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Scale-up of industrial biodiesel production to 40 m^3 using a liquid lipase formulation†

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Abstract

In this work, we demonstrate the scale-up from an 80 L fed-batch scale to 40 m^3 along with the design of a 4 m^3continuous process for enzymatic biodiesel production catalysed by NS-40116 (a liquid formulation of a modified *Thermomyces lanuginosus* lipase). Based on the analysis of actual pilot plant data for the transesterification of used cooking oil and brown grease, we propose a method applying first order integral analysis to fed-batch data based on either the bound glycerol or free fatty acid content in the oil. This method greatly simplifies the modelling process and gives an indication of the effect of mixing at the various scales (80L to 40m^3) along with the prediction of the residence time needed to reach a desired conversion in a CSTR.

Suitable process metrics reflecting commercial performance such as the reaction time, enzyme efficiency and reactor productivity were evaluated for both the fed-batch and CSTR cases. Given similar operating conditions, the CSTR operation on average, has a reaction time which is 1.3 times greater than the fed-batch operation.

We also showed how the process metrics can be used to quickly estimate the selling price of the enzyme. Assuming a biodiesel selling price of 0.6 USD/kg and a one-time use of the enzyme (0.1 % (w/w_{oil}) enzyme dosage); the enzyme can then be sold for 30 USD/kg which ensures that that the enzyme cost is not more than 5 % of the biodiesel revenue. This article is protected by copyright. All rights reserved

Keywords: Continuous bioprocess, Scale-up, Fed-batch, Enzymatic Biodiesel

Introduction

Over the past decade, biodiesel has attracted considerable attention as a renewable, biodegradable substitute for petroleum based diesel fuel (Brask et al., 2011; Meher et al., 2006; Ranganathan et al., 2008). The term biodiesel refers to the mono alkyl ester of long chain fatty acids derived from vegetable oils or animal fats, for use in compression-ignition engines (Al-Zuhair, 2007). The advantage of biodiesel compared to other alternative biofuels is that it can be blended at any level with petroleum-based diesel to be used in existing diesel engines and infrastructure with practically no changes (Christopher and Zambare, 2014).

The commercial production of biodiesel greatly depends on the starting feedstock. Recently, there has been significant research interest in bio-based gas-to-liquid (GTL) methods to take advantage of the significant portion of unconventional shale gas, tight gas and flared associated gas in North America, Australia and China (Fei et al., 2014; Wood et al., 2012). The idea is to use methanotrophic bacteria which convert the methane in the natural gas into microbial lipids (Fei et al., 2014; Hanson and Hanson, 1996). Such lipids could then be converted to renewable diesel via hydrotreatment (Helwani et al., 2009; Jin et al., 2014). However, bio-based GTL methods are still in their infancy and hence, the main route for the industrial production of biodiesel today is by use of an alkali catalyst to convert high quality vegetable oils and methanol (Ganesan et al., 2009; Gog et al., 2012). Unsurprisingly, the main cost in this production process is the feedstock (Atadashi et al., 2013; Behzadi and Farid, 2007). Hence, to circumvent the use of high cost feedstock and to improve the process economics, several industrial companies that produce biodiesel have recently converted to using an enzymatic process, using a lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) since the enzyme allows the use of alternative, lower cost feedstock. The ability to treat material such as used cooking oil and animal fats gives this process a niche role in being able to turn waste into fuel. In this way, the biodiesel producer has the ability to treat a wide range of low quality/cost feedstocks that have a high free fatty acid (FFA) content that would otherwise require additional pre-treatment steps if a conventional alkaline catalyst were to be used (Fjerbaek et al., 2009; Hwang et al., 2014). Compared to using an alkaline catalyst, lipases are able to esterify the free fatty acid (FFA) (Figure 1a), as well as transesterify the acyl-glycerides (Figure 1b) contained in waste oils to esters (Adlercreutz, 2013; Al-Zuhair, 2008; Nielsen et al., 2008; Pedersen et al., 2014). Also there is efficient substrate utilization by the biocatalyst. For the lipase catalysed process, only 50 % excess methanol (molar basis) is needed to reach over 95 % biodiesel yield (Fjerbaek et al., 2009). An alkaline catalysed process on the other hand uses over 100% excess methanol which substantially increases the downstream recovery cost of the methanol (Meher et al., 2006). The “wet” methanol recovered in the downstream process can have over 10 wt % water (Al-zuhair et al., 2011). The traditional alkaline catalysed process would require significant drying of the methanol before it could be recycled. The enzyme catalysed process on the other hand could utilise this “wet” methanol reducing the processing cost.

Previously reported enzymatic processes for biodiesel production have often employed an immobilized enzyme catalyst (Du et al., 2008; Tan et al., 2010). However, these processes face several challenges. The cost of an immobilized lipase formulation is typically such that it must be reused many times. Investing in immobilized enzyme that needs to be reused and relying on it being stable, with highly varying and often dirty feedstock, is very risky. Compared to using an immobilized lipase, a liquid lipase formulation substantially lowers the cost of the biocatalyst for a given catalytic activity, to the point where one-time use of the catalyst is a viable option (Cesarini et al., 2013). This means that, in a situation where the biocatalyst were to lose significant activity due to poor process operation, the economics of the liquid lipase catalysed process is much less affected compared to using an immobilized biocatalyst. Additionally, in terms of retrofitting conventional alkaline catalysed process, no changes to the rectors need to be made given that the biocatalyst is in

a liquid formulation. The use of a liquid lipase as the biocatalyst thus offers several advantages that contribute to making an economically viable and attractive biodiesel process.
Previous work using a lipase for biodiesel production has been carried out by feeding methanol over the course of the reaction (fed-batch operation) so as to minimize the inhibition and deactivation of the biocatalyst by methanol (Al-Zuhair, 2005; Fedosov et al., 2013; Fjerbaek et al., 2009; Nordblad et al., 2014). In terms of modelling the fed-batch operation, the common trend reported in the scientific literature has been to use virgin oils or "simulated" used cooking oil when mechanistically modelling the transesterification reaction at the lab scale (< 250 mL) (Al-Zuhair et al., 2009; Fedosov et al., 2013; Ricca et al., 2010). Likewise, in our previous work at laboratory scale (2 L), using pure rapeseed oil, we showed how mechanistic modelling can give great insight into how to operate the process (Price et al., 2014). However, we also found that it is quite challenging to model the reaction when we applied our kinetic model to real industrial feedstocks such as used cooking oil (UCO) and brown grease (BrG). We suggest this is due to the presence of other components (e.g. surfactants) found in the oil which the model doesn't take into consideration and would be quite complex to model mechanistically.
In this work we have used pilot plant (80 L and 4 m^3) and plant data (40 m^3) from Viesel Fuel LLC to analyse the performance of enzymatic transesterification (biocatalyst, NS-40116 a liquid formulation of a modified *Thermomyces lanuginosus* lipase) of UCO and BrG. We then propose a simplified modelling approach to predict the reaction performance across scales for UCO and BrG, and show the development of a continuous enzymatic biodiesel process.
The simplified modelling approach was based on integral analysis (assuming first order behaviour) of fed-batch data for UCO and BrG. For UCO, the analysis was based on the bound glycerol content (BG, mass % of the glycerol moiety in the unconverted acyl glycerides) over the course of the reaction. For BrG the analysis is based on FFA instead of BG (BrG had a FFA content > 55%).
Recent reviews of the state of the art in enzymatic biodiesel production failed to mention the commercial viability of using a liquid lipase as a catalyst (Christopher and Zambare, 2014; Hwang et al., 2014). Hence, along with the simplified modelling strategy, we also report the key process metrics for the reaction (final BG and FFA value achieved (mass %), reactor productivity ($g.L_{reactor}^{-1}.h^{-1}$) and biocatalyst yield ($g_{product}.g_{biocatalyst}^{-1}$)) that influence the commercial viability of the process. Also, we show how the process metrics can be used by the enzyme producer to competitively price the enzyme; given that in this case the selling price of the enzyme is based on the value added to the product, compared to the actual cost of manufacturing the enzyme.

Materials and Methods

Chemicals and Biocatalyst

For the pilot and plant runs the UCO feedstock had a typical FFA content of < 15 % (w/w_{oil}), with the acyl glycerides (triglyceride (TAG), diglyceride (DAG) and monoglyceride (MAG)) making up the remainder. The BrG feedstock had a typical FFA content > 55 % (w/w_{oil}), with the acyl glycerides making up the remainder. Methanol (97.8%) was used as an acyl donor and an experimental thermostable enzyme formulation NS-40116 (NS) from Novozymes A/S (Bagsværd, Denmark) was used to catalyse the reaction.

Pilot plant fed-batch runs

The fed-batch pilot plant runs were performed in 80 L and 4 m^3 tanks. The reactor was charged with the oil feed, water (1-2 % (w/w_{oil})) and enzyme (0.067-0.2 % (w/w_{oil})) (Note, for BrG as feedstock approximately 50 % of the water was replaced with glycerol). Mineral acids in the oil were neutralized as described previously (Brask et al., 2014). Methanol was continuously fed to the reactors and was maintained beneath 20 mass % methanol in the heavy phase (comprised of water, glycerol, methanol and enzyme). Mixing was performed by using hydrodynamic mixers with a

specific power input of 1.0 W/L and 0.12 W/L respectively (Note: given the use of hydrodynamic mixers, the specific power input is estimated from the Hydraulic Pump Power/working volume in the reactor (Perry, 2007)). An external heat exchanger was used to maintain an average temperature of 35 ^{o}C in the tanks.

Plant fed-batch run

The fed-batch plant run was performed in a 40 m^3 reactor with a working volume of approximately 34 m^3. The reactor was charged with the oil feed, water (1-2 % (w/w_{oil})) and enzyme (0.64 % (w/w_{oil})). Methanol was continuously fed to the reactors and was kept below 20 mass % methanol in the heavy phase. Mixing was carried out using hydrodynamic mixers with a specific power input 0.15 W/L. An external heat exchanger was used to maintain an average temperature of 35 ^{o}C in the tanks.

Pilot plant CSTR runs

When the fed-batch reaction in the pilot plant neared completion the 4 x 4 m^3 tanks were set in series and operated continuously (see Figure 2). The oil flowrate was 2.04 ± 0.11 L/min throughout the reaction process. Mixing was performed by using hydrodynamic mixers where the enzyme (0.1 % (w/w_{oil})) and water (1-2 % (w/w_{oil})) were continuously fed into the suction side of the hydrodynamic mixer's pump. Methanol was continuously fed to all reactors and was maintained beneath 20 mass % methanol in the heavy phase.

Sample analysis

Throughout the reaction 15 mL samples were taken at regular intervals to monitor the progress of the reaction. The 15 mL samples were centrifuged at 500 rpm for 2 min to give an upper oil phase and a lower polar phase. A sample from each phase is then analysed using Quality Trait Analysis (QTA) based on infrared spectrometry. The infrared spectra of the samples were read from a Bruker Tensor 27 FT-IR (Massachusetts, USA) and analysed by the Eurofins QTA central processor. For the upper oil phase results are returned for the TAG, DAG, MAG and FFA in mass % and for the lower polar phase results are returned for water, methanol and glycerol in mass %. It should be noted at the time of writing the reported calibration ranges for TAG, DAG, MAG and FFA in the upper oil phase are 0-9.9, 0-5.5, 0-2.9 and 0-80 mass % respectively. Subsequently the BG value was calculated according to ASTM D6584 using Equation 1.

$$BG = 0.2591MAG + 0.1488DAG + 0.1044TAG \quad (1)$$

Additionally, the fatty acid methyl ester (FAME) is determined only at the end of the reaction using a GC-2014 gas chromatography (Shimadzu, Kyoto, Japan) to ensure the biodiesel is within specification (>96.5 mass % FAME).

Results and Discussion

First order analysis and evaluation of the apparent rate constant (k_{app})

It is well documented in the scientific literature that the enzyme catalysed transesterification reaction to produce biodiesel (FAME) proceeds via the Ping-Pong Bi-Bi mechanism (Cheirsilp et al., 2008; Fedosov et al., 2013; Fjerbaek et al., 2009; Tran et al., 2014). However, we noticed from our results that the majority of the reaction could in fact be described solely by first order kinetics, greatly simplifying analysis. To easily visualise the first order behaviour, an integral analysis test as described by Levenspiel was used (Levenspiel, 1999). In our previous work we evaluated the use of Callera™ Trans L (a liquid formulation of a modified *Thermomyces lanuginosus* lipase) on the

transesterification of rapeseed oil to produce FAME (Price et al., 2014). Based on these data, a plot of $\ln(BG_o/BG)$ vs. t, for the transesterification of rapeseed oil using 50 % excess methanol, 0.55 % (w/w_{oil}) Callera™ Trans L and 5 % (w/w_{oil}) water, was prepared and is presented in Figure 3a. Interestingly, there is a curvature at the start (0-5 hrs) which suggests a more complex reaction mechanism. However, we also noticed that after 30 % conversion of the BG, the reaction appeared to be first order with respect to BG a indicated by the linear portion of the plot. This simplification is most likely be due to the fact that methanol was fed throughout the reaction to maintain the methanol concentration in the heavy phase. Hence, as the reaction proceeded and BG was consumed, the reaction appears to be pseudo-first order with respect to BG at the lower BG concentrations. In this way an apparent rate constant, k_{app} for the reaction can easily be determined from the gradient of the line. Alternatively the BG vs. t data could be fitted to Equation 2.

$$BG = BG_o' e^{-k_{app}t} \quad (2)$$

In both cases a k_{app} value of 0.128 h^{-1} was found as illustrated in Figure 3a and Figure 3b.
This analysis was then applied to the pilot plant data (80 L and 4m^3 reactor) and is illustrated in Figure 4 for the transesterification of UCO using 20 % excess methanol and 0.1 % (w/w_{oil}) NS-40116. The 80 L and 4m^3 reactor showed a k_{app} value of 0.067 h^{-1} and 0.029 h^{-1} respectively. It can clearly be seen that the reaction rate in the 80 L reactor is more than double the reaction rate in the 4m^3 reactor. The main difference between the two systems is the specific power input for mixing which is approximately 1 W/L and 0.12 W/L in the 80 L reactor and 4m^3 reactor respectively. We suggest that at the higher power input in the 80 L reactor, there is a smaller average droplet size (giving a larger specific interfacial area). This is also seen in the work reported by Al-Zuhair and co-workers, where increased agitation (power input) caused the average droplet size to get smaller (Al-Zuhair et al., 2004). Hence, given that the enzyme is interfacially activated, this means there is more enzyme at the interface able to react, which results in a faster rate of reaction (Hermansyah et al., 2006; Jurado et al., 2008; Nordblad et al., 2014; Reis et al., 2009).
The effect of increasing the enzyme concentration on the k_{app} value in the 80 L reactor with UCO as feedstock is illustrated in Figure 5. Below 0.15 % (w/w_{oil}) NS-40116, k_{app} is directly proportional to the enzyme concentration. At 0.2 % (w/w_{oil}) NS-40116 the proportionality was broken, at which point we suggest the available interfacial area limits the reaction rate.
The same first order analysis used for UCO can also be applied to BrG. The key to the method is using a measurement that best characterizes the changes in the oil over the course of the reaction. Since BrG has a higher FFA content than UCO, the first order analysis in this case was based on residual FFA content. This is illustrated in Figure 6 for the transesterification of BrG in the 80 L reactor using 20 % excess methanol and 0.1 % (w/w_{oil}) NS-40116, where a k_{app} value of 0.031 h^{-1} was estimated. The apparent rate found for the BrG feedstock is almost half that of UCO feedstock. This may be due to the composition of the heavy phase since the high FFA feedstock contains more glycerol than water (so as to minimize the hydrolysis reaction), and hence has an adverse effect on the reaction rate. A similar observation was also reported by Pedersen and co-workers where they reported a reduction in the initial reaction rate when water was substituted with glycerol in the heavy phase (Pedersen et al., 2014). In this case, although the desired end point FFA value of 0.25 mass % FFA was not met in the reaction step, it can easily be achieved at the end of the downstream operation (e.g. by using a caustic wash step) to produce biodiesel that is within specification.
As illustrated in the various cases the estimation of the k_{app} value serves two purposes. First, one is able to characterise the apparent rate of reaction for a given type of oil based on the degree of mixing (specific power input) in the reactor. For example, the power input to the 4 m^3 and 40 m^3 can easily be increased by a factor of 4 (while still having a reasonable power input < 1W/L). This should make more efficient use of the enzyme activity, by reducing the reaction time of the fed-batch reaction. Second, one is able to characterise the apparent rate of reaction for different types of oil. For

example, when screening new oil feedstock's to use in the plant a small fed-batch reaction could be performed to evaluate how the new feedstock will perform at production scale.
The above method works for the fed-batch case and in the following we extend this to continuous biodiesel production where we present the results for the continuous operation and associated model-based simulations.

Continuous operation and model-based simulations
The steady state BG values in the pilot plant along with the BG predictions made using various values of τ are illustrated in Figure 7. The series of CSTRs were simulated using Equation 4

(following section) which assumes first order behaviour for the entire reaction. Likewise, a QTA measurement of 0.39 mass % BG was used to signify that the BG was within specification.
In the pilot plant CSTR run, it is possible to meet the BG specification using 4 tanks with a residence time of approximately 22.5 hours in each tank (overall τ of 90 hrs) and having a k_{app} of 0.029 h^{-1}. The simulations of the plant data, based on the pilot plant estimations also correspond quite well to the actual plant data. Nevertheless, the prediction for the first tank is quite poor, most likely due to using the first order assumption for the entire reaction and is a drawback of the method.
Furthermore, it was observed that adding a tank to the series and maintaining the same total residence time (5 tanks with a τ of 18 hrs in each tank) results in a 9 % reduction of BG in the process output. Investing in one more tank incurs added capital expenditure but gives the added flexibility to handle higher BG feedstock. Alternatively, by having a shorter residence time of 15 hrs in each tank (overall τ of 60 hrs) and increasing k_{app} from 0.029 to 0.06 h^{-1} the BG specification could also be achieved. However, this comes at a cost, given that the mixing power input would need to be increased for k_{app} to go from 0.029 to 0.06 h^{-1}. Likewise, it should be noted that increased mixing may have an impact on the enzyme stability which still needs to be evaluated. Nevertheless, this processing option has an overall τ which is 33 % less compared to the pilot plant CSTR run, which directly translates to an increase in the plant throughput of 33%.
Overall, the method greatly simplifies the modelling process and allows the quick evaluation of different CSTR processing options.

Proposed methodology for process operation and evaluation
Based on the results presented in the previous sections a generic methodology for process operation and evaluation is illustrated in Figure 8. The methodology shows how to evaluate reactor performance across various scales along with using the method to predict continuous operation. The method is decomposed into four main steps; feedstock evaluation, reaction, calculation and analysis/validation/prediction.
Feedstock evaluation: For any new oil feedstock that is obtained, it is important to characterize the inbound raw materials. Some of the critical feedstock measurements are pH, FFA, BG, insolubles, solid particle size, moisture, melting point, metal and sulphur content. However, in step (1) of the methodology, the important values are the FFA and BG content in the oil, given that these values dictate how much methanol is needed to complete the reaction in step (2) and the type of analysis that would be performed in step (3).
Reaction: The transesterification of the oil is performed as a fed-batch operation and the BG and FFA concentration measured over the entire course of the reaction along with the total time of the fed-batch reaction (t_{FB}). This is done at the various scales using the same initial conditions for the

reaction (temperature, oil:alcohol molar ratio, water content and enzyme amount). However given that the reaction system is biphasic and that the biocatalyst is interfacially activated, having the same type of mixing (more specifically the same liquid-liquid interfacial area) at the various scales is also required. Using the specific power (W/L) for mixing is a good guide, but is rarely ideal for scale-up,

since differences in density and recirculation time will affect droplet coalescence and dispersion (Paul et al., 2004).

Calculation: From the analysis performed in step (1), it is already known if the BG or FFA is at a higher concentration at the start of the reaction (C_o [mass %]). Hence, given that the latter part of the reaction can be described by assuming the reaction is first order in C (concentration of BG or FFA); either step 3a or 3b is chosen based on the component (BG or FFA) which is in higher concentration. The experimental C vs. t data can then be fitted to Equation 3.

$$C = C_o' e^{-k_{app} t} \quad (3)$$

Where C_o' [mass %] accounts for start of the reaction not being first order and k_{app} [1/hr] is the apparent rate constant for the reaction.

Analysis and prediction: With k_{app} obtained in step (4) we now have a second process metric (t_{FB} being the first). In step (5a) if the values of k_{app} at the various scales are significantly different for the same reaction conditions it then gives a strong indication that mixing may be an issue. For example, if k_{app} is much smaller in the plant compared to the pilot plant, the mixing intensity should be increased. However, this may not be practical. Therefore, to be able to use the pilot plant results to make predictions about plant scale operation may require the reduction of the mixing intensity in the pilot scale, using a scale-down approach. After mixing intensity is adjusted, there will then be a need to go back to step (2) and perform the reaction at the pilot/plant scale. Once the values of k_{app} are similar in the pilot plant and the plant it then gives confidence to be able to use the pilot plant reactor to perform feedstock evaluation and testing of the different types of oil feedstock (step (5b)).

In step (5c), assuming first order behaviour for the entire reaction, then BG_0', k_{app} and the residence time in the CSTR (τ) can be used to predict for CSTR operation, the BG value in each reactor n, as shown in Equation 4.

$$BG_n = \left(\frac{BG_0'}{(1+\tau k_{app})^n}\right) \quad (4)$$

BG_0' and k_{app} are obtained from Equation 2 and plots of BG vs number of tanks for various values of τ can then be used to quickly determine the operating conditions necessary to run the continuous process along with evaluation of the process.

Process metrics for the fed-batch and continuous system

This data presented in this study present a unique opportunity to compare and analyse some of the commercially important process metrics for the fed-batch and continuous enzymatic biodiesel reaction. Such a comparison can also help answer some important questions form an industrial perspective. For example, assuming a biodiesel producer has 4 reactors of the same capacity, which operation mode, (fed-batch or CSTR) will be most beneficial? For the analysis, the process metrics

are determined from actual pilot plant data for the fed-batch and CSTR operation using 0.1 mass % enzyme (a single use of the enzyme is assumed where the mass is based on the weight of the enzyme formulation).

As seen in Table 1, to reach a similar BG value at the end of the reaction, the residence time in the CSTR will need to be greater than the reaction time in the fed-batch, since for any positive order reaction $\tau > t_{FB}$ (Levenspiel, 1999). Hence, the reactor productivity (RP) in the CSTR (8.50 $g_{product}.L_{reactor}^{-1}.h^{-1}$) is 17 % smaller than in the fed-batch case (9.96 $g_{product}.L_{reactor}^{-1}.h^{-1}$) and is due to the increase in reaction time in the CSTR to reach similar conversion in the fed-batch (τ =1.17 t_{FB}). The two reaction systems show similar biocatalyst yield. Of course operating at a higher biocatalyst loading will increase the reactor productivity (reducing the time that the reaction takes to reach completion) at the expense of increasing the biocatalyst cost contribution (Lima-Ramos et al., 2014). Hence there is a trade-off between the reactor productivity and the biocatalyst yield. Likewise, simply increasing the biocatalyst loading for this case without increasing the mixing power (increasing the liquid-liquid interfacial area) means that there would be an inefficient use of the enzyme as indicated in Figure 5. Next we evaluate the plant throughput operating four fed-batch reactors compared to four CSTR's (same capacity). Here we have assumed a 90 % operation time and the current pumping capacity for filling and empting the fed-batch tanks (t_{FE}) with a downtime between of 6 hrs between batches is used. The metrics show that the throughput in the pilot plant fed-batch case (964 $Ton_{product}.yr^{-1}$) is better than the CSTR case (852 $Ton_{product}.yr^{-1}$) by 13 %. The fed-batch case will have better reactor productivity provided $t_{FB} + t_{FE} < \tau$. Hence, in terms of plant throughput for small to medium scale biodiesel production (< 50,000 $Ton_{product}.yr^{-1}$ (Skarlis et al., 2012)) the fed-batch operation will outperform the CSTR operation. However, the ability to produce biodiesel continuously is quite desirable. The CSTR operation has the advantage of continuous steady state operation and also the ability to handling cheaper, high melting point substrates given that the biodiesel produced in the first reactor could act as a solvent. Further, an initial addition of water is not necessary in CSTR operation since it can rely on the water formed from the esterification of FFA in the oil. Consequently the final FFA is lower and a lower operating cost for water processing can be expected.

Enzyme reuse

Given the determination of the process metrics we now show how it can be used by an enzyme producer to quickly estimate a selling price for the enzyme. The current analysis of the process metrics was based on a single use of the enzyme. However, this is a quite inefficient use of the enzyme given that the enzyme has been shown to have sufficient stability to be reused 5 times (Brask et al., 2014). Therefore to evaluate the economic viability of reusing the enzyme for the fed-batch case, we introduce the biocatalyst yield as a function of reuse ($Y_{biocatalyst_n}$, $g_{product}.g_{biocatalyst}^{-1}$) as shown in Equation 5.

$$Y_{biocatalyst_n} = \frac{\sum_{n=0}^{end} r^n . Y_{fame}}{\sum_{n=0}^{end} E_0 . r^n . x_a}, x_a = \begin{cases} 1 & n = 0 \\ x_a & n \neq 0 \end{cases} \quad (5)$$

Where r is the fraction of the reactor available (in this case given glycerol takes up approx. 10 % of the reactor volume then r = 0.9), n is the number of times the enzyme is reused, Y_{fame} is the fractional yield of biodiesel (0.975 is used and it is assumed that it doesn't change), E_0 is the initial

amount of enzyme added (% w/w_{oil}) and x_a is the fraction of enzyme added to each successive batch to account for the activity loss (0.2 is used in this scenario).

Using Equation 5 and assuming that all the enzyme is contained in the heavy phase and that this heavy phase is reused from batch to batch, we can estimate the biocatalyst yield. Figure 9a illustrates that an upper limit exists for the biocatalyst yield and the reactor productivity dependent on reuse of the enzyme. The biocatalyst yield approaches an upper limit as n increases given the diminishing reactor volume due to the reuse of the heavy phase. Likewise, the reactor productivity is affected by the diminishing reactor volume and is not constant when n is increased (for r = 1 RP is constant for any value of n). Given the biocatalytic yield and the reactor productivity, the cost contribution of the enzyme $\left(\frac{USD}{L_{reactor}.h}\right)$ can be estimated from Equation 6.

$$\frac{\sum_{i=1}^{n} RP_i}{Y_{biocatalyst_n}}.Cost\ Biocatalyst = \frac{USD}{L_{reactor}.h}.(6)$$

This is illustrated in Figure 9b. In this case we have assumed that the biodiesel selling price is 0.6 USD/kg and the enzyme cost does not correspond to more than 5 % of the revenue. In order to ensure that the cost contribution of the enzyme is not exceeded, the selling price of the enzyme cannot exceed 65 USD/kg, assuming the enzyme is to be reused 3 times or more. Likewise, for a one time use of the enzyme the price cannot exceed 30 USD/kg. This method serves as quick way for the enzyme producer to estimate the maximum selling price of the enzyme during the initial development phase. Practical experience has shown that when reusing the enzyme, the reaction time for each fed-batch varies slightly and above 3 to 4 recycles of the enzyme the separation of the enzyme from the heavy phase can become troublesome due to the emulsion formed. Hence an analysis of the cost to recover the enzyme and how the recycling of impurities affects the reaction process also needs to be considered when doing a detailed cost analysis but is beyond the scope of this current work.

Conclusions

A new modelling approach has been proposed which could serve as an important tool for predicting plant performance especially when variable feedstock quality has an impact on the process. The first order analysis enables the quick determination of commercially important process metrics which could then be used to evaluate process operation and scale up. Additionally the graphical first order analysis of concentration dependences is a powerful tool for probing reaction behaviour. However for these types of analysis there is a need for sufficient high-quality data over the entire course of the reaction (Blackmond, 2005). Although, the range of the measurement and the accuracy of the method are still under development, the QTA measurements do appear to enable reproducible, rapid measurements to analyse the biodiesel reaction over the course of the reaction.

The commercial process metrics show that for small to medium scale biodiesel production, that fed-batch operation shows better throughput compared to CSTR operation. This is an important result given that for most locations the available waste oils and fats feedstock would limit the size of the biodiesel plant to small/medium scale. However, it stands to reason that the reactor operation (fed-batch or CSTR) will depend not only on the size of the plant but the type of feedstock, the available equipment/investment and expertise of the plant personnel.

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Abbreviations

BG, BG_o	bound glycerol, initial bound glycerol content in oil [mass %]
BrG	brown grease
C	concentration of BG or FFA, [mass %]
CSTR	continuous stirred tank reactor
DAG	diglycerides [mass %]
E_o	initial amount of enzyme added [% w/w_{oil}]
FAME	fatty acid methylesters (biodiesel) [mass %]
FFA	free fatty acids [mass %]
k_{app}	apparent first order rate constant [h^{-1}]
MAG	monoglycerides [mass %]
n	number of times the fed-batch reaction is performed [-]
NS	experimental thermostable enzyme formulation NS-40116
QTA	quality trait analysis
r	fraction of the reactor available [-]
RP	reactor productivity [$g_{product}.L_{reactor}^{-1}.h^{-1}$]
TAG	triglycerides [mass %]
t_{FB}	total time of the fed-batch reaction [h]
t_{FE}	downtime plus the filling and empting time [h]
UCO	used cooking oil
x_a	fraction of enzyme added to each successive batch [[$g_{enzyme}.g_{Eo}^{-1}$]]
$Y_{biocatalyst}$	biocatalyst yield [$g_{product}.g_{biocatalyst}^{-1}$]
Y_{FAME}	fractional yield of biodiesel [$g_{FAME}.g_{oil}^{-1}$]

Greek Symbols

τ	residence time in CSTR [h]

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Tables

Table 1 Process metrics for operating 4 x 4 m^3 reactors as CSTR's compared to fed-batch

	CSTR	**Fed-batch**
Overall Residence time [hr]	89.4	76.5
Biocatalyst yield ($g_{product}.g_{biocatalyst}^{-1}$)	962	962
Reactor productivity ($g_{product}.L_{reactor}^{-1}.h^{-1}$)	8.50	9.96
Plant throughput* ($Ton_{product}.yr^{-1}$)	852	964

* The empty/filling time of 6hr for the fed-batch is included.
All other metrics exclude the empty/filling time

List of figures

Figure 1 Simple depiction of the lipase (E.C.3.1.1.3) catalysed reactions in biodiesel (Alkyl Ester) production. R^1, R^2 and R^3represent linear fatty acid chains with 12 to 24 carbon atoms which can be saturated or unsaturated. R is the alkyl group of the alcohol.

Figure 2 Process flow diagram for the first two tanks in the pilot plant.

Figure 3 Shown is lab data for the transesterification of rapeseed oil using 50 % excess methanol, 0.55 wt.% Callera™ Trans L and 5 wt. % water. a) Integral first order analysis to determine portion of the reaction that exhibits first order behaviour. b) Plot showing how BG varies over the course of the reaction. In both cases a k_{app} of 0.128 h^{-1} is found.

Figure 4 Results from the 80 L reactor (y_2 - ●), 4m^3 reactor (y_2 - ♦, error bars are the standard deviation of three replicate process runs) and the 40 m^3 reactor (y_1 - ■) for the transesterification of UCO. A k_{app} value of 0.146 h^{-1}, 0.067 h^{-1} and 0.029 h^{-1} is found for the 40 m^3, 80 L and 4m^3 reactor respectively. In the 40 m^3 reactor 0.64 wt. % NS is used while 0.1 wt. % NS is used in the 80 L and 4m^3 reactor.

Figure 5 Effect of enzyme concentration on k_{app} in the 80 L (●) reactor, 4m^3 reactor (♦) and the 40 m^3 reactor (■) with UCO as feedstock.

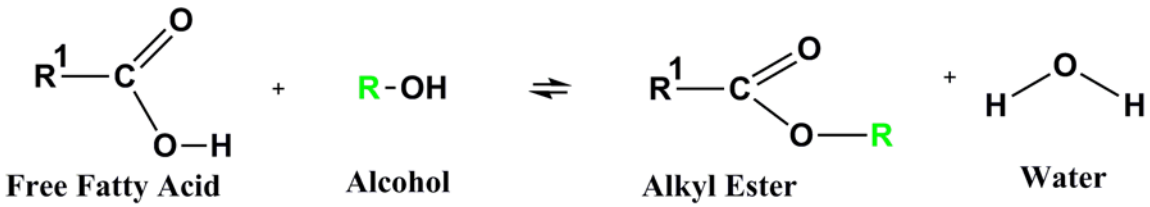
Figure 6 Results from the 80 L reactor (●) for the transesterification of BrG using 20 % excess methanol and 0.1 wt.% NS-40116. A k_{app} value of 0.031 h^{-1} is found. Shows the same type of analysis can be achieved for BrG

Figure 7 Plot of BG as a function of the number of tanks, for varying residence time and k_{app} assuming first order behaviour for the entire reaction. It is also shown that experimentally that it is possible to meet the BG specification using 4 tanks (x) with an approximate τ of 22.5 hrs which the model also predicts. Note τ is the residence time in each tank and error bars are the standard deviation of the measurement over 10 hrs of steady state operation.

Figure 8 Methodology in scaling up and moving from fed-batch into continuous production. The metric k_{app} found in step 4 is used in step 5 for process evaluation and to predict CSTR performance by plotting BG vs the number of tanks for various residence times (τ_{CR}).

Figure 9 a) Illustration of how the biocatalyst yield (♦) and the reactor productivity (●) varies with reuse of the enzyme. b) Given the biocatalyst yield and the reactor productivity the cost contribution of the enzyme can be estimated to ensure that the enzyme cost is not more than 5 % of the revenue (--).

a) Esterification



b) Transesterification

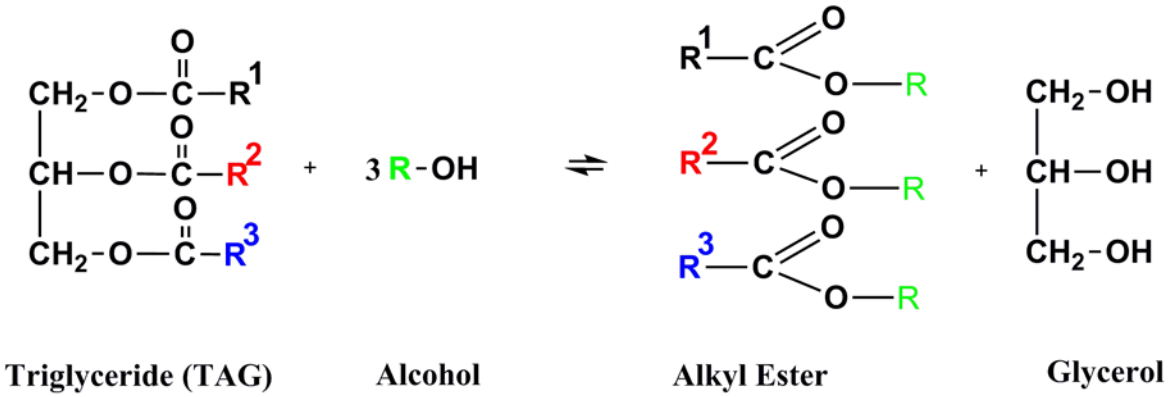
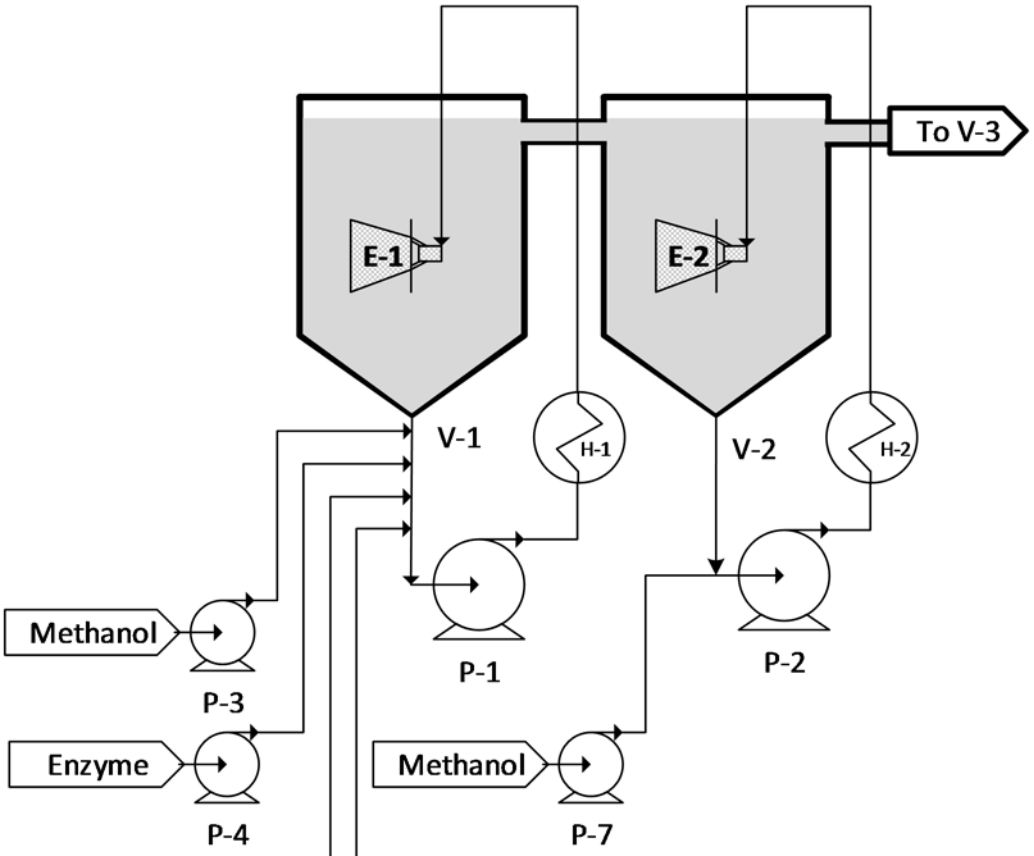
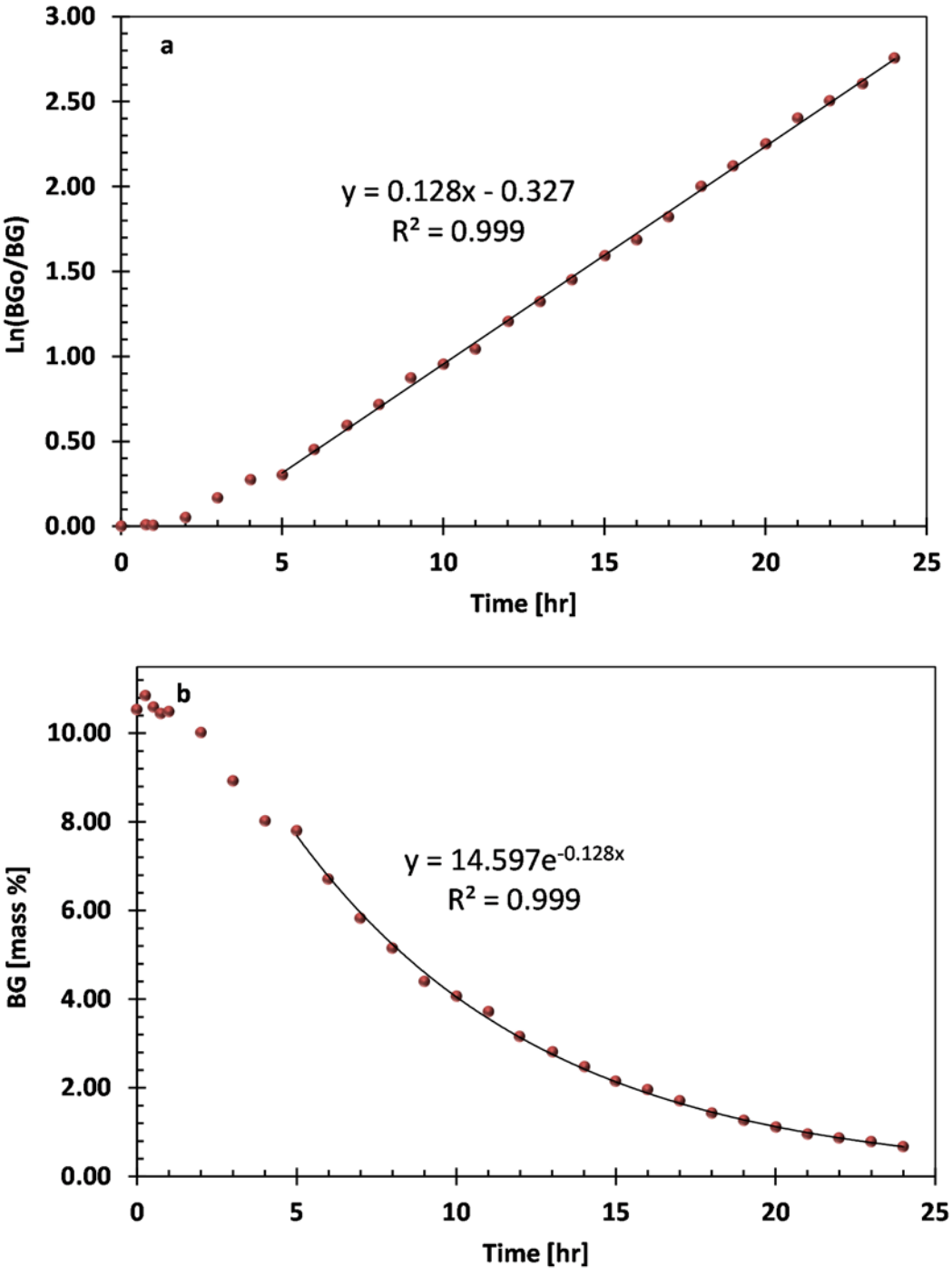


Figure 1



ID	Description	ID.	Description
E-1	Eductor 1	P-4	EnzymePump Tank 1
E-2	Eductor 2	P-5	Water Pump Tank 1
H-1	Heat Ex. Tank 1	P-6	Oil Pump Tank 1
H-2	Heat Ex. Tank 2	P-7	Methanol Pump Tank 2
P-1	Eductor Pump Tank 1	T-1	Tank 1
P-2	Eductor Pump Tank 2	T-2	Tank 2
P-3	Methanol Pump Tank 1		

Figure 2



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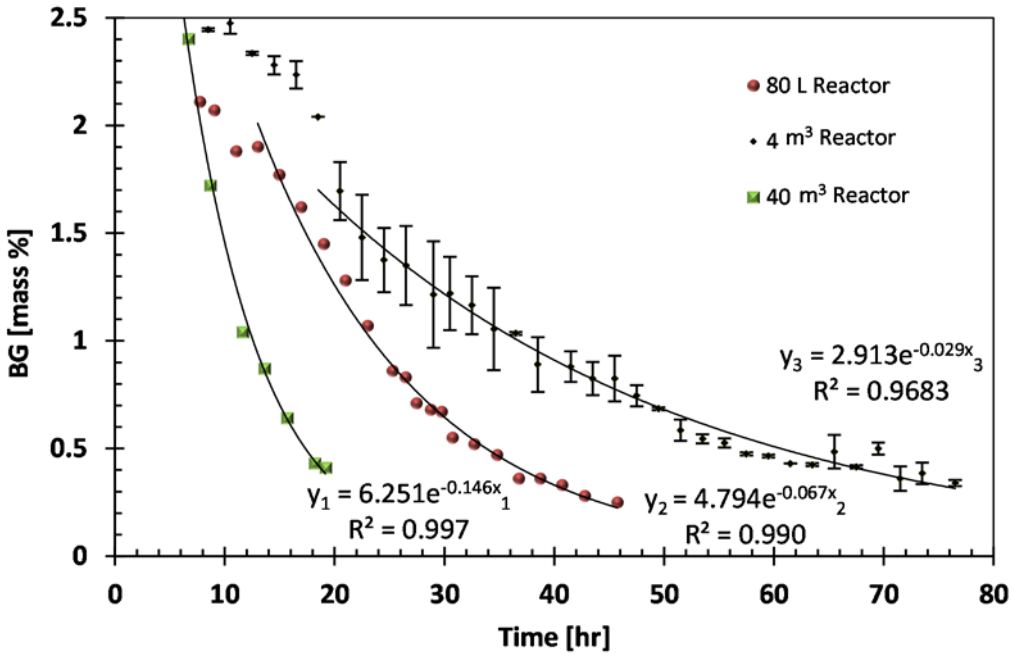

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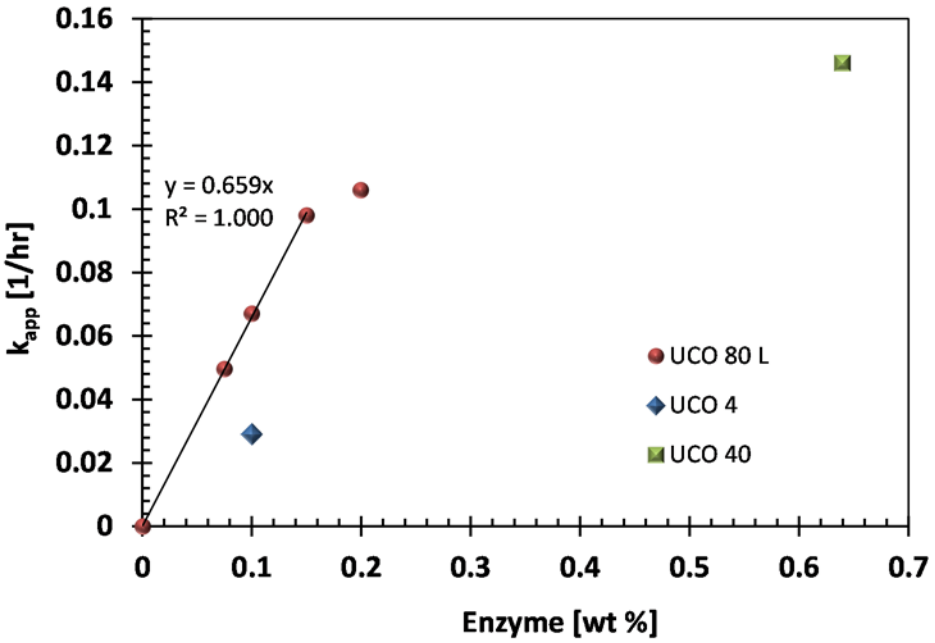
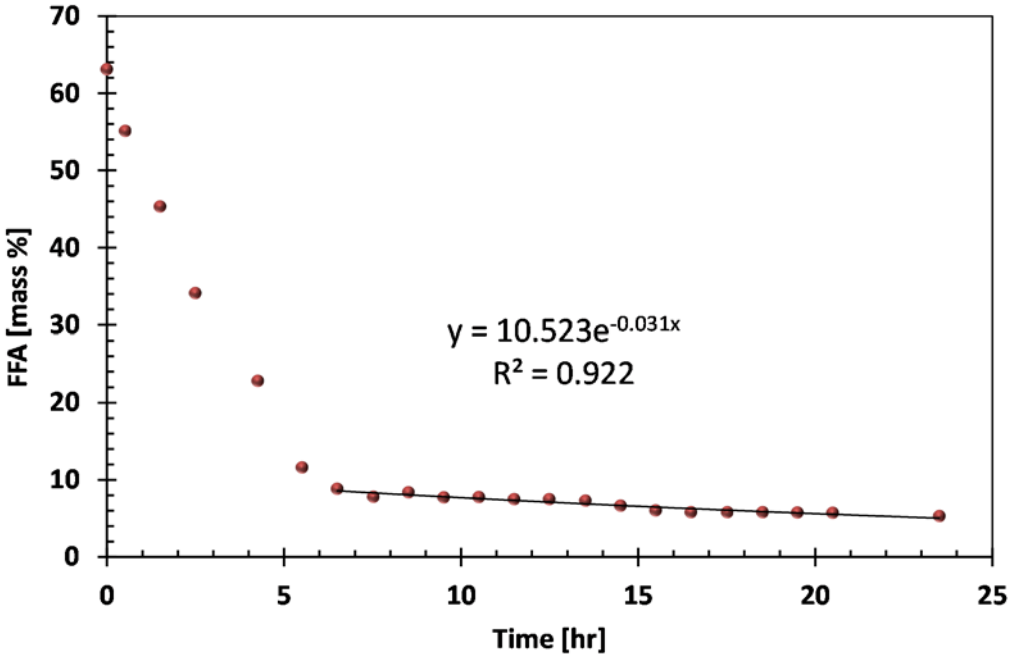


Figure 5



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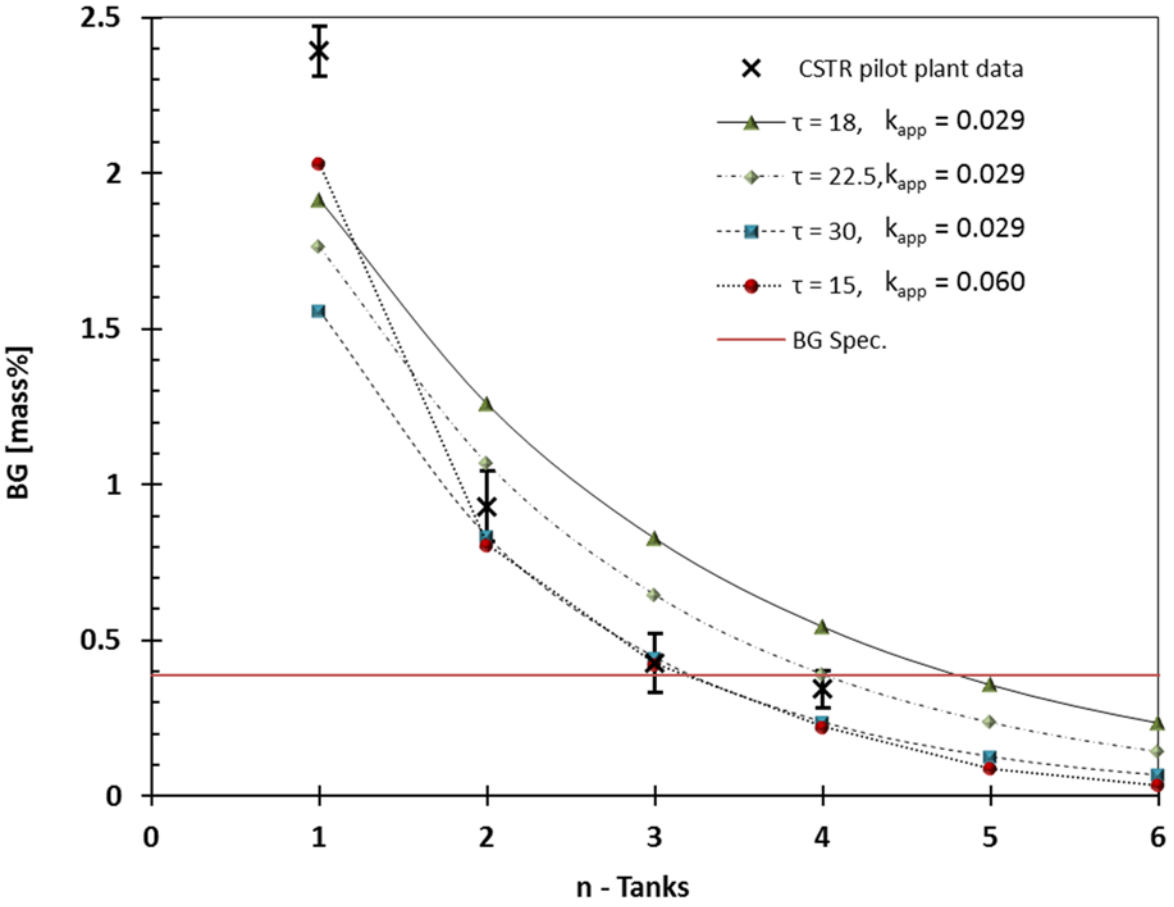

Figure 7

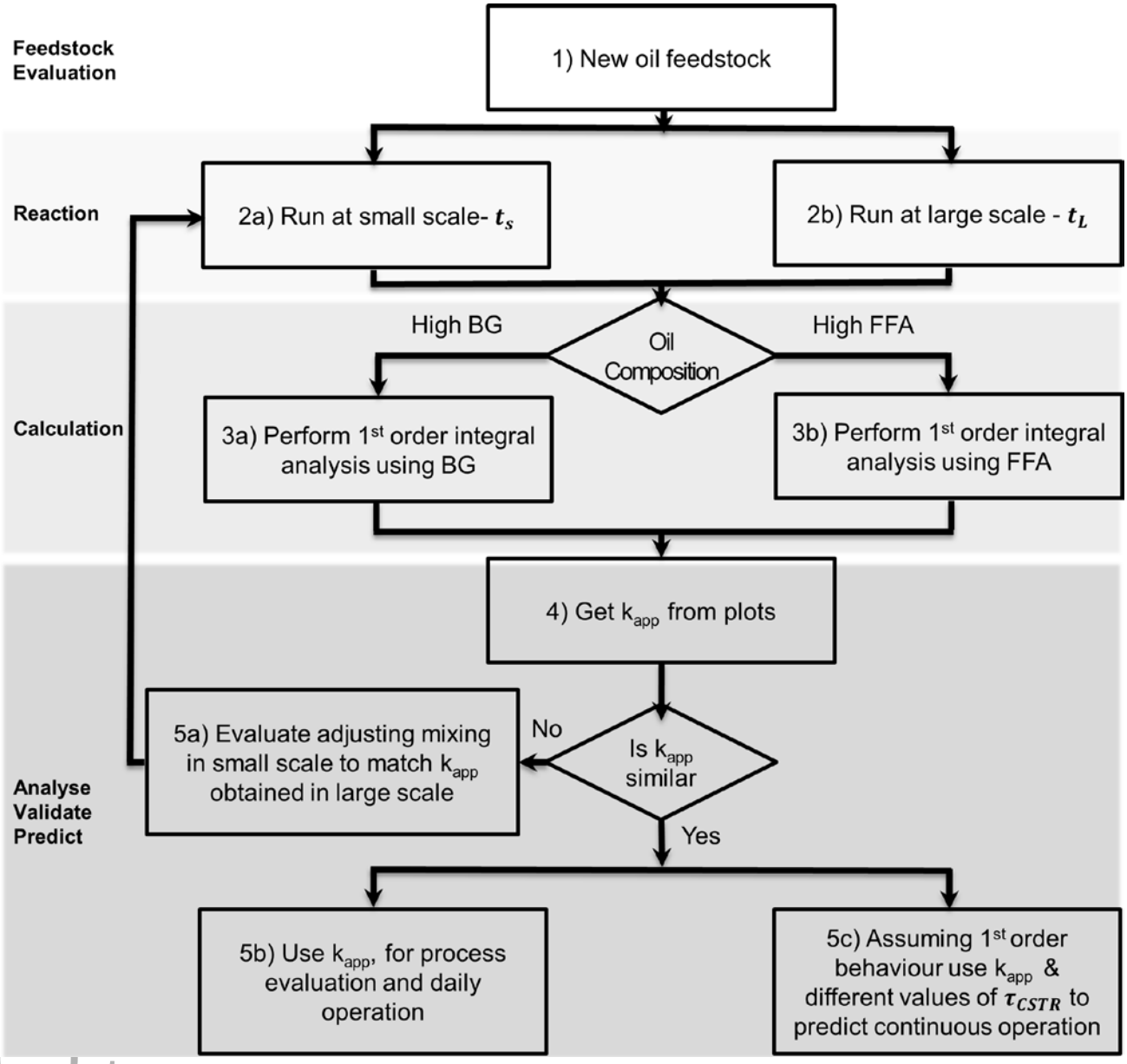

Figure 8

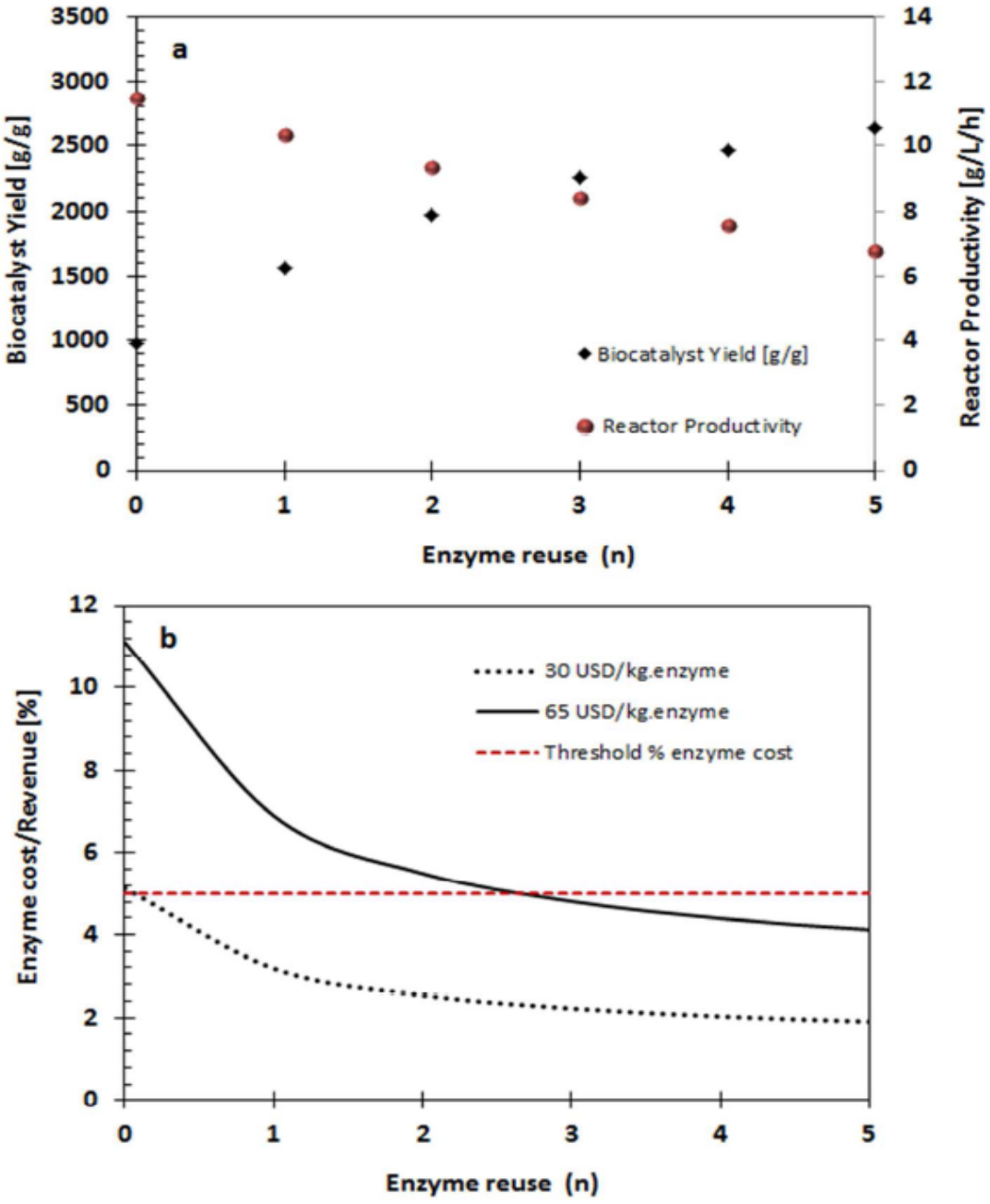

Figure 9