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Production of Fatty Acid Methyl Esters via the In Situ Transesterification of Soybean Oil in Carbon Dioxide-Expanded Methanol

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Abstract The production of fatty acid methyl esters (FAME) by direct alkali- and acid-catalyzed in situ transesterification of soybean flakes in CO_2-expanded methanol was examined at various temperatures and pressures. Attempts to synthesize FAME from soy flakes via alkaline catalysis, using sodium methoxide as a catalyst, in gas-expanded methanol were unsuccessful. However, performing the reactions in 54 mL of a 1.2 N sulfuric acid-methanol mixture containing 50% mole fraction CO_2 resulted in an 88.3 ($\pm$1.5)% conversion of the triacylglycerol (TAG) in 22.5 g soy flakes to FAME within 10 h. Decreasing the total volume of the liquid phase by one-third from 54 mL to 36 mL, while keeping the mmol of acid and all other variables constant, resulted in an 88.2($\pm$5.0)% conversion of triglyceride to FAME. Quantitative HPLC analysis showed that 99.8 and 92.3% of the total mass balance for FAME, TAG, and free fatty acid (FFA) could be accounted for in the reactions performed at the liquid-phase volumes of 54 and 36 mL, respectively. The overall results show that the introduction of CO_2 into the system increases the rate of reaction by as much as 2.5 fold in comparison to control reactions without CO_2. Additionally, we observed a direct correlation between FAME yield and the concentration of acid catalyst. We

also determined by nitrogen analysis that at least 82.9% of protein is retained in the lipid-free soy flakes after acid-catalyzed in situ transesterification in CO_2-expanded methanol.

Introduction

The production of biodiesel, which chemically is a fatty acid alkyl ester, has increased greatly in recent years due to their emergence as a widely accepted fuel additive and replacement for petroleum-based diesel fuel [1]. One main reason for their wide acceptance is that, unlike petroleum-based diesel, alkyl esters are derived from renewable resources and have low environmental impact. Furthermore, fatty acid alkyl esters have better fuel properties, such as higher flashpoint, higher cetane number, and excellent lubricity, than their petroleum-based counterparts. The widely used process for the production of biodiesel is the transesterification of triacylglycerols (TAGs), which are available from a number of natural resources, agricultural products such as vegetable oils and animal fats, and industrial byproducts such as restaurant greases and trap grease [1].

In the US, the most commonly used vegetable oil for the production of biodiesel is degummed and partially refined soybean oil. This oil is typically recovered from soybeans by mechanical extrusion or by solvent extraction. Hexane is a very efficient and commonly used solvent for the extraction of soybean oil [2]. If a method for biodiesel production that directly uses the original oil-bearing source as a starting material can be devised, then the oil extraction step can be circumvented. As a result, the overall economy

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of biodiesel production may be improved. Lowering the cost of feedstocks is desirable because feedstock prices contribute up to 80% of the total costs associated with the production of biodiesel. Furthermore, bypassing the hexane extraction step eliminates the need to use and thus dispose of an organic solvent.

Several researchers have successfully synthesized alkyl esters directly from flaked, ground, or whole seeds via alkaline- or acid-catalyzed in situ transesterification reactions [3–11]. Others have performed direct methanolysis of oil seeds and soy flakes over a packed bed of immobilized enzymes in a stream of supercritical CO_2 [12]. However, in situ transesterification reactions of oil-borne solid starting materials require as much as a 560-fold molar excess of methanol relative to the amount of TAG, compared to a 6-fold molar excess of methanol for the conventional transesterification of refined or partially refined oils. The energy required to recover, by distillation and condensation, the excess unreacted alcohol after the reaction renders the process economically noncompetitive in comparison to conventional biodiesel production from isolated oil. There is thus a need for research to reduce the amount of methanol required to perform in situ transesterifications.

Other attempts to eliminate the hexane extraction step in oil recovery have employed supercritical-CO_2 (sc-CO_2) extraction of soybean seeds [13]. These studies show no decrease in operating costs; however, the use of heat transfer integration decreased operating costs of sc-CO_2 extraction to a level similar to those of conventional extraction plants. A one-pot in situ transesterification reaction whereby the oil-borne solid starting materials are simultaneously subjected to sc-CO_2 extraction and transesterification reaction can be envisioned. However, the disadvantages of conducting reactions in sc-CO_2 are that it is nonpolar and cannot solubilize many reagents or catalysts. Additionally, reactions performed in sc-CO_2 only increase the reaction rates of mass transfer-limited reactions and they require high process pressures (>100 bar) and large reactor volumes. Using conventional organic solvents could alleviate these problems; however, as with hexane, typical organic solvents pose a usage and disposal requirement that is stricter than CO_2. One compromise would be to dissolve CO_2 in reduced amounts of traditional organic solvents to form a mixture with a liquid phase potentially capable of increasing the reaction rate. Such a gas-expanded solvent mixture could allow a better solubilization of reactants or catalysts than sc-CO_2 alone, while simultaneously reducing the requirement for conventional organic solvents.

Previously, researchers have used gas-expanded solvents to synthesize methyl esters from an alcohol and extracted TAGs or free fatty acids (FFAs) [14, 15]. The yields from and catalyst for the proprietary protocol are

unknown; however, this technology may be suitable for in situ transesterifications. In a gas-expanded liquid, a gas such as CO_2 is used as a co-solvent that is dissolved in an organic solvent such as an alcohol, ketone, ether, or acetate. Although many of these co-solvent systems have been evaluated, carbon dioxide-methanol systems have been studied the most [16–20].

The goal of this study was to explore the possibility that in situ transesterification would occur in a gas-expanded system. Our results show not only that the in situ transesterification reaction could occur in a gas-expanded solvent system, but also that the methanol requirement was reduced for the reaction.

Materials and Methods

Materials

Soy flakes, prepared for industrial scale hexane extraction, were graciously provided by the Archer-Daniels-Midland Co. (Decatur, IL). Moisture content upon receipt was 2.81%. A sample of these was dried to 1.6 wt.% in a gravity convection oven at 75 °C and extracted with hexane for 4.5 h in a Soxhlet apparatus to determine initial oil content. The average oil content of three samples was determined to be 23.6 ($\pm$0.4)% (mass basis) after subjecting the hexane mixture to rotary evaporation and subsequent high pressure liquid chromatography (HPLC) analysis to determine TAG content. HPLC analysis did not detect the presence of FFA.

Carbon dioxide (from a tank equipped with a siphon tube) was obtained from Airgas, Inc. (Radnor, PA). Methanol (HPLC grade) and hexane (HPLC grade. 95% n-hexane) were purchased from J.T. Baker (Phillipsburg, NJ). Sodium methoxide (30% in methanol) was a kind gift from Degussa (Niederkassel, Germany). Glacial acetic acid (USP grade) and sulfuric acid (96.1%) were from Mallinckrodt (Paris, KY). 1-Monoolein, 1,3-diolein and FFAs (palmitic, stearic, oleic, linoleic and linolenic acids) used to generate standard curves for HPLC were obtained from Sigma-Aldrich (St. Louis, MO). These FFAs were run as a mixture in amounts proportional to their abundance in soybean oil. Standard curves for HPLC analysis were also generated with a mixture of FAME standards (RM-1) obtained from Supelco (Bellefonte, PA).

Transesterifications

For alkali-catalyzed in situ transesterification reactions, 22.5 g flaked soybeans and various volumes of sodium methoxide diluted to a total volume of 54 mL in methanol were added to a 300-mL stainless steel Parr reactor (Model

452HC, Parr Instrument Company, Moline, IL). For acid-catalyzed reactions, the same amount of soy flakes was mixed with various volumes of sulfuric acid diluted to total volumes of 27, 36 or 54 mL in methanol in the 300-mL reactor. The reactor was equipped with a mechanical stirrer, thermocouple, siphon tube, catalyst basket, and exhaust valve.

To attain the desired mole fraction of CO_2 in the liquid phase, pressures were adjusted as necessary according to vapor–liquid equilibrium data [16, 19, 20]. CO_2 was introduced by manually bleeding the gas into the vessel for pressures below 7.38 MPa or by using an Isco 500D syringe pump (Lincoln, NE) to attain higher pressures. Experiments were performed at room temperature, 57 °C, or 121 °C in the presence and absence of CO_2 with stirring at 300 rpm for 0.5, 3.0, 6.5 and 10.0 h under steady-state conditions.

At the completion of each reaction, the mixtures were allowed to cool and depressurized. The liquid phase contents of the reactor were decanted into a centrifuge bottle. The flakes were washed three times by suspension in 30 mL of methanol each time, and washes were combined with the initial liquid phases in the centrifuge bottle. The flakes were then subjected to hexane extraction in a Soxhlet apparatus. The combined methanol washes and initial liquid phase in the centrifuge bottle were centrifuged (15 min, 4,000×g, room temperature), the supernatant was isolated, and the volume was recorded. The theoretical maximum FAME yield was calculated based on the TAG content determined by Soxhlet extraction. Accordingly, assuming that the supernatant contained 100% of the theoretical maximum of FAME, it was diluted, theoretically, to 1 g FAME/22.5 mL methanol. A 1 mL aliquot was neutralized with 1 mL 0.1 N acetic acid (if base-catalyzed) or 1 mL 0.1 N sodium hydroxide (if acid-catalyzed) in methanol. The alcohol was removed under nitrogen on a N-EVAP 112 nitrogen evaporator (N-EVAP, Berlin, MA) at 40 °C for 20 min. The residual material was redissolved in 10 mL of a mixture of hexane, acetic acid and isopropanol (98.9:1.0:0.1; v/v/v). An aliquot of this mixture was analyzed by HPLC using a Sedex75 (Cranbury, NJ) evaporative light-scattering detector (ELSD) to determine amounts of FAME, FFA, and TAG. Samples were injected into a LiChromosorb 5 diol G100 × 3.0 mm column by Varian (Walnut Creek, CA) and eluted with a mobile phase mixture of hexane:acetic acid (1,000:1). Reactions were conducted in duplicate.

Residual diacylglycerol (DAG), and monoacylglycerol (MAG) levels were determined by HPLC on a 3 × 100 mm LiChrosorb SI 60-5 column (Chrompack Inc., Raritan, NJ). The mobile phase programs for HPLC involved gradients of isopropanol and water in hexane/0.6% glacial acetic acid as previously described [21].

Post reaction soy flakes were subjected to Soxhlet extraction as previously described and analyzed by HPLC to quantify the amount of FAME and unreacted oil that remained on the flakes.

Nuclear Magnetic Resonance (NMR)

To determine if NaOMe was being converted to its carbonic acid derivative during the base-catalyzed in situ transesterification, white powders resulting from NaOMe that was exposed to CO_2 in the catalyst basket of a reactor and NaOMe dissolved in methanol and then expanded with CO_2 (1,000 psi) for 30 min were analyzed by solution-state NMR. The methanol was removed by the pressure of the CO_2 flowing through the catalyst basket and by rotary evaporation, respectively. Spectra were recorded at 4.7 Tesla on a Gemini NMR Spectrometer (Varian, Inc., Palo Alto, CA) at 23 °C, using a 5-mm broad-band probe. All samples were dissolved in d_2-H_2O. The 1D proton (^1H) spectra (200 MHz) were acquired with a spectral width of 3,000 Hz, a 90-degree pulse angle and a 1.0-second relaxation delay. The inverse-gated ^{13}C spectra (50 MHz) were acquired with a spectral width of 12.5 kHz, a pulse angle of 45°, a relaxation delay of 2 s, 38 K data points, and 36 K transients. ^1H spectra: (H_2O-d_6, 200 MHz): δ 3.31 (s, –OCH_3); ^{13}C spectra: (H_2O-d_6, 50 MHz): δ 48.8 (–CH_3, 1.026), δ 163.6 (–C=O, 1.000),

Protein Analysis

Nitrogen content (wt.%) was determined by loading and combusting 150 mg of reacted or unreacted soy flakes using a FlashEA 1112 Series Nitrogen/Protein Analyzer (Thermo Electron Corporation, Waltham, MA). Once the percentage nitrogen content was determined, protein content could be determined by multiplying the % nitrogen by 6.25, the protein factor for soy [22].

Results and Discussion

Acid- and alkali-catalyzed in situ transesterifications in CO_2-expanded methanol have been studied. Alkali-catalyzed transesterifications are typically faster than acid-catalyzed transesterifications. They are also less corrosive and are commonly used in industrial processes [23]. Therefore, we began our studies with alkali-catalyzed transesterification reactions.

Alkali-Catalyzed Transesterifications

The results of in situ alkali-catalyzed transesterifications in the absence of gas expansion have been previously

reported by Haas et al. [6, 24]. Those investigators found that approximately 93% of the lipid in flakes with a moisture content of 7.4% could be converted to FAME at 60 °C with an 8 h incubation period using MeOH/TAG/NaOH at a molar ratio of 226:1:1.6 [24]. They further observed that reductions in water content of the flakes resulted in product yields of 97% (2.6% water content) and 100% (0.0% water content) FAME, and that the methanol requirement was also drastically reduced by as much as 60% when totally dry flakes were used.

This paper aims to investigate the possibility that the methanol requirement of in situ transesterification could be further reduced, or the rate of the reaction increased, by introducing CO_2 into the system at temperatures and pressures that would allow the CO_2 to dissolve into the methanol. It is known that the dissolution of CO_2 in methanol resulted in a less polar solvent system [25, 26]. Because Haas et al. [6] previously showed through Soxhlet extraction of flaked soybeans with methanol that free triglycerides were not extracted into or otherwise detected in the liquid phase at any stage of in situ transesterification of soy flakes, we hereby postulated that the creation of less polar media would increase the transport rate of the triglyceride out of the soy flakes into the liquid phase where it could be converted into FAME; thereby, increasing the rate of reaction.

Control reactions revealed that our system was adequate for the in situ transesterification of soy flakes. When soy flakes with 4.0% moisture were transesterified using MeOH/TAG/NaOMe at a molar ratio of 226:1:1.6, FAME was produced at 86.6% of maximum theoretical yield in the absence of CO_2 in 5 h at 57 °C. HPLC analysis of a Soxhlet extract of the reacted flakes revealed that no TAG remained in the flakes. The recovered mass balance for total FAME, TAG and FFA fluctuates throughout the entire data set as determined by quantification of the corresponding peaks generated by HPLC. These fluctuations are likely the result of the short, broad, overlapping TAG and FFA peaks, which could distort the calculation of the amounts of these chemical species. Conversely, no other peaks overlapped the tall and sharp FAME peaks, lending to less room for error. In an attempt to locate other species of reaction, a second HPLC and gradient method was employed to generate HPLC data for representative samples from the data set. The presence of 2.9(±0.5)% (DAG) and 0.2(±0.2)% (MAG) was confirmed under our best reaction conditions (Table 1, lines 9 and 15).

Reactions were then performed to determine the effects of CO_2 on the system. Carbon dioxide was introduced at temperatures and pressures that could achieve 10%, 30% and 70% molar ratios with methanol. In all cases, the amount of FAME produced dropped dramatically (<15% of maximum theoretical yield) in CO_2-expanded methanol.

When the moisture content of the flakes was reduced from 4.0 to 1.6% and the reaction was then performed using MeOH/TAG/NaOMe at a molar ratio of 226:1:1.6, FAME was produced in 91.0% of the maximum theoretical yield in the absence of CO_2 in 5 h. HPLC analysis of a Soxhlet extract of the reacted flakes again revealed that no TAG remained in the flakes. However, once CO_2 was introduced into the system at temperatures and pressures that were able to achieve a 10% molar volume of CO_2 dissolved in methanol, the amount of FAME produced after 5 h decreased to 62.2%. HPLC analysis of a Soxhlet extract of the reacted flakes revealed that 23.7% of the TAG remained. Increasing the base concentration from 0.1 N to 0.3 N increased the FAME production to 67.6% while decreasing the amount of the unreacted TAG to 16.8%. A base concentration of 0.6 N further increased FAME production to 84.8% and an HPLC analysis of a Soxhlet extract of the reacted flakes showed no TAG in the solid residue suggesting the complete conversion of available TAG in the flakes used in the experiment.

Further experiments revealed that transesterification was occurring in our system faster than previously believed. In fact, in the absence of CO_2, FAME production as high as 64.7%, could be achieved in 30 min under standard reaction conditions. Typically, the solid substrate, methanol, and catalyst were allowed to equilibrate for approximately 30 min at the desired reaction temperature before CO_2 was introduced into the system. Consequently, previous results were misleading because the bulk of the observed transesterification reaction had occurred before CO_2 was ever introduced. Using a catalyst basket, which is a capped storage reservoir for solid or liquid reactants or catalysts, further proved that CO_2 not only failed to improve the alkali-catalyzed reaction but it also inhibited the reaction completely. The catalyst basket was located in-line with the tubing that introduced the CO_2 into the Parr reactor. Inside the reactor were only soy flakes and methanol that had been warmed to 57 °C. As CO_2 entered the system, it supplied enough pressure to open the cap on the catalyst basket thus allowing simultaneous introduction of CO_2 and catalyst. Upon opening the reactor, a powdery residue was apparent around the catalyst basket suggesting that the CO_2 caused the catalyst to precipitate out of solution. HPLC analysis showed no FAME was produced using this method.

To determine if the sodium methoxide was being converted to its carbonate in the presence of CO_2, a sealed reactor was pressurized with 1,000 psi CO_2 and allowed to mix for 30 min with 0.1 N NaOMe in 54 mL of methanol at room temperature. The vessel was depressurized and the methanol was removed by rotary evaporation. The white powder that resulted from the rotary evaporation and the white powder from the catalyst basket were both shown by

Table 1 Acid-catalyzed, in situ transesterification of 22.5 g soy flakes (1.6% moisture) in gas-expanded methanol at 121 °C

	MeOH vol. (mL)	CO_2	Time (h)	H_2SO_4 Conc. (N)	H_2SO_4 (mmol)	FFA (wt%)	TAG (wt%)	FAME (wt%)	Mass balance (wt%)
1	54.0	−	3.0	1.2	33.8	0.1 (±0.3)	68.8 (±3.2)	33.1 (±1.6)	102.0 (±4.6)
2		+	3.0	1.2	33.8	0.0 (±0.0)	31.6 (±4.0)	55.2 (±4.4)	86.8 (±6.5)
3		−	6.5	1.2	33.8	0.8 (±.75)	47.1 (±1.65)	37.6 (±7.6)	85.5 (±6.4)
4		+	6.5	1.2	33.8	0.1 (±0.05)	23.1 (±3.3)	67.7 (±4.8)	90.9 (±1.5)
5		−	10.0	0.0	0.0	25.4 (+5.2)	49.2 (+3.0)	12.4 (+2.8)	87.0 (+5.2)
6				1.2	33.8	0.5 (±0.2)	61.9 (±2.1)	41.5 (±3.7)	103.9 (±4.3)
7		+	10.0	0.0	0.0	8.0 (±2.3)	70.1 (±3.2)	12.4 (±2.8)	90.5 (±5.2)
8				0.6	16.9	0.0 (±0.0)	51.0 (±7.6)	32.5 (±4.6)	83.5 (±0.9)
9				1.2	33.8	0.0 (±0.0)	0.2 (±0.2)	88.3 (±1.5)	88.5 (±1.7)
10	36.0	−	3.0	2.0	33.8	2.1 (±0.3)	57.6 (±3.6)	33.0 (±3.7)	92.7 (±2.3)
11		+	3.0	2.0	33.8	0.6 (+0.2)	53.4 (±2.7)	25.3 (±4.6)	79.3 (±5.4)
12		−	6.5	2.0	33.8	2.1 (±0.6)	62.1 (±1.8)	33.5 (±5.5)	97.7 (±2.3)
13		+	6.5	2.0	33.8	0.1 (±0.0)	39.0 (±2.9)	46.4 (±2.6)	85.5 (±3.4)
14		−	10.0	2.0	33.8	2.0 (±0.4)	55.5 (±2.0)	34.8 (±3.7)	92.3 (±2.5)
15		+	10.0	2.0	33.8	0.7 (±0.2)	6.6 (±0.8)	88.2 (±5.0)	95.5 (±5.7)
16	27.0	+	6.5	2.4	33.8	0.3 (±.25)	53.9 (±5.0)	52.2 (±5.6)	106.4 (±0.9)
17				3.6	50.6	1.5 (±0.1)	23.3 (±4.1)	45.0 (±6.2)	69.8 (±10.4)
18		−	10.0	2.4	33.8	0.3 (±0.0)	58.7 (±2.4)	39.2 (± 5.2)	98.2 (± 1.2)
19		+	10.0	1.2	16.9	0.9 (±0.6)	56.2 (±2.3)	31.0 (±2.6)	88.1 (±0.3)
20				2.4	33.8	0.2 (±0.0)	41.2 (±6.9)	46.2 (±8.5)	87.6 (±1.7)
21				3.6	50.6	0.5 (±0.5)	26.8 (±5.1)	59.9 (±10.7)	87.2 (±5.15)

All percentages represent a fraction of the maximum theoretical yield. Mass balance refers to the combined, theoretical mass% of FAME, TAG, and FFA. A total of 54 mL MeOH, 22.5 g soy flakes, and 1.2 N H_2SO_4 represents a molar ratio of 217:1:5.6 (MeOH/TAG/H_2SO_4) for reactants and catalyst

^{13}C NMR to have strong carbonyl peaks with integrations revealing approximately 50% conversion of sodium methoxide to its carbonic acid derivative. Therefore, it is assumed that the formation of the carbonic acid of NaOMe increases the acidity of the system and reduces the pH below what is necessary for an alkali-catalyzed transesterification to occur.

Acid-Catalyzed Transesterifications

We performed acid-catalyzed reactions for the conversion of TAG to FAME using MeOH/TAG/H_2SO_4 at a molar ratio of 217:1:5.6 at 121 °C and show the results in Table 1. This molar ratio represents standard reaction conditions on the scale of 54 mL methanol, 22.5 g soy flakes (1.6% moisture), and 1.2 N H_2SO_4 for the reactants and catalyst. Initially, we had used MeOH/TAG/H_2SO_4 at a molar ratio of 221:1:2.8 that was calculated based on the molar ratio of MeOH/TAG/NaOMe of 226:1:1.6 used for the alkali-catalyzed reactions [6] and then adjusted to 0.5% wt. H_2SO_4 as suggested by Freedman et al. [23] for an acid-catalyzed transesterification reaction. Due to low conversion of TAG to FAME at this ratio of catalyst to substrate after 10 h, however, we decided to double the catalyst concentration, yielding the results
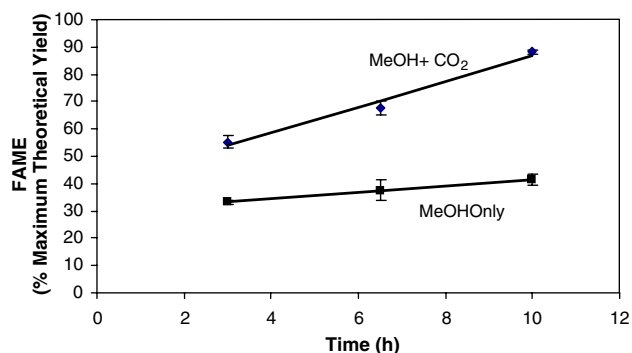
shown in Table 1, line 9. Using the molar ratio of 217:1:5.6 (MeOH/TAG/H_2SO_4), we determined by HPLC analysis that no TAG was converted to FAME during the time required to raise the temperature of the reaction from room temperature to 75 °C.

Freedman et al. [23] conducted reactions at 117 °C. From available vapor–liquid equilibrium data, the closest temperature to 117 °C that could still maintain a CO_2-expanded MeOH system was 121 °C. [16–20]. Lower temperatures were not explored because acid-catalyzed transesterifications typically require temperatures above 100 °C [23]. A 50% molar volume of CO_2 in methanol was chosen at random primarily to serve as a starting reference point whereby future experiments can be designed to evaluate higher and lower mole fractions.
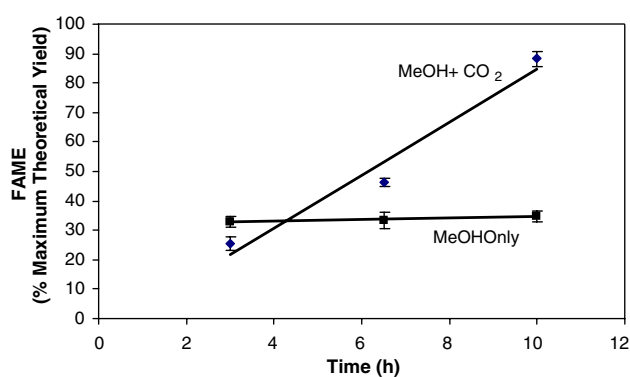
The results of the in situ transesterification of soy flakes in gas-expanded methanol are shown in Table 1. There is no example of acid-catalyzed in situ transesterifications with flaked soybeans in the literature with which to compare this data but a published account of the acid-catalyzed in situ transesterification of ground soybeans is available [11]. Using 1.5 N H_2SO_4 in methanol, at a molar ratio of 285:1:9 (MeOH/AG/H_2SO_4), it was reported that 22% of the TAG was converted to FAME after 3 h at 65 °C.

Within error, experiments performed in 36 mL of methanol for 3 h and in 27 mL of methanol for 10 h showed no increase or decrease in FAME production when CO_2 was introduced into the system. However, all other data comparing experiments performed in the absence and presence of CO_2 show that when all other variables are held constant, the introduction of CO_2 into the system significantly increases FAME production. Figures 1 and 2 illustrate this very clearly as there is little change in FAME production over time in the absence of CO_2. For example, in the absence of CO_2, transesterification in 36 mL of methanol produces 33.0% FAME after 3 h and proceeds no further even after 10 h. Yet, the introduction of CO_2 into the system increases FAME production by factors of 1.4 (after 6.5 h) and 2.5 (after 10 h) in experiments performed in 36 mL methanol. For experiments performed in 54 mL of methanol, the introduction of CO_2 increases FAME production by factors of 1.7 (after 3 h), 1.8 (after 6.5 h) and 2.1 (after 10 h). The observed increase in FAME production upon the addition of CO_2 could be the result of increasing the total volume of the liquid phase and the increased solubility of TAG in an expanded liquid phase that is less polar than methanol. The inherent acidity of the CO_2-expanded methanol could also assist the acid-catalyzed transesterification.

The amounts of FAME detected at 10 h for reactions performed in 54 and 36 mL of methanol in the presence of CO_2 represent 99.8 and 92.3%, respectively, of the total mass detected for those sets of reactions. Reactions performed in 27 mL of methanol were less successful than reactions performed in 36 and 54 mL of methanol when all other conditions were held constant. After 10 h in the presence of CO_2 and 2.4 N catalyst, only 46.2% of the theoretical yield of FAME was observed when using 27 mL of methanol. Attempts to increase FAME production by increasing the catalyst concentration to 3.6 N only resulted in 59.9% total FAME, a 1.3-fold increase. Increasing the acid concentration had previously resulted in

Fig. 2 In situ transesterification of 22.5 g soy flakes (1.6% moisture) in 2.0 N H_2SO_4 in methanol at 121 °C in the presence and absence of CO_2
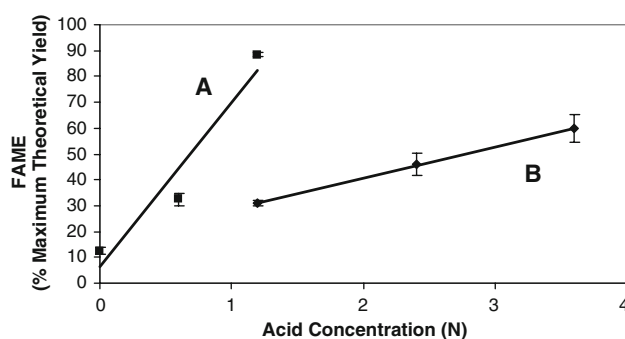
Fig. 3 In situ transesterification of 22.5 g soy flakes (1.6% moisture) in various concentrations of H_2SO_4 in total reaction volumes of 54 mL (a) or 27 mL (b) at 121 °C for 10 h

a corresponding increase in FAME production (Table 1). Figure 3 shows the only two examples from the data set with more than two such data points.

The data in Table 1 also reveals that the introduction of CO_2 into the system decreases the production of FFA in all cases studied (Table 1). The apparent inhibition of FFA production by CO_2 is quite advantageous since the presence of residual FFAs in FAME would increase the acid number, thermal instability and corrosive properties and decrease the oxidative stability of the fuel.

Protein Analysis

Preserving the protein content is essential in determining the suitability of the lipid-free flakes for animal feed after the in situ transesterification reaction. Data have shown that the meal derived from alkali-catalyzed, in situ transesterifications of soy flakes is a suitable feed component [27]. In our experiments, the protein content of soy flakes dried to 1.6% moisture was determined to be 48.8 ± 0.2 wt%. This is typical of protein content found in commercially available soybean meal [28]. Considering that the flakes used in this experiment still contained an average of 23.6% oil, the

Fig. 1 In situ transesterification of 22.5 g soy flakes (1.6% moisture) in 1.2 N H_2SO_4 in methanol at 121 °C in the presence and absence of CO_2

theoretical maximum amount of protein for the transesterified, defatted flakes would be 63.9%. After being subjected to acid-catalyzed in situ transesterification for 6.5 and 10.0 h in CO_2-expanded methanol, the protein content of the remaining meals was determined to be 55.9 ± 2.8 and 53.0 ± 2.0 wt%, respectively, suggesting a retention of protein content of at least 87.5% (6.5 h experiments) and 82.9% (10 h experiments).

Summary

Alkali- and acid-catalyzed in situ transesterification of soy flakes were performed in gas-expanded methanol to explore the possibility of lowering the amount of methanol requirement. It became evident that sodium methoxide could not be used as the catalyst due its conversion to sodium carbonate by the added CO_2. Alternatively, sulfuric acid was used as the catalyst and, unlike alkali-catalyzed in situ transesterifications, the addition of CO_2 did not inhibit the reaction. It was observed that in the acid-catalyzed system, the total volume of the liquid phase can be lowered by one-third (54–36 mL) without a significant decrease in FAME production.

It was also shown that the addition of CO_2 into the system increased the rate of acid-catalyzed reactions. In fact, the addition of CO_2 increased the rate of the reaction by as much as 2.5-fold in experiments performed in 36 mL methanol after 10 h. The study further showed that catalyst concentration was an important factor in FAME production under our reaction conditions, as higher concentrations of acid typically resulted in an increased yield of FAME.

The economics of this process and the variations thereof have yet to be examined. However, nitrogen analysis shows that 87.5% (6.5 h experiment) and 82.9% (10 h experiment) of the protein is left intact after soy flakes have been subjected to acid-catalyzed in situ transesterifications. Maintaining the value of the lipid-free flakes as a viable component of animal feed is important to the economics of FAME production by in situ transesterification.

Using a physical pretreatment method, Haas et al. [29] have presented data showing a reduction in the methanol requirement in non-gas expanded systems to <2 mL per 5 g of soy flakes. This significantly reduces the molar ratio of methanol:TAG to <35:1. We anticipate that a similar pretreatment might also reduce the methanol requirement in the present gas-expanded acid-catalyzed in situ transesterification systems.

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