# Alkaline *in situ* transesterification of *Chlorella vulgaris*

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**Abstract**

*In situ* transesterification, or "reactive extraction", of lipids in algal biomass has the potential to greatly simplify and reduce costs of the production of algal biodiesel, as it reduces the number of unit operations by contacting the biomass directly with the alcohol and catalyst required to convert lipids to their alkyl esters (biodiesel). A design of experiments was conducted to understand the impact of process variables in the production of Fatty Acid Methyl Ester (FAME) from *Chlorella vulgaris* microalgae. Three process variables (catalyst ratio, solvent ratio and reaction time) were studied, based on their process significance. The maximum FAME recovery of 77.6±2.3wt% was obtained at a reaction time of 75 minutes, using a catalyst:lipid (NaOH) molar ratio of 0.15:1 and a methanol:lipid molar ratio of 600:1. Additional experiments were performed at the optimum methanol ratio (600:1) to compare results obtained using an alkaline catalyst with an acid catalyst. In terms of time, the alkaline catalyst (sodium hydroxide) outperformed the acid catalyst (sulphuric acid) obtaining higher conversions at lower reaction times. Nevertheless, using an acid catalyst ratio of 0.35:1 for longer reaction times resulted in higher conversions, up to 96.8±6.3wt%, and may have facilitated the breakage of microalgae cell walls. In conclusion, the alkaline *in situ* transesterification of algal biomass can achieve high conversion in less time than an acid catalyst, using a lower ratio of catalyst. The final selection of the type of catalyst will depend on the characteristics (batch vs continuous) and cost of the *in situ* transesterification including catalyst and methanol costs, and the downstream processes required to obtain a saleable biodiesel.

**Key words**: *in situ* transesterification, microalgae, biodiesel, catalyst, experimental design

## 1. Introduction

The UN's Intergovernmental Panel on Climate Change report states that in the last 30 years carbon dioxide emissions have risen by 80% [1]. The increased levels of greenhouse gases have had several environmental impacts, the most important being global warming. In 2008 the transport sector in the European Union contributed 21% to total greenhouse gas emissions [2]. This contribution could decrease by using biodiesel instead of petrol-diesel [3]. Biodiesel has an established market in Europe, as it is already commercially produced and used with existing distribution and storage infrastructure. Biodiesel has competitive combustion efficiency [4], and can be obtained from a wide range of sustainable biomass, such as crops that can grow on marginal land (e.g. jatropha), used fryer oil, waste streams, agricultural residues and microalgae.

Using microalgae to produce biodiesel has several advantages over production from terrestrial plant crops. Microalgae are fast-growing photosynthetic microorganisms that can complete an entire growing cycle in few days, and can be cultivated in fresh water, sea water, or wastewater. Microalgae can be used to sequestrate carbon dioxide and can produce lipids at up to 77wt% of total biomass [5]. One option for producing biodiesel from microalgae is to convert algal lipids to fatty acid alkyl esters via transesterification [6, 7]. Other alternatives are thermal cracking and microemulsions [4]. The transesterification occurs as a series of three reactions: triglycerides (lipid compounds) are sequentially converted to diglycerides, monoglycerides and, finally glycerol (by-product), with the alkyl ester (the "biodiesel") being produced at every step. This is achieved by reacting the lipids with an alcohol which usually requires an acidic or alkaline catalyst. Methanol is the most commonly used reactant in industry, as it is readily available and is relatively inexpensive [8]. The use of methanol yields Fatty Acid Methyl Esters (FAMEs). Alternatively, ethanol can be used; however this is

more expensive than methanol and does not always produce a consistently measurable product [9]. The transesterification can be performed using a homogenous or heterogeneous, acid or alkaline, catalyst. Examples of homogeneous acid catalysts are sulphuric or hydrochloric acid; while sodium or potassium hydroxide/methoxide are homogenous alkaline catalysts.

Conventionally, the oil is extracted and refined from microalgae prior to conversion to FAME. Several studies have focussed on transesterification of microalgae oil using alkaline catalysts [10, 11] or acid catalysts [12-16]. Vijayaraghavan and Hemanathan [10] showed, using fresh water microalgae oil, ethanol and potassium chloride, that microalgal biodiesel quality was comparable to biodiesel from conventional sources. Hossain *et al.* [11] showed that an alkaline reaction can reach a conversion to biodiesel of 90%, when using oil from microalgae *Spirogyra sp.* and *Oedigonium sp.*, sodium hydroxide, and methanol. Miao and Wu [12] studied the acid transesterification of microalgae oil using a high molar ratio of sulphuric acid (2.25 M) at different temperatures and methanol ratios. They obtained a maximum conversion of 68% at 30$^{o}$C, using a methanol ratio of 45:1. Xu et al. [14] obtained biodiesel from *Chlorella protothecoides* oil after 4 h, using 100% acid catalyst, 56:1 molar ratio of methanol, and a temperature of 30 °C. Li et al. [15] also used *C. protothecoides* oil, obtaining 98% oil to biodiesel conversion within 12 h at a temperature of 38$^{o}$C using 100% lipase as catalyst and 3:1 molar ratio of methanol to oil.

All the previous studies strictly require microalgae oil extraction and purification by mechanical or chemical methods. Alternatively, the oil extraction step can be eliminated by performing the reaction directly in the lipids contained in organic matter, a process known as *in situ* transesterification (Fig. 1). Johnson and Wen [16], Wahlen *et al*. [17], Ehimen *et al.*

92 [18] and, Haas and Wagner [19] have recently evaluated acid-catalysed *in situ*
93 transesterification. Johnson and Wen [16] tested biodiesel production from algae
94 *Schizochyrtium limacinum* SR21 using different solvents (methanol, chloroform, hexane and
95 petroleum ether). They obtained a maximum 68% yield of FAMEs when chloroform or
96 hexane were added to methanol using 1.5 mol of sulphuric acid and 132:1 mol of methanol
97 and solvent at 90$^{o}$C for 40 min. Ehimen *et al.* [18] tested *Chlorella* algae at different
98 temperatures, alcohol molar ratios, reaction times and moisture contents in the production of
99 biodiesel. Their study showed a maximum lipid to FAME conversion of around 88% after a
100 reaction time of 2 hours, using 0.04 mol of sulphuric acid, 500:1 mol of methanol and a
101 temperature of 90$^{o}$C. Xu and Mi [20] conducted an alkaline *in situ* transesterification of
102 *Spirulina* sp. in order to test different types of co-solvents.

103 In this study we evaluated FAME production by alkali-catalysed *in situ* transesterification of
104 microalgae *Chlorella vulgaris* at different reaction times, methanol ratios, and catalyst
105 concentrations. Past studies have not thoroughly investigated the use of alkaline catalysts for
106 microalgae, due to their high free fatty acid (FFA) contents. For example, Haas and Wagner
107 reported an FFA content of 35.1 wt% in microalgae biomass [19]. An alkaline catalyst is
108 normally not recommended for feedstocks containing more than 2 wt% of FFA per total
109 lipids, due to increased soap and water formation [6]. However, the amount of FFA in
110 microalgae can also be low, as it varies according to the type of strain and growing conditions
111 [21]. If FFA is low, then alkaline catalyst are the most likely option as the transesterification
112 reaction proceeds faster than with an acid catalyst, reducing reactor size, and therefore capital
113 cost. Alkaline catalysts are also less corrosive to equipment than acid catalysts [22]. Most
114 importantly, past evidence from *in situ* transesterification of oilseeds shows that alkaline
115 catalyst have a higher tolerance for water than conventional processes [23]. This is important

as microalgae biomass will contain water, and it can be very costly to dry it to the very low levels required for biodiesel production.

## 2. Methodology

### 2.1 Microalgae *Chlorella Vulgaris*

Dried *Chlorella vulgaris* was purchased from Chlorella Europe (London, UK). The lipid content of *Chlorella vulgaris* was measured by mixing 1 g of powder with 45 mL of a homogenized mixture of methanol and chloroform (1:2, v/v). After overnight extraction, samples were vacuum-filtered with Whatman 2E filter paper into an acetone-washed separating funnel and then transferred to another clean glass test tube. A weak salt solution consisting of potassium chloride (KCl; 0.88v%) was added at 25% of the starting volume [24]. The mixture was shaken gently and two layers were left to separate. The top layer mixture was removed using a Pasteur pipette and drained to waste; the bottom layer mixture was transferred into a weighed clean test tube. The solvent was removed by evaporation at room temperature for several days until achieving constant weight. The amount of FFA present was determined by titration using method ASTM D5559 [25].

Identification of FAME from the crude algae oil was conducted by using the one-step lipid extraction method and FAME preparation described by Garces and Mancha [26]. A methylating mixture was prepared containing methanol-toluene:2,2-Dimethoxypropane:sulphuric acid (39:20:5:2 by volume). The methylating mixture (3.3 mL) was mixed with heptane (1.7 mL) and added to 0.2 g of microalgae followed by vigorous shaking and incubation in a water bath at 80$^{o}$C for 2 h. After this, the sample was cooled down and the upper layer formed in the mixture was separated and analysed using gas chromatography (see section 2.4).

## 2.2 Experimental design

In order to have a systematic approach to data collection and analysis, a design of experiments (DOE) was used. Although the transesterification reaction to produce biodiesel is seen as a simple reaction mechanism, there are multiple parameters that affect the process such as temperature, mixing rate, solvent and catalyst ratios, reaction time, biomass, and pH. Using a DOE is a more effective procedure to evaluate some, if not all, the parameters involved when compared to the traditional one-at-a-time methodology because it can study several parameters at the same time with the lowest possible number of observations [27].

The series of experiments to evaluate the performance when using an acid or alkaline catalyst were first set up to follow a $3^3$ factorial design. The fixed variables were: temperature ($60^oC$), grams of algae (7g) and mixing rate (380 rpm). A temperature of $60^oC$ was used as this is the standard temperature used in industry, and a high mixing rate of 380 rpm ensured that the process was not limited by external mass transfer. The changing variables were: solvent (methanol to oil molar ratios of 300:1, 400:1 and 600:1), catalyst (NaOH to oil molar ratios of 0.05:1, 0.15:1, 0.25:1) and reaction time (5 min, 15min and 45 min). Once the first results were obtained an additional experiment was conducted to evaluate a fourth level of the alkaline catalyst (0.35:1) and methanol (800:1) ratios at the same three reaction times. The overall experimental design needed a total of 63 observations in duplicate. The final weight of FAME obtained was the response variable in the experimental design and acid *in situ* transesterification.

## 2.3 Procedure for *in situ* transesterification

Apart from the experimental design, further acidic ($H_2SO_4$) and alkaline (NaOH) *in situ* transesterifications were conducted at a methanol to oil molar ratio of 600:1 to allow comparison of catalyst performance at different reaction times and catalyst concentrations.

All *in situ* transesterifications whether alkali (NaOH) or acid- catalysed ($H_2SO_4$) were carried out in 50ml centrifuge tubes. The tubes were filled with 7 g of algae and then pre-heated in the oven at 100°C for 1hour to remove any moisture due to storage. When using sodium hydroxide (NaOH) as catalyst, granules were pre-dissolved in methanol at a concentration of 100 g/L to form sodium methoxide. The required amounts of catalyst (NaOH or $H_2SO_4$) and methanol were added to each tube consecutively to begin the experiment and avoid any reaction delays between experiments. The transesterification reaction was performed at a constant temperature of 60$^{o}$C and a stirring rate of 380 rpm using a shaking incubator (IKA KS 4000 icontrol). Once the reaction was complete, 0.5 mL of acetic acid (for reactions using an alkaline catalyst) or 0.5 mL of water (for reactions using an acid catalyst) was added to each tube to neutralise the catalyst and stop the reaction. After, the tubes were stored in a refrigerator (at 5$^{o}$C) to reduce the temperature, and then the algae residues were separated from the bulk liquid by centrifugation (SCI QUIP sigma 3-16p) for 5 min at 4000 g. The bulk liquids (containing methanol, FAME and by-products) were stored in pre-weighed tubes. The final weight of the bulk liquid was recorded for each tube and the FAME concentration was measured by gas chromatography (see section 2.4).

2.4 Analytical techniques

Analysis of total FAME yields from the *in situ* transesterification was performed using gas chromatography (GC, Hewlet Packard 5890) adjusted to the following conditions: carrier gas: helium, 7psi; air pressure, 32psi; hydrogen pressure, 22psi; a capillary column was used with a head pressure of 4.5psi. Samples of 250mg were mixed with 1 mL of an external standard solution (C17:0 Sigma Aldrich 51633, 10 mg/ mL) in 2 mL vials. One microlitre of the mixture was injected to the GC and data was collected using DataApex Clarity software, UK. The mass of FAME obtained in the biodiesel rich phase from experiments was calculated by multiplying the weight of the final biodiesel mixture obtained times the FAME concentration

measured by GC. Dividing the mass of FAME obtained by the maximum FAME available in the lipids gave the FAME yield.

In order to characterise the compounds in the FAME chromatogram a grain FAME mix (Sigma Aldrich 47801, 10 mg/mL) and a series of pure FAME compounds (C16:0, C17:0, and C18:2) were analysed at the same GC conditions as the FAME samples.

## 3. Results and discussion

### 3.1 Alkaline *in situ* transesterification

The microalgae cultures were shown to contain 26.9±0.4wt% lipids of total biomass. This lipid ratio was in accordance with the manufacturer's specifications, and is relatively low, as the commercially available *Chlorella* is used as a protein-rich nutrient. Within the total lipids, a maximum FAME mass of 791 mg was obtained for the 7 g of dried microalgae used in all experiments. FFA accounted for 3.2±0.2wt% of total lipids; this value is in the low range, and agrees with values reported by Widjaja *et al.* when using *Chlorella* biomass dried at 100$^{o}$C [21]. Fig. 2 shows the mass of FAME obtained at the different conditions of the experimental design. A significant FAME conversion was achieved very rapidly: in just 5 minutes, there was a 55wt% FAME conversion (of total FAME mass) when using the highest methanol/NaOH ratios (Fig. 2a). After 15 minutes the FAME yield increased further, achieving 60wt% at the highest methanol/NaOH ratios (Fig. 2b). Finally, at 45 minutes, (Fig. 2c), the yield achieved maximum conversion for the range of times studied in the experimental design. Figs. 2b and 2c also show that increasing the methanol ratio from 300:1 to 600:1, decreases the amount of catalyst needed to reach an increased conversion of FAME yield. It can also be observed that using low (300:1) or high (800:1) ratios of methanol plus a high ratio of catalyst (0.35) decreases the FAME yield beyond 15min (Fig. 2b and Fig. 2c). A low yield is obtained when using a low methanol ratio and a high ratio of catalyst probably

due to production of soap instead of FAMEs, as sodium hydroxide produces water upon dissolution in methanol. On the other hand, when using high ratios of methanol, the FAME conversion may have decreased due to the dilution of the catalyst.

The percentage FAME yield reached a maximum of 71±1wt%, after 45min, at a catalyst ratio of 0.35 and solvent ratio of 600:1. This value is higher than the maximum conversion of algal biomass obtained by Johnson and Wen [16] of 66wt% but lower than the 88wt% maximum conversion reported by Ehimen et al. [18], both using acid catalysis. As the maximum conversion was reached at the highest time evaluated in the initial set, the amount of FAME obtained could still be increasing (see next section).

### 3.2 Identification of optimal reaction time

The experimental design indicated that using a solvent ratio of 600:1 gave the highest lipid to FAME conversion, and that using a catalyst ratio of 0.25 was as efficient as using a catalyst ratio of 0.35 (see section 4.3). However, the highest FAME products were given at the highest evaluated time of 45 min. In order to find an optimum time an additional set of experiments were conducted at 75 min and a longer period of time (20 h). Fig. 3 shows that 75 min gave the highest FAME product for catalyst ratios higher than 0.15. At this plateau value, the highest FAME yield was 77.6±2.3wt% of total FAME mass. The maximum yield obtained when using microalgal biomass was lower than the maximum yields reported for alkaline catalysed sunflower, *Jatropha curcas* and cotton seed (>90%) by *in situ* transesterification, but higher than when using primary sewage sludge and an acid catalyst (66%)[28].

At a reaction time of 75 min there was no difference between different catalyst ratios of 0.15, 0.25 or 0.35. Therefore the optimum conditions are found to be when using a catalyst ratio of

0.15 (to minimise catalyst consumption), combined with a reaction time of 75 min and a solvent ratio of 600:1.

### 3.3 Analysis of experimental design

The balanced data of the DOE was statistically evaluated in order to identify interactions among variables for the *in situ* transesterification of microalgae. This is facilitated by using the "p-value" and "t-value" factors. As a rule, large magnitudes of t and small magnitudes of p ($p \leq 0.005$) indicate that the parameter significantly affects the process. Linear and combined effects of the parameters were tested at 95% significance. The effect of all the individual parameters were significant giving a $p = 0.000$. However, F values differed and indicated that catalyst ratio (92.4), time (44.4) and solvent ratio (29.9), in that order, affected the FAME yield most. In this experiment, initial solvent ratios used were already high therefore the statistical analysis indicates that if the solvent ratio was further decreased, the effect on FAME conversion will be the minimum, from the parameters evaluated. Results from studying interaction between parameters showed that the combined effects of solvent*catalyst, time*catalyst, and time*solvent, were also significant ($p \leq 0.005$).

Fig. 4 gives a graphical representation of interaction between variables. It can be observed that for the lowest alcohol and catalyst ratios (300:1 and 0.05:1) the FAME yield was a relatively weak function of the reaction time in the range examined (Fig. 4 b and g). This indicates that a catalyst ratio of 0.05 is too low for the reaction to complete. For all other ratios, the rate of change of the FAME yield between 5 and 15 min was higher than between 15 and 45 min. As expected, the rate of FAME conversion decreases with time. It can also be seen that an increase in catalyst molar ratio from to 0.35:1 (curves in Fig. 4b) caused a higher rate of change, than an increase in methanol molar ratio from 300:1 to 800:1. This is due to the fact that the catalyst ratio is being increased 6 times the initial value while the methanol

ratio is only increased by a factor of 2.6. Fig. 4d shows that an alcohol ratio of 800:1 gave the highest rate of change through time, going from 180 mg to 420 mg between 5 and 45 min; however the mass of FAME was low. A high rate of change should be expected when the solvent is used in high concentrations. The increased methanol requirement for obtaining sufficient levels of conversion is a disadvantage compared to conventional transesterification. *In situ* transesterification typically requires molar ratios of 100s:1 compared with using around 6:1 ratios in transesterification of pure, liquid triglycerides [15] .

Fig. 4e indicates that increasing the catalyst ratio from 0.05:1 to 0.25:1 linearly increases the amount of FAME up to methanol ratios of 600:1. The main effects plot (Fig. 5) also indicated that a solvent ratio of 600:1 produced the highest FAME yield. A methanol ratio of 800:1 decreased the FAME obtained at catalyst ratios of 0.25:1 and 0.35:1 (Fig. 4e and Fig. 4h). As previously mentioned, this could be due to the dilution of the catalyst at high solvent ratios. Using a high alcohol ratios also affected reproducibility; experiments using 800:1 methanol ratios had the lowest similarity (Fig. 4, graph f).

The change in FAME yield observed when changing catalyst ratios in the main effects plot (Fig. 5) shows a logarithmic curve indicating that there is an optimum catalyst ratio for the FAME yield. There was no significant difference (ANOVA, $p = 0.343$) between FAME yields obtained at 0.25:1 and 0.35:1 catalyst ratios after 45 min (Fig. 4, graph g). Additionally, after 75 min the yields obtained at 0.15, 0.25 and 0.35 catalyst ratios did not show significant differences. This suggests that to obtain the maximum yield within 45 min using a catalyst ratio of 0.25:1 should be optimum, while leaving the reaction for 75 min decreases the catalyst ratio needed to 0.15:1 for maximum yield.

A further evaluation of the data was conducted by analysing residuals. The residuals followed a linear trend in the normal probability plot and had a normal frequency distribution. This validates the statistical analysis. Graphs j to l in Fig. 4 shows the variation between

experiment 1 and 2. There was no significant difference between experiments which confirmed the reproducibility of the data and this can also be observed in the main effects plot (Fig. 5).

### 3.4 Comparison of acid and alkaline *in situ* transesterification

Fig. 6 shows the mass of FAME obtained when using an alkaline or acid catalyst at the previously found optimum methanol ratio of 600:1. Higher yields were obtained with an alkaline catalyst than an acid catalyst over the lengths of time tested. Using a catalyst ratio of 0.05:1 produced low FAME yields (<100 mg) independent of the type of catalyst. When using an alkaline catalyst (Fig. 6a), after 5min of reaction, the FAME yield increased approximately linearly as the catalyst ratio increased. At 15 min and 45 min, the yield of FAME was the highest at a 0.25:1 catalyst ratio. As the reaction continued, the FAME yield increased more rapidly at low concentrations of catalyst until achieving a plateau. All catalyst molar ratios achieved approximately the same plateau after 45 min, apart from concentrations lower than 0.15.

Regarding the acid catalyst (Fig. 6b), ratios of 0.15:1 produced FAME yields lower than 200 mg (<25% conversion). During the first 75 min, an increase of the acid catalyst ratio up to 0.35 increased the FAME obtained to ~320 mg (approximately 50% conversion). Clearly the acid reaction is much slower on a mole of catalyst basis. To determine the maximum yield that could be obtained by the acid catalyst, the reaction was allowed to proceed for a long period of time (20 h). In these conditions the highest FAME yield obtained was 96.9±6.3wt% (corresponding to 766±50 mg), achieved at the highest catalyst ratio of 0.35.

### 3.5 Characterisation of fatty acid methyl esters

The FAMEs obtained when using an alkaline or acid catalyst are shown in Table 1. When acid or alkaline catalysts were used the compounds, the most abundant species were palmitic

acid (C16:0), stearic acid (C18:0), elaidic acid (C18:1n9t), linoleic (C18:2n6c) and linolenic acids (C18:3n6 and C18:3n3). Only FAMEs produced using an alkaline catalyst contained gamma linolenic acid (C18:3n6). On the other hand, increased concentrations of myristic acid (C14:0) and myristoleic acid (C14:1n9 *cis*) appeared when using an acid catalyst on the microalgae (or the crude oil). Crude oil contained increased quantities of short chain compounds whereas FAME obtained by *in situ* transesterification exhibited increased concentrations of long chain compounds (C18:1). From the identified FAME compounds, it was found that the profiles were mainly composed of unsaturated fatty acids ranging from 56% to 71%, which is in accordance with previous literature [29, 30]. In particular, the FAME profile was similar to that reported by Couveia and Oliveira [30], where palmitic (C16:0), elaidic-oleic (C18:1), and linoleic (C18:3) were the major fractions obtained from *Chlorella* biomass. The differences between the acid- and alkali-extracted FAMEs from *Chlorella vulgaris* have a range of (possibly interrelated) causes, including: differing extents of conversion at these conditions, differences in the catalysts' extraction of lipids from the cell membrane and differences in the conversion of FFA.

## 4. Conclusions

This research was intended to define the values of the processing parameters required to produce biodiesel from microalgae in the most efficient manner. *In situ* transesterification of algal biomass is an example of process intensification as it reduces the number of steps required to obtain biodiesel by combining lipid extraction and transesterification into one step. This study evaluated essential process parameters for the alkaline *in situ* transesterification of microalgae to FAMEs via an experimental design. In the range of the three parameters evaluated (methanol ratio, catalyst ratio and time) the highest FAME yield was obtained at a reaction time of 75 min, using a methanol ratio of 600:1, and a catalyst ratio of 0.15:1. It was shown that a conversion of 77.6±2.3wt% can be achieved using an alkaline catalyst at

considerably lower reaction times than when using an acid catalyst. However, the methanol ratio was extremely high, as for oilseed biodiesel production by *in situ* transesterification, and methods to reduce the amount of methanol will need to be proposed for this process to become economic, as a substantial energy cost will be incurred by recycling the methanol. On this evidence, for this feedstock, alkaline catalysis is significantly more rapid than acid catalysis, but has a lower yield. The final selection of the type of catalyst to be used will depend on the characteristics of the algae biomass (particularly amount of FFA content) and the final downstream processes necessitated by the conversion rate achieved, and removal of the catalyst itself. The FAME profiles of the acid and alkali-catalysed processes were shown to differ somewhat, but the major FAMEs produced from *Chlorella vulgaris* were palmitic acid, elaidic acid, oleic acid, and linoleic acid.

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## Figures

**Fig.1 Comparison between *in situ* transesterification and conventional transesterification.** Green squares indicate initial and final products, blue squares are main processes required, and grey squares indicate by-products obtained.

**Fig. 2 Fatty acid methyl esters (FAME) obtained at different catalyst ratios, solvent ratios, and reaction times.** Reaction times for the different graphs were: a) 5 min, b) 15 min, and c) 45 min.

**Fig. 3 Mass of FAME obtained at different reaction times**. Labels indicate different catalyst ratios of sodium hydroxide per mol of lipid. A fixed methanol ratio of 600:1 was used for all data points.

**Fig. 4 Interaction plot of results.** Values observed are means of duplicate experiments. Values plotted are means of duplicate experiments.

**Fig. 5 Main Effects plot.** Mean data obtained for the factors (time, solvent, catalyst and experiment) studied in the experimental design.

**Fig. 6 Effect of using different catalyst ratios on FAME yield**. *In situ* transesterification was done using either: (a) Alkaline catalysis or (b) acid catalysis. Values obtained at methanol:oil 600:1, and temperature 60$^{o}$C.