It is already known that the thermal stability of CALB is highly dependent on the concentration of methanol, and this is a further area of optimization. Likewise, in other experiments (data not shown) different FAME qualities (ranging from biodiesel to crude FAME stemming from sludge palm oil) have been examined. It was generally found that higher FAME quality gave higher yield, indicating that the apparent equilibrium is influenced by the FAME quality. It may also be that the acid titration method systematically results in overestimation of FFA concentration due to the presence of acidic byproducts in lower quality FAME. Finally, both processes are en-route to pilot scale testing.

7. Concluding remarks

We have presented a novel enzymatic FFA esterification reaction utilizing liquid CALB enzyme along with glycerol at low water concentrations. The system is capable of reducing FFA concentrations in crude biodiesel to levels close to, or within, specification. On this basis, we then proposed two comprehensive processes for the conversion of residual FFA in crude biodiesel stemming from the enzymatically-catalyzed biodiesel production (Eversa Transform transesterification). One system uses a stepwise reaction-flash. The other uses a stirred bubble reactor system, where dry methanol gas is bubbled through the FAME, thereby reducing the water concentration, yielding a continuous shift in equilibrium towards esterification of FFA. Both of the proposed processes have been found capable of significantly reducing waste generation and will reduce (or eliminate) the need for caustic washing. Soap recovery requirements will be lowered, and as a result, the productivity of existing and future biodiesel plants can be improved. Hopefully, this will result in a positive shift towards more sustainable biodiesel production.

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1 **Appendix**

2 Table A1: Physical and Chemical Data used for modelling of SBR.

	Value	**Unit**		
Density Reaction (FAME)	900	kg/m^3		
Density condenser (MeOH)	791	kg/m^3		
Water molar mass	18.02	g/mol		
Methanol molar mass	32.04	g/mol		
FAME molar mass	269	g/mol		
Universal gas constant	8.31447	J/(mol*K)		
Physical properties of pure substances (Kobuchi et al., 2011)				
	Water	**Methanol**	**FAME**	**Unit**
Molar volume at 25 °C	18.01	40.7		[cm3・mol-1]
Molar volume at tb	18.08	42.08		[cm3・mol-1]
Solubility at 25 °C	47.9	28.2		[(J・cm-3)^0.5]
Normal boiling point	100.001	64.511		[℃]
Constants of Antoine's equation (Dortmund 2019)				
A	807.131	8.0897		
B	1730.63	1582.27		
C	233.426	239.07		

Binary interaction parameters (Kobuchi et al. 2011)			
ε(i,j)	0.0959	0.1512	
Interaction energy parameters (Felice et al. 2008)			
λ(i,j)	5550	3338	[J・mol-1]

Latex Code for equations

Model:

```
\begin{equation}
    \dv{C_{R,i} (t)}{t}=\frac{Q_g\ \text{M}_{i}\ \rho_R}{R\ V_R}\ \left(\frac{\pi_{C,i}}{T_C}-
\frac{\pi_{R,i}}{T_R}\right)\ \quad \left[\frac{\text{wt}\%}{\text{s}}\right]
\end{equation}
```

Extended Raoult's Law:

```
\begin{equation}
    \pi_{j,i}=x_{j,i}\ p_i^o
\end{equation}
```