Biodiesel production by *in situ* transesterification

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Biodiesel is conventionally produced by transesterification of vegetable oils using an alcohol (usually methanol) and a catalyst (usually hydroxides or methoxides of sodium or potassium). The process usually uses pre-extracted oil as the raw material, which is usually produced by pressing the oil-bearing seeds, often followed by solvent extraction to extract any remaining oil. Alternatively, biodiesel can be produced via '*in situ* transesterification' or 'reactive extraction'. In this process, oil-bearing seeds are grindd, then reacted directly with the alcohol and catalyst, thereby eliminating the need for pre-extracted oil, and its associated capital and intensive running cost production methods. Various parameters play important roles in determining the conversion, reaction rate and quality of the biodiesel in *in situ* transesterification. These include: catalyst type, seed moisture content, agitation intensity, molar ratio of alcohol to oil, reaction temperature, catalyst concentration, seed fragment particle size and alcohol type. This article gives an overview of *in situ* transesterification, the parameters that have a significant effect on this process and the advantages and disadvantages of this process.

Since Dr Rudolf Diesel first demonstrated his compression ignition combustion engine using peanut oil at the 1900 Paris exhibition [1], the use of vegetable oil as a transport fuel has been known to be feasible. However, petroleum fractions that were compatible with the diesel engine became less expensive than vegetable oils, so vegetable oil-based fuels were not commercially viable. Over the last 20 years, factors such as geopolitical tension in the Middle East, leading to price volatility in crude oil and fears for security of supply, and the realization of global warming, have combined to stimulate interest in vegetable oil-based diesel fuels. Research into **biodiesel** prior to 1990 centered on using raw or modified vegetable oils [2–5]. Despite these fuels successfully passing shorter engine performance tests (less than 10 h duration), problems began to emerge after longer periods of use. The major problems (according to Pryde [4]) were:

- Coking and trumpet formation on the injectors, to such an extent that the fuel atomization does not occur properly or is even prevented altogether by total blockage of the injector;

- Carbon deposits;

- Oil ring sticking

- Thickening and gelling of the lubricating oil as a result of contamination with vegetable oil.

These problems occur because of the higher viscosity of the vegetable oil, reduced volatility and the reactivity of unsaturated hydrocarbon chains. These problems were addressed by Peterson *et al.*, when they assessed winter rape oil in diesel engine [6]. They suggested that polyunsaturated fatty acids in the oil tend to polymerize and subsequently form gums in the engine chamber. The gums cause the carbon deposit and sticking problems. Darcey *et al.* also reported that the use of blended crude sunflower oils in diesel engines resulted in solid contamination in the lubricating oil [3].

To reduce these problems, Goering and Fry [7] and Ziejewski *et al.* [8] tried to decrease the viscosity of the vegetable oils by creating 'microemulsions'. The process effectively reduced the viscosity of the vegetable oil. Furthermore, use of the hybrid oil obtained resulted in reduced engine wear [7]. However, deposits of carbon

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on the injector tips, intake valve and tops of cylinder liners were increased [7,8]. Incomplete combustion and abnormal increases in the viscosity of lubricating oil was also reported [8].

Thermal cracking [9,10] and **transesterification** [11,12] were also reported in this period (pre-1990). Thermally cracked fuels, after fractionation, were shown to have similar properties to diesel fuel [10]. The drawback of this technique was that the processes were too expensive for modest throughputs, because they were very energy-intensive [13]. In transesterification however, researchers believed they had found the right process. Not only was the quality of biodiesel produced comparable to that of petroleum diesel, but the process could also be operated at low temperature (typically 60°C) and low pressure, resulting in relatively low energy consumption. In addition, the fuel performed well in engine tests [12]. However, it was also discovered that ester yields were reduced due to the existence of gums and extraneous material in the crude vegetable oil [11]. Research into *in situ* **transesterification** was also reported at this time, particularly by Harrington and D'Arcy-Evans [14,15]. Both reported experiments on *in situ* transesterification of sunflower seed oil. Among the noteworthy conclusions was the claim that this process yielded fatty acid esters that were qualitatively similar to those produced by the liquid-phase transesterification, and at a greater yield [14,15].

In the 1990s, most research was concerned with the transesterification of vegetable oil. The effects of the various process parameters and a range of raw materials were reported throughout the decade. Problems arose when the market price of these edible vegetable oils increased. This caused the profitability of the process to decrease. It was suggested that transesterification was only profitable at vegetable oil prices below US$400 per metric ton [16]. To overcome this problem, alternatives to the usual raw materials were investigated and, as a consequence, a myriad of inedible oils were investigated. The focus of current research is to introduce, low-cost and often inedible oils. Apart from new raw materials, researchers also began to investigate new processes to reduce processing costs. Algae began to receive attention as a new raw material [17,18] as it has the potential to provide the possibility of 20-fold increases in oil yield per hectare.

This review critically discusses *in situ* transesterification, which is a process of producing alkyl ester directly from oil-bearing material, usually grindd **oilseeds**. Since its introduction by Harrington and D'arcy Evans [14], numerous researchers have investigated the performance and feasibility of this process. However, whether it can replace the current transesterification technology remains to be seen. The possibility of producing biodiesel via *in situ* transesterification can only be materialized once the process as a whole has been fully characterized [19]. In conventional transesterification, the raw material usually comes from edible oil, where oil was extracted from the seeds using the conventional extraction processes of crushing, perhaps followed by solvent extraction. Haas *et al.* estimated that the cost of refined soybean oil in biodiesel transesterification accounted for 88% of the total production cost [20]. It was also asserted by Haas that biodiesel produced by *in situ* transesterification is more expensive than biodiesel produced via conventional transesterification, due to the large amounts of methanol required for the process. The production cost of biodiesel by *in situ* transesterification of soya was estimated at US$3.14 compared with 0.38 by transesterification [19].

Ongoing research into *in situ* transesterification may render it more economically attractive. Inedible oils, such as *Jatropha curcas* and *Pongamia piñata,* may be grown on 'marginal' land, and, for this reason and because they have greater yields per hectare than soya and canola, may prove to be inexpensive feed. *J. curcas* for example, contains phorbol esters, which are co-carcinogen and toxic. The *Jatropha* oil obtained by cold pressing and solvent extraction still contains such substances. However, biodiesel produced from *Jatropha* oil by *in situ* transesterification has been shown not to contain such chemicals [21]. The application of *in situ* transesterification therefore not only **intensifies** the process, but removes the need for a specific step to extract these toxic substances in the downstream processing, reducing the exposure of workers to such substances.

Definition

In situ transesterification is the direct transesterification of grindd oil-bearing seeds. The seed fragments are reacted with alcohol and catalyst, producing alkyl fatty acid esters. This should be contrasted with conventional biodiesel transesterification, where the raw materials are pre-extracted from oil-bearing seeds.

Figure 1 summarizes the two processes: in situ transesterification has fewer steps than conventional processing. The crushing and solvent extraction steps that are required in the conventional process, but not in in situ transesterification are usually the most capital and
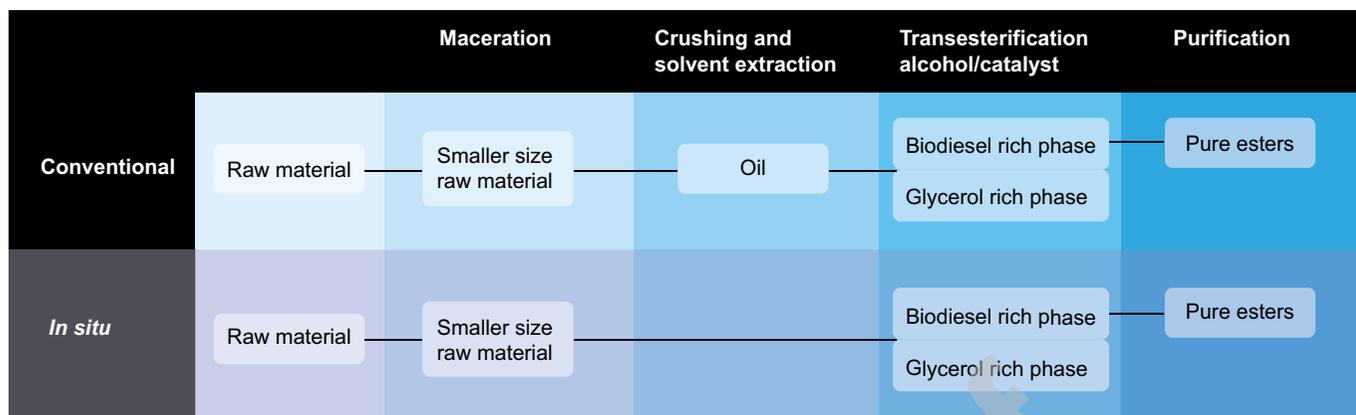
Figure 1. Conventional versus *in situ* transesterification.

running cost-intensive.

To measure the efficiency of *in situ* transesterification, most publications used the terms yield and purity. The majority of researchers define yield as the percentage of biodiesel-rich phase over oil content in raw materials, which is determined by hexane Soxhlet extraction. Purity is defined as the percentage of methyl esters in product obtained from the purification stage. This percentage is usually calculated from a gas chromatogram result.

Variables in *in situ* transesterification

▪ Raw materials

Traditional oil-bearing seeds, such as rapeseed and sunflower seed, or even materials such as distiller's dried grains with solubles (DDGS) [22] and jatropha seed [23,24], have been studied by researchers. The fatty acid profiles of the oils produced by these materials vary substantially and, consequently, process parameters differ. Even though fatty acid profiles are known to influence biodiesel properties such as cetane number and cold filter plugging point [25], no researchers have studied this with respect to *in situ* transesterification. The *in situ* approach however, can be applied to almost any lipid-bearing material **(Table 1)** [14,22,23,26–32].

▪ Catalyst

It is well-documented that *in situ* transesterification is unable to proceed without catalyst [26,33]. Short-chain alcohols, particularly methanol, are poor solvents for lipids. Zeng *et al.* observed that methanol alone was capable of extracting only 4.5% (seed mass) of oil from 20 g soybean, compared with 45% when using n-hexane [34]. Acid or alkali catalysts in *in situ* transesterification help to break oilseeds' cell walls, thereby allowing methanol to access the oil in cotyledon cells. Ren *et al.* investigated the *in situ* transesterification of canola using scanning electron microscopy (SEM) and light microscopy [35]. Lipid staining showed that this reactive

Table 1. Different raw materials used by researchers in *in situ* transesterification.			
Raw Material	**Oil (%)**	**Fatty acid composition (%)**	**Ref.**
Sunflower seed	38	16:0(6.8), 18:0(5.0), 18:1(19.6), 18:2(68.6)	[14]
Soybean	23	16:0(12.0), 18:0(5.0), 18:1(25.0), 18:2(52.0), 18:3(6.0)	[26]
Distiller's dried grains with soluble	8.8	16:0(12.9), 18:0(1.6), 18:1(28.5), 18:2(55.5), 18:3(1.4)	[22]
Meat and bone meal	9.1	16:0(25.2), 18:0(19.7), 18:1(35.6), 18:2(1.9), 18:3(0.3)	[22]
Palm oil pulp	80	12:0(0.3), 14:0(0.8), 16:0(44.3), 18:0(5), 16:1(0.2), 18:1(39.1), 18:2(10.1), UK(0.2)	[40]
Cottonseed	31.6	16:0(28.7), 18:0(0.9), 18:1(13.0), 18:2(57.4)	[27]
Rapeseed	42	16:0(4.0), 18:0(1.9), 18:1(62.1), 18:2(32.0)	[28]
Jatropha curcas	54	16:0(16.0), 18:0(7.0), 18:1(45.0), 18:2(32.0)	[23]
Wastewater sludge (primary sludge)	2	16:0(42.0), 18:0(14.0), 18:1(28.0), 18:2(10.0), 20:1(6.0)	[29,30]
Microalga (*Schizochytrium limacinum*)	51†	14:0(2.06), 16:0(35.5), 18:0(0.81), 22:5(8.58), 22:6(53.05)	[31]
Microbial biomass (*Lipomyces starkeyi*)	50†	14:0(0.4), 16:0(33.0), 17:0(0.4), 18:0(4.7), 16:1(4.8), 18:1(55.1), 18:2(1.6)	[32]
Microbial biomass (*Rhodosporidium toruloides*)	58†	14:0(0.7), 16:0(24.3), 17:0(0.6), 18:0(7.7), 16:1(1.1), 18:1(54.6), 18:2(2.1), uk(8.9)	[32]
Microbial biomass (*Mortierella isabellina*)	53†	14:0(1.2), 16:0(28.2), 18:0(1.0), 16:1(5.8), 18:1(55.5), 18:2(5.8), 18:3(2.4), uk(0.1)	[32]
†Total lipid extraction. UK: Unknown.			

extraction followed a 'shrinking core' model, as the mass of cells containing lipid within the cell could clearly be seen to shrink as the extraction progressed. Sodium hydroxide can dissolve cell walls, but they did not find any evidence for this for cells inside the particles, when examining sections via light microscopy.

The total mass yield of extract by *in situ* transesterification (40.9%) was found to be greater than that obtained from transesterification (30.3%) [14]. It was claimed that this was due to the capability of *in situ* transesterification to extract materials that were not extracted from the seed by hexane [14]. As a nonpolar solvent, hexane can only extract nonpolar substances, such as triglycerides. When acidified/alkaline methanol was used instead of hexane, both polar and nonpolar substances were extracted from the seed. Dufreche *et al.* also claimed that a higher percentage of material was extracted (19.39) when using methanol rather than when using hexane (1.94) when they extracted sewage sludge [29]. The sharp increase in methanol extraction in this case was due to the presence of large amounts of phospholipids, in the form of microorganism cell membranes, in the sewage.

Acid catalysts

Acids, in particular sulfuric acid, were the preferred catalysts in earlier (1980s) research into *in situ* transesterification for biodiesel production. Harrington and D'arcy-Evans were the first researchers working on sulfuric acid as a catalyst in *in situ* transesterification [14,15]. Acid catalysis has often been investigated for the treatment of raw materials with high levels of free fatty acids (FFAs). Alkaline catalysts will react with FFAs to produce soap and glycerol, decreasing the amount of the catalyst, or consuming it alltogether. Furthermore, soap acts to emulsify the product, rendering the separation between alkyl esters and glycerol more difficult. Acid catalysis, in contrast, does not promote saponification. Mondala *et al.*, for instance, used sulfuric acid as the catalyst for conversion of their raw material, municipal sewage sludge, as it contained 65 wt% FFA [30]. Turkay *et al.*, who investigated extraction from rice bran, used acid catalysts, because the acidity of rice bran oil was unpredictable, and usually high [36–38].

Most researchers report high conversion of methyl esters when using acid catalysts. Harrington and D'arcy-Evans achieved 98% conversion of sunflower seed oil to fatty acid methyl ester (FAME) using a methanol/sulfuric acid mixture. Siler-Marinkovic and Tomasevic, who also worked with a sunflower seed/methanol/sulfuric acid system, observed conversion over 90% over a wide range of experimental condition [39]. Shuit *et al.* reported that 90% of the oil was extracted from *J. curcas* seed when using acid-catalyzed *in situ* transesterification, and

100% of it was converted to FAME [23]. Obibuzor *et al.* also reported high conversion (97%) of oil to FAME from reactive extraction of oil palm waste pulp using a methanol/sulfuric acid mixture [40]. Acid catalysis also worked efficiently in reactive extraction of oleaginous microbial biomass. Lipid contents from three different oleaginous biomass, *L. starkeyi*, *M. isabellina* and *Rhodosporidium toruloides* were successfully converted to FAME at 97, 91 and 98%, respectively. Liu *et al.* investigated the *in situ* transesterification of cellular biomass of yeast and fungi using an acid catalyst and methanol [32]. They found that both sulfuric acid and hydrochloric acid could produce moderate ester yields, which were 60 and 53%, respectively. Significantly lower yields (10%) were achieved when phosphoric acid was used, but the authors do not offer an explanation for this.

Researchers also observed that the reaction time was longer when using an acid catalyst rather than an alkaline catalyst. Shuit *et al.*, for instance, found that 90% conversion of *J. curcas* using sulfuric acid required 24 h [23]. Obibuzor *et al.* obtained more than 90% conversion in 12 h when using reactively extracted palm oil pulp waste with sulfuric acid [40].

Alkaline catalysts

The first *in situ* transesterification using an alkaline catalyst was reported by Haas *et al.* [41]. The experiment was conducted using soybean flakes as a raw material. The combination that produced the highest percentage of methyl ester was 12.5 ml of methanol, 0.18 N of sodium hydroxide. This was equivalent to 226:1:1.6 molar ratio of methanol/oil/NaOH. Comparing this value with the conventional transesterification experiments (6:1:0.22) by Freedman *et al.* [42], it is clear that *in situ* transesterification requires more methanol and more catalyst.

When comparing the effectiveness of both acid and alkali catalysts, Haas *et al.* listed three advantages in favour of using alkaline catalysts [41]:

- Instead of using pulverized beans as described in previous literature [14,15,26], *in situ* transesterification with an alkali catalyst only requires soybeans to be flaked;

- The amount of reagent required is reduced and milder process condition are required;

- Higher yields of methyl ester are observed.

The advantages of the first two options can be clearly seen in the literature. For instance, a molar ratio of 553:1 methanol to oil was used by Harrington and D'Arcy-Evans [14] (sunflower seed/methanol/sulfuric acid) to achieve 97% conversion, whereas using an alkaline catalyst, Georgogianni *et al.* used 163:1 molar ratio (sunflower seed/methanol/sodium hydroxide)

and achieved 95–97% conversion [43]. However, for the third option, it seems that both types of catalysts produced a comparable yield of FAME, but not at the same rate. The reaction time required when using sulfuric acid to produce 97% yield was 4 h, while sodium hydroxide only needed 2 h to produce the same yield. Furthermore, at 40 min, 94% of oil had already been converted to methyl ester.

The conversion of oil to methyl esters was typically very high when using methanol and sodium hydroxide. Among researchers that successfully achieved high yield of conversion were Georgogianni *et al.* [27,43], on sunflower seed/methanol/sodium hydroxide (97%) and cottonseed/methanol/sodium hydroxide (97%), and Haas *et al.* [41] on soybean/methanol/sodium hydroxide (88%). Qian *et al.* also achieved over 95% conversion in reactive extraction of cottonseed using methanol and sodium hydroxide [33]. Alkaline catalyst reactive extraction has also been applied to a number of nonoilseed feedstocks. To investigate whether *in situ* transesterification was applicable to all lipid-bearing materials, Haas' group performed experiments on DDGS and meat and bone meal (MBM). Both raw materials contained low percentages of oil, but via alkaline methanolysis, the oil fractions of DDGS and MBM were successfully converted to methyl ester at

91 and 93% conversion, respectively. Dufreche *et al.*, using acid catalysis, noted that *in situ* transesterification of sewage sludge achieved 6.23% conversion compared with 0.38% when hexane extraction/acid transesterification was used [29]. Even the second highest conversion (3.44% – achieved when a mixture of hexane, methanol and acetone used to extract the oil) was 2.79% lower than *in situ* transesterification. Clearly, this is a significant difference, and could make the difference in determining the economic viability of low oil content feedstocks.

Table 2 lists different raw materials, catalyst and solvents used by researchers to produce biodiesel through *in situ* transesterification [15,22–24,26,27,30,32,34,39,41,43–45]. The selection of catalyst very much depends on the feedstock properties, especially the content of FFA.

▪ Moisture content
In conventional transesterification, the presence of water in the process would cause soap formation and frothing. This would result in increased viscosity, gel formation and difficulty in separation between the glycerol and alkyl ester-rich phases [13]. In addition, the saponification process will consume triglyceride, thereby reducing the potential yield of the methyl ester. In reducing the moisture content prior to the

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Raw material	Solvent	Catalyst (mol/l)	Molar ratio solvent:oil	Reaction time (h)	Temp. (°C)	Conversion (oil basis) (%)	Notes	Ref.
Sunflower	Methanol	H_2SO_4 (0.75)	532:1	5	65	93		[15]
Sunflower	Methanol	H_2SO_4 (0.7)	300:1	4	64.5	98.2		[39]
Soybean	Methanol	H_2SO_4 (0.75)	281:1	10	65	23.3		[26]
Soybean	Methanol	H_2SO_4 (0.75)	150.1	3	121	83	CO_2 cosolvent	[44]
Jatropha curcas	Methanol	H_2SO_4 (0.2)	300:1	24	60	99.8	Hexane cosolvent	[23]
Microbial biomass	Methanol	H_2SO_4 (0.2)	830:1	20	70	96.8 (*Lipomyces starkeyi*) 91.0 (*Mortierella isabellina*) 98.1 (*Rhodosporidium toruloides*))		[32]
Primary sewage sludge	Methanol	H_2SO_4 (0.9)	1400:1	24	75	66		[30]
Soybean	Methanol	NaOH (0.09)	543:1	8	23	88		[41]
DDGS	Methanol	NaOH (0.4)	655:1	1.2	35	91.1		[22]
MBM	Methanol	NaOH (2.0)	550:1	0.2	35	93.3		[22]
Cottonseed	Methanol	NaOH (0.4)	673:1	0.3	60	95	Ultrasound	[27]
Cottonseed	Ethanol	NaOH (0.4)	613:1	0.7	80	98	Ultrasound	[27]
Sunflower	Methanol	NaOH (0.4)	476:1	0.7	60	97	Ultrasonic	[43]
Sunflower	Ethanol	NaOH (0.4)	434:1	0.7	80	98	Ultrasonic	[43]
Sunflower	Methanol	NaOH (0.2)	101:1	13	20	98	DEM cosolvent	[34]
Jatropha curcas	Methanol/ethanol mix	NaOH (0.02)	512:1	1	60	87		[24]
Jatropha curcas	Methanol	NaOH (0.04)	100:1	1	60	70		[45]

DDGS: Distiller's dried grains with solubles; DEM: Diethoxymethane; MBM: Meat and bone meal; Temp.: Temperature.

reaction, Haas *et al.* found that the amount of alcohol required for the process was lessened significantly [46]. They reported 60% reduction of methanol and 56% reduction of sodium hydroxide when soybean flakes were dried in a convection oven until it had a water content of 0%. Experiments at 2.6% water content samples reduced the methanol and sodium hydroxide requirement by 40 and 33%, respectively.

In situ transesterification has been shown to require higher alcohol to oil ratio than conventional transesterification. Even though the application of *in situ* transesterification eliminates the need for pre-extracted oil, it is asserted by Haas [20] that it is more expensive than biodiesel produced by conventional transesterification. The reduction of water however, was able to reduce the estimated biodiesel production cost from US$3.14 to 1.02 per gallon [19]. The same trend was also reported by Qian *et al.* [33]. Methyl ester conversion was found to increase significantly from 80 to 98% when the moisture content was reduced from 8.7 to 1.9%. Further reduction of moisture content however had very little effect on the conversion.

By contrast, research at Newcastle on *in situ* transesterification of grindd rapeseed using methanol/sodium hydroxide has shown that drying the seeds from 6.7 to 0 wt% water does not reduce the solvent requirement, nor increase the yield of ester [28]. It was found that the ester yield only reduced when there was more than 2 wt% water in the solvent. This indicates that, for rapeseed at least, the drying step may be unnecessary, which should reduce the cost of biodiesel produced by this method, as drying can incur substantial running costs. The mechanism is simply that the methanol extracts the water rapidly, followed by reaction within the seed, thereby separating the water from the reaction. Saponification could still occur in the bulk liquid phase, but the water concentration is greatly reduced.

▪ **Mixing intensity**

Georgogianni *et al.* observed the effect of low-frequency ultrasound on *in situ* transesterification reaction [27,43]. In both studies, the authors compared the difference between the use of a mechanical stirrer (600 rpm) and low-frequency ultrasound (24 kHz) as a means of agitation. When the experiments were conducted on *in situ* transesterification using methanol, no significant difference was noticed. Both agitation methods gave high conversions of methyl ester after 20 min of reaction. However, when ethanol was used, the application of ultrasound produced high conversions more rapidly than mechanical stirring. At 40 min 98% conversion was achieved with ultrasound, whereas mechanical stirring resulted in a lower yield (88%). Both works on ethanol reported the same results, despite using different raw materials (sunflower and cottonseed). They asserted that the reason behind this phenomenon was that ultrasound produced less soap, because no stirring action was required. No further experiments were conducted to confirm the hypothesis. However, saponification occurs as a result of the reaction between sodium hydroxide and FFA and, as any reaction, its occurrence will depend to some extent upon the degree of mixing, but is unlikely to be dependent on the form of mixing, so this point is debatable. It may be that, as ethanol is a better solvent for triglycerides than methanol, more of the reaction takes place in the liquid phase, rather than in the seed, leading to a sonochemical enhancement for ethanol, but not for methanol.

Zeng *et al.* studied *in situ* transesterification of sunflower seeds with diethoxymethane (DEM) as co-solvent [34]. They found that agitation speed had no influence on biodiesel yield or FAME purity, which is as would be expected for an effective co-solvent.

▪ **Molar ratio of alcohol to oil**

All researchers agree that the required molar ratio of alcohol to oil in *in situ* transesterification was extremely high compared with the conventional transesterification of vegetable oil. For example, Siler-Marinkovic and Tomasevic [39] used a 300:1 ratio in their experiments with sulfuric acid catalyst, while Haas *et al.* [41] applied a 543:1 ratio for sodium hydroxide. The typical conditions used for conventional transesterification are 6:1 [42]. Calculations performed by Haas' group indicated that the amount of methanol involved in this process was the main reason for the high price of biodiesel produced by this method [19]. This increase is mainly due to the fact that the purification of the biodiesel became more complicated and costly.

As in transesterification, insufficient alcohol leads to incomplete reaction. Kildiran and co-workers achieved only 23.3% (oil basis) conversion from total oil dissolved in methanol when they used a 281:1 molar ratio of methanol to oil [26]. On the contrary, works by Harrington and D'arcy Evans [15] and Siler-Marinkovic and Tomasevic [39] reported conversions of 93.2 and 98.2%, respectively, when they used methanol-to-oil ratios of 370 and 300:1. Distinctively, conversion also decreases above a certain molar ratio. Siler-Marinkovic and Tomasevic [39] used 1.81-times less alcohol than Harrington and D'arcy Evans [15], but maintained all other parameters, and observed that the conversion increased from 93 to 98%.

Interestingly, researchers are now trying to find ways to reduce the amount of alcohol required. The use of co-solvent in conventional transesterification is known to improve the solubility of alcohol, thereby increasing the rate of reaction [47]. Qian *et al.* [33] discussed the

feasibility of using petroleum ether as a co-solvent in the process. The amount of oil extracted from seed and dissolved in methanol increased from 95% in 1 h without co-solvent, to 98% with petroleum ether/ methanol mixture. However, petroleum ether/methanol was only effective below a volume ratio of 1:3. The concentration of oil was reported to be diluted when the ratio exceeded 1:3.

The application of co-solvent in the *in situ* transesterification had also been investigated at length by Zeng *et al.* [34]. They demonstrated that using DEM as a co-solvent reduces the amount of methanol required. At a 58:1 molar ratio of DEM/oil, only 101:1 molar ratio of methanol/oil was required to produce a 96% yield of crude biodiesel. For comparison, the highest yield achieved by researchers working with sunflower seeds was 97%, but the methanol/oil molar ratio was 476:1 [43].

The most recently reported attempt to lower the alcohol/oil ratio was by using CO_2 as a co-solvent [44] at temperatures and pressures at which methanol acts as a less polar solvent, which should increase the rate of triglyceride extraction, and therefore overall reaction rate. However, the addition of CO_2 only gave a positive result when it was being used with an acid catalyst (in this case sulfuric acid) rather than an alkali. Sodium carbonate was detected in the system, suggesting that the methoxide was converted to carbonate in the presence of CO_2, thereby reducing the amount of catalyst and therefore rate of reaction. Using sulfuric acid, the authors claimed that the total volume of methanol can be lowered by one-third without adversely affecting methyl ester yield. Not only did it lower the methanol volume, but the rate of reaction increased by as much as 2.5-fold.

The reason for the requirement for a large molar excess of alcohol in *in situ* transesterification may be that the rate determining step is the diffusion of the alcohol into the particles. A high molar ratio would be required to overcome substantial mass transfer resistances for the reaction to proceed at an appreciable rate. Further evidence for this is the increase in rate with decreasing particle size (see later section on particle size).

▪ Temperature
Haas *et al.* compared *in situ* transesterification (soybeans/methanol/sodium hydroxide) reaction performance at two different temperatures: 60°C and room temperature (23°C) [41]. Both temperatures were sufficient to yield high percentages of methyl ester. However, the reaction at room temperature required more methanol than the reaction at 60°C. At the lower reaction temperature, the optimal molar of alcohol-to-oil ratio was 2.4-times higher than at the higher temperature. The same pattern was also observed in the study in Newcastle. Increasing temperature from 30 to 60°C

increased the initial rate of ester formation, but the time needed to reach equilibrium (60 min) was comparable (rapeseed/methanol/sodium hydroxide)[28].

In conventional transesterification, Noureddini and Zhu observed that the temperature influenced mass transfer as well as conversion [48]. The mass transfer region period became shorter (from 55 to 20 min) when the temperature was increased (from 30 to 60°C). This effect was very obvious when the reaction was conducted at low mixing intensity (Re =3100) but became insignificant at high mixing intensity (Re = 6200). This indicates that, at higher mixing intensities, the external mass transfer resistance is removed and the rate is no longer dependent on temperature. Temperature should not have a strong effect on *in situ* transesterification, as the reaction is believed to be largely mass transfer controlled. This is in agreement with the results reported by Haas *et al.* [46]. The conversions achieved with the reaction (DDGS/methanol/sodium hydroxide) at three different temperatures (35, 45 and 55°C) were almost the same and the reactions completed at the same time (180 min).

Liu and Zhao [32], on the other hand, reported a considerable increase in reaction rate with increasing temperature when an acid catalyst was used (biomass/methanol/sulfuric acid). For a 20-h reaction using 0.2 M sulfuric acid, the yield of ester increased progressively from 44.8 to 74.5 to 85.1 to 96.8% when the temperature was increased from 40 to 50 to 60 to 70°C, respectively. As transesterification with an acid catalyst is generally much slower than with an alkaline catalyst, it is conceivable that an increase in temperature will produce a more significant effect in a acid catalyst than a alkaline catalyst.

It should be noted that it is very likely that optimal temperature is a function of feedstock. Different feedstocks will have different internal structures, therefore different effective diffusivities, and this may explain some of the apparent contradictions in findings in the literature.

▪ Catalyst concentration
Catalyst concentration has been identified as the most important factor in determining the rate of conventional transesterification by some researchers [49,50]. However, in *in situ* transesterification, Zeng *et al.* found that while the catalyst concentration did not affect methyl ester yield, it did influence the purity of the methyl ester [34]. For instance, reactive extraction (sunflower seed/methanol/sodium hydroxide) at low catalyst concentration (molar ratio of oil/catalyst = 0.3:1) achieved 93% conversion with 30% purity while at high concentration (molar ratio of oil/catalyst = 0.4:1), the conversion was 95% with 98% purity.

This is however, in contrast with Qian *et al.*, who

reported that the conversion of oil to methyl ester was increased from 33 to 97% when the concentration of sodium hydroxide was increased from 0.05 to 0.1 mol/l (cottonseed/methanol/sodium hydroxide) [33]. Nonetheless, 0.05 mol/l in this case is equivalent to a 0.2:1 molar ratio of catalyst/oil, which is low compared with the levels in the experiments by Zeng *et al.*'s experiments. The smaller amount of catalyst used by Qian *et al.* [33] may be the reason that they have observed the opposite result from Zeng and co-workers. In addition, the rate-determining step is the diffusion of the alcohol into the particles. Therefore, the fact that different feedstocks were used may contribute to the contradiction between these findings, as different oilseeds have different internal structures.

▪ Particle size

As the particle size of the seeds plays a very important factor in conventional solvent extraction [51,52], it should be similarly important in *in situ* reactive extraction. Kildiran *et al.* [26] investigated two sizes of soybeans seed (<1 and <0.5 mm) at three different reaction times. At 1 h reaction time, a greater than 1 mm particle size gave the highest percentage of oil dissolved in ethanol. However, when the reaction time became longer (i.e., at 3 and 5 h), smaller particle sizes (<0.5 mm) produced better yields.

Ren *et al.* investigated the effect of particle size in rapeseed *in situ* transesterification [35]. Light microscopy and SEM analysis of seed samples showed that at the smallest particle size all the lipids were removed from the seed particle in 1 h. At larger particle sizes, some lipids remained in the center of the particles at this time,

and it was evident from experiments with light microscopy and lipid staining that there was a 'shrinking core' of oil-bearing material. As the particle size of the rapeseed fragments increased from 300–500, to 500–850, to 1000–1400 μm, the 1-h conversion decreased from 86 to 65 to 43%, respectively. The results clearly suggest that, for rapeseed at least, the transport of the methanol into the seed particles was the rate-determining step.

▪ Alcohol type

At least five types of monohydroxy alcohols have been evaluated as reagents in *in situ* transesterification. Ozgul and Turkay investigated the possibility of changing the reagent [38]. They evaluated methanol, ethanol, propanol and butanol as a reagent in the *in situ* transesterification of rice bran oil. The solubility of the oil increased with the alcohol chain length. However, they noted that, even though the amount of oil dissolved increased, alkyl ester content decreased. The reduction in polarity of the alcohol molecule as the chain length increases enables it to increasingly stabilize emulsions formed during the course of the reaction. The emulsion formed can persist and adversely affect conversion.

▪ Biodiesel quality

One of the most important factors to be considered in the development of *in situ* transesterification is whether the process can provide the market with quality biodiesel and meet the requirement of governing bodies. Two of the most accepted standards are ASTM D6751 and EN 14214.

Haas and Scott examined methyl ester produced from soybean flakes via *in situ* transesterification and compared it with the ASTM D6751 standard [46]. The methyl ester passed all the tests except for the acid number test, which required additional washing before it passed the test a second time. **Table 3** shows the comparison reported by Haas and Scott against another standard, EN 14214.

Future perspective

As the world will always depend on transport, the search for alternative and greener fuel sources is poised to become more intense. 'Second-generation' biodiesel, and biofuels in general, should have fewer conflicts with food supplies and more negative lifecycle carbon balances.

In situ transesterification has

Table 3. Comparison of soybean flake methyl ester obtained via *in situ* transesterification against ASTM D6751 and EN 14214.

Property	Soybean methyl ester	ASTM D6751	EN 14214
Flash point (°C)	160	>130	>101
Water and sediment (vol%)	0	0.05	0.05
Carbon residue (wt%)	<0.010	0.05	0.3
Sulfated ash (mass%)	0.000	0.020	0.02
Kinematic viscosity (cSt, at 40°C)	4.017	1.9–6.0	3.5–5.0
Sulfur (wt%)	0.00035	0.05	0.001
Cloud point (°C)	0.0	Report	Not specified
Cetane number		>47	>51
Copper corrosion	Class 1a	Class 3	Class 1
Acid number (mg KOH/g)	0.04	0.80	0.50
Free glycerine (wt%)	0.000	0.02	0.02
Total glycerine (wt%)	0.071	0.240	0.25
Phosphorus (wt%)	0.000	0.001	0.001
Reduced pressure distillation (temperature at 90% recovery,°C)	350	360	NA

cSt: ; NA: Not applicable.

developed significantly since first reports in 1985 [14]. Technical feasibility has been demonstrated for a range of feedstocks, catalysts and alcohols. The challenges for researchers in this area now are to find methods of rendering this process profitable. This process should, in principle, be implemented on a large scale: biodiesel is after all a bulk commodity and should be produced at these scales to realize economies of scale. However, certain features of the process may allow producers in rural areas to produce their own fuel. The relative simplicity of the process may allow oilseed growers to move along the complete length of the value chain of the product, by removing their dependence on often large-scale centralized crushing and solvent

extraction facilities. Process economics change with scale, feedstock, time and geography, and there may well be niche applications for this process already. One obvious niche is as a 'bolt-on' to existing biofuel plants, or plants producing oil-bearing waste, which have very different process economics from purpose-built processes.

In situ transesterification presents researchers with huge challenges in order to make it profitable. The most important challenges must be to reduce the volume of alcohol in the reaction. To overcome this obstacle, future research on *in situ* transesterification should concentrate in three areas. The first will be to study a wide range of chemicals as possible co-solvents, in order to reduce

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Executive summary

In situ transesterification
- *In situ* transesterification is an alternative method of producing methyl ester transport fuels.
- The process directly uses oil-bearing materials rather than pre-extracted oil, as in conventional transesterification.
- The process eliminates processes such as crushing, solvent extraction and degumming, and perhaps drying, which are key processes in conventional biodiesel production.

Raw materials
- Any lipid-bearing material can be a substrate for biodiesel production via the *in situ* transesterification approach, but there are likely to be significant changes in rate and conversion due to varying internal structures of the feedstock.

Catalysts
- The process does not proceed in the absence of a catalyst.
- Both acid and alkali catalysts can be used for *in situ* transesterification
- Selection of a catalyst depends on the properties of the raw materials, especially the free fatty acid content (acid catalysts should be used for higher free fatty acid contents).

Moisture content
- Extraction of some oilseeds is strongly affected by moisture content. Reducing moisture content can reduce the methanol requirement.
- Other oilseeds do not seem to require drying prior to *in situ* transesterification.

Mixing intensity
- The mixing intensity has been shown to have little effect on the process, probably because the rate-limiting step is intraseed diffusion, rather than external mass transfer. Further clear evidence of this being the rate-limiting step is that: reducing particle size clearly increases the rate of extraction; and for rapeseed, a 'shrinking core' of oil has been shown to exist in the seed fragments as the reaction progresses.

Molar ratio of alcohol to oil
- The current greatest obstacle to realization of this process is that the higher methanol/oil molar ratios that seem to be required militate against the economic viability of this process, due principally to the energy costs involved in recovering the methanol for reuse, and to some extent the increased equipment size. Various methods of reducing the methanol requirement or reducing its impact on the process economics are currently under investigation.

Temperature
- It is feasible to perform *in situ* transesterification either at room temperature or at high temperature without compromising on the reaction rate and conversion.
- It is possible that optimal temperature is a function of feedstock.

Catalyst concentration
- The effect of catalyst concentration on *in situ* transesterification is unclear. There must be a catalyst, otherwise the reaction will not proceed at all, but the literature gives conflicting results as to the dependence of the rate upon catalyst concentration at realistic conditions.

Particle size
- Smaller seed particle size increases rate of reaction, indicating that the rate is controlled by internal mass transfer (studies on rapeseed).

Alcohol type
- Various monohydroxy alcohols can be used in the process, but the usage of higher alcohols reduces the biodiesel's purity, and these alcohols tend to be more expensive. However, when using the most usual alcohol, methanol, the quality is comparable to that of conventional biodiesel.

Biodiesel quality
- Biodiesel produced by this method can meet the ASTM D6751 and EN 14214 biodiesel standards.

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the ratio of reactant to oil. The second area is to investigate the process path itself, to involve more intensified unit operations, such oscillatory baffle reactors (already proven for conventional transesterification [53] and being investigated at Newcastle for reactive extraction) and microreactors. The discovery of novel processes or unit operations might make the process more economically viable. The third area that needs more understanding is the *in situ* transesterification reaction mechanism. The mechanism of the process is crucial in predicting the various effects of process parameters on the system and in providing insight into possible further improvements. For example, if the reaction occurs inside the seeds, parameters such as mixing intensity in bulk solvent will not cause significant improvement to the kinetics of the process, and the seed particle size becomes critical.

For the process to be acceptable and profitable, all output streams from the plant must be used. These plants should not simply be biofuel production facilities, rather they should be biorefineries. Studies on the meal left after the process also must be intensified to provide another source of income. The meal can be sold as animal feed, if it still contains nutritional materials. Ren *et al.* found that, for rapeseed subjected to *in situ* transesterification, the carbohydrates and protein remained unaffected by the process and were intact inside the meal [35]. Barrows *et al.*, who conducted a field trial on soybean meal from *in situ* transesterification as fish-food, observed that the rainbow trout fed with this meal gained weight in a normal manner, as with control fish (fed with industrial hexane-extracted soybean meal) [54].

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