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Simplifying the Process of Microalgal Biodiesel Production Through In Situ Transesterification Technology

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Abstract Crop-based biofuels, including biodiesel, has sparked international concerns during recent years. Microalgae have been strongly advocated as the most promising substitute for oil crops. However, the commercialization of microalgal biodiesel is hindered by the high costs of feedstock and conventional production processes. This paper elucidates a simplified, scalable production process under conditions of least energetic demand, which integrates oil extraction and conversion into one step through in situ transesterification. Introducing a co-solvent is the key to success. Criteria for co-solvents applicable to the microalgal biodiesel industry are proposed. The overall biodiesel yield (OBY) of *Spirulina* was determined for benchmarking purposes, using the Bligh and Dyer protocol for oil extraction, and transesterification with potassium hydroxide. The performance in in situ transesterification of the selected co-solvents toluene, dichloromethane and diethyl ether, as well as the solvent combinations petroleum ether/toluene, toluene/methanol and dichloromethane/methanol, was evaluated by OBY. Among all the co-solvents tested, the toluene/methanol system, 2:1 by volume ratio, demonstrated the highest efficiency, achieving a biodiesel yield of 76% of the OBY for the first in situ transesterification cycle and 10% for the second in situ transesterification cycle.

Keywords Microalgal biodiesel · In situ transesterification · Co-solvent criteria · Simplified process

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Introduction

Biodiesel is a mixture of fatty acid methyl esters (FAME) that is generally derived from triglycerides (TAG) through transesterification. Nowadays, this renewable oil is produced commercially from all sorts of crop oils, and used to power a variety of vehicles, including airplanes [1]. Soybean oil and rapeseed oil are the most common feedstock in the United States and Europe, respectively [2]. The global output of biodiesel has increased in the past 5 years, reaching up to 12 billion liters in 2008 [3].

It has been advocated for decades that biofuels such as ethanol, hydrogen and biodiesel, could replace petrodiesel, owing to their clean and sustainable characteristics. However, as shown in Fig. 1, the price of crop-based biodiesel has yet to beat that of petrodiesel, even with government subsidies. Furthermore, in April 2008, United Nations (UN) officials claimed that the US and the EU took a "criminal path" by contributing to the global food crisis through use of food crops to produce biofuels of bioethanol and biodiesel.

Although countries that use biodiesel are not expected to take full responsibility, international concerns about the upward pressure on global food prices and intensified competition for cropland currently make the use of crop-based biodiesel a somewhat politically embarrassing situation [4].

Microalgae are far superior to oil crops in that they require no cropland occupation, have a short life cycle, and high oil productivity per hectare. The Aquatic Species Program (ASP) initiated by the US Department of Energy in 1978 is regarded as the most comprehensive research effort aimed at algae-to-fuel in history. As a potential biodiesel feedstock, microalgae are now enjoying a resurgence in attention decades after the termination of the ASP.

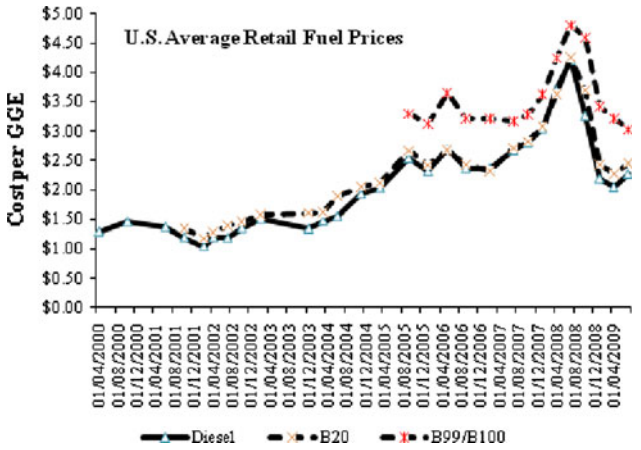

Fig. 1 Average United States retail prices of crop-based biodiesel and petrodiesel. Data taken from the Alternative Fuels and Advanced Vehicles Data Center of US Department of Energy (http://www.afdc.energy.gov/afdc/fuels/biodiesel.html)

A final report on the ASP, released in 1998, discussed the feasibility of the use of microalgae because, in 1995, the estimated price of microalgal biodiesel was US $40–60/barrel and petrodiesel was just US $20/barrel [5]. Fifteen years have passed; however, the commercialization of microalgal biodiesel still faces the enormous obstacle of expensive production costs. Philip T. Pienkos and Al Darzins, from the US National Renewable Energy Laboratory, estimated a cost of microalgal oil (not biodiesel) at US $105/barrel for high productivity (50 g/m^{-2}/day^{-1} at 50% TAG), whereas the price for petroleum was less than US $50/barrel in December 2008 [6]. Obviously, the price of microalgal biodiesel has yet to beat that of petrodiesel. In order to reduce microalgal biodiesel costs, progress in any aspect of the comprehensive project will benefit the retail price of the end product. Genetic manipulation, biomass culturing and cheaper harvesting techniques would all help with cost reduction. Another promising approach would be to optimize conventional productive processes, which can be briefly described as an extraction-transesterification process.

The conventional energy-consuming process in a crop-based biodiesel plant is demonstrated in Fig. 2 [7]. Crude oil is first extracted by an organic solvent (usually hexane) from an oil-crop feedstock (e.g., soybean) and is then delivered to the reactor for transesterification. In this two-step process, although hexane is efficient in oil extraction and alkali-catalyzed transesterification, which is advantageous in high speed (a matter of seconds or minutes) [8] and mild heating conditions (~60°C), three energy-intensive operations are involved, namely, hexane vacuum evaporation, process heating, and stirring during transesterification. Among the three operations highlighted, stirring may not be as costly as process heating or methanol recovery in a standard biodiesel production process; however, no stirring or a better manner of stirring should be considered as a potential process cost-saving strategy [9]. The reason why these costly operations are required is that, on the one hand, hexane is methanol-immiscible, and so should be removed from crude oil before adding methanol for transesterification; on the other hand, methanol is oil-immiscible, and so needs stirring to homogenize the reactants during transesterification. Undoubtedly, if a

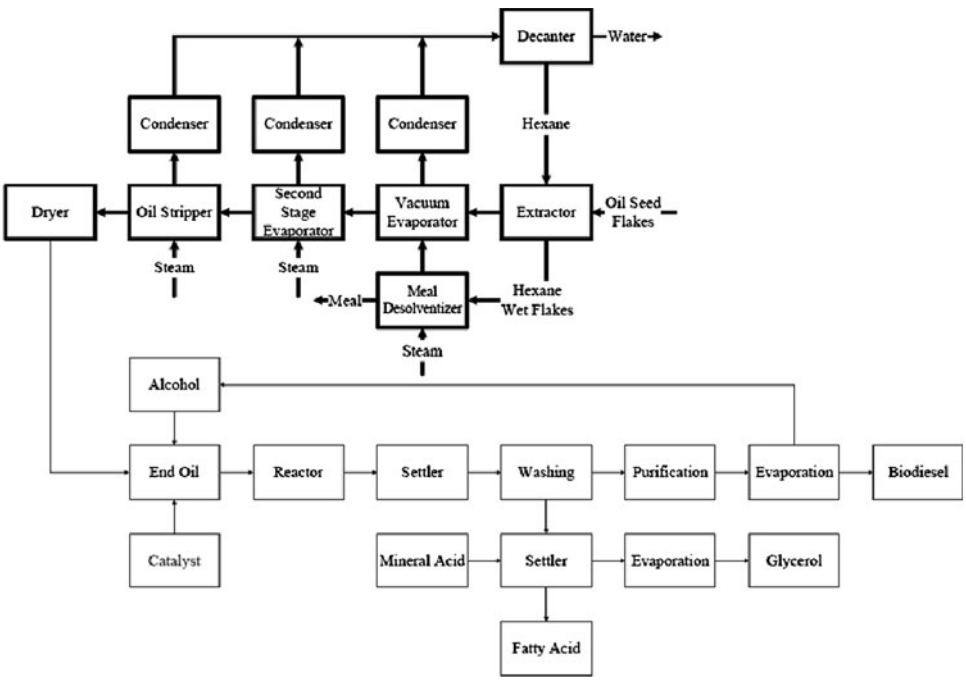

Fig. 2 Conventional process for biodiesel production in the extraction-transesterification mode (adapted from Zappi et al. [7])

"super agent" that can function like hexane and, at the same time, is miscible with both methanol and oil could be found, evaporation, heating and even stirring would no longer be necessary. Combining oil extraction and transesterification in one step, i.e., "in situ transesterification", with the assistance of a "super agent", would simplify the process and greatly reduce operational costs.

To date, literature on in situ transesterification generally covers two types of efforts: (1) in situ transesterification using oil seeds without co-solvents, and (2) in situ transesterification using virgin oils with the assistance of co-solvents. The former has been reported [10–12] for cotton seeds, sunflower seeds and vegetable oils, as opposed to microalgae. Georgogianni [10, 11] investigated sodium hydroxide catalyzed in situ transesterification of cottonseed oil and sunflower seed oil with methanol, using low-frequency ultrasonication and mechanical stirring at 60°C, respectively. The results showed similar ester yield to those obtained by conventional transesterification [9, 10]. Haas et al. studied sodium hydroxide catalyzed in situ transesterification of soybean oil at 23°C and 60°C for 8 h, the former temperature giving a higher predicted biodiesel yield than the latter [12]. Co-solvent assisted in situ transesterification at 60°C has also reported for several organic solvents. Boocock et al. demonstrated that the use of tetrahydrofuran (THF) or methyl tertiary butyl ether resulted in a single-phase reaction and speeded up methanolysis considerably [13]. THF was particularly chosen because it can be co-distilled with methanol at the end of the reaction due to its boiling point of 67.8°C. In fact, THF has been adopted as a co-solvent for biodiesel production by the BIOX Corporation (www.bioxcorp.com) [14], which employs animal fats or palm oil as feedstock. Regarding the feedstock of microalgae, a recent study by Ehimen et al. investigated the variables affecting sulfuric acid catalyzed in situ transesterification using *Chlorella* oils, including alcohol volume, temperature, reaction time, biomass moisture content and stirring. The results showed that an increase in temperature favors biodiesel yield and intermittent stirring has nearly the same effect as continuous stirring [9].

This paper intends to present a third way of investigating co-solvent assisted in situ transesterification by using microalgal biomass directly rather than microalgal oils. Our efforts focus on co-solvent assisted in situ transesterification under conditions of least energetic demand, namely, without stirring and heating. It should be noted that this paper does not mean to propose no stirring or heating in production. There is no doubt that stirring and heating would benefit the reaction speed and biodiesel yield greatly. However, these factors were not addressed in this study because the introduction of the co-solvent to the reaction system provided a spontaneous monophasic production system, in which stirring and heating were no longer essential demands. This lent an opportunity to explore the feasibility of microalgal biodiesel production by an in situ transesterification technique with the lowest possible energy consumption. Ultimately, it is the ratio of product yield/energy input that is closely tied to final profits.

For this purpose, the critical aim of this study was to seek a co-solvent that is qualified to act as a "super agent". Therefore, criteria for co-solvents applicable to the process of in situ transesterification in microalgal biodiesel industry were first proposed. Several co-solvents were selected using these criteria. Microalgal biomass of dried *Spirulina* was used as the feedstock. The overall biodiesel yield (OBY) of *Spirulina* was determined using the Bligh and Dyer method [15] adopted for lipid extraction in the ASP [5]. The performance of co-solvent assisted in situ transesterification was evaluated by the OBY. It should be pointed out that the OBY, created according to the classical laboratory protocols, was just for benchmarking. As the draft National Algal Biofuels Technology Roadmap released recently by the US DOE points out:

> 'the methodology generally used for algal lipid analysis—which is largely based on solvent extraction and gravimetric analysis—has yet to be standardized, and thus, the values published in the literature should be regarded, at best, as only an estimation of the lipid content.'

Materials and Methods

Materials

Dry *Spirulina* powder was purchased from an algae company in Shenzhen, China. Potassium hydroxide, methanol, hydrochloric acid, organic solvents and conical polypropylene tubes of 50 ml were laboratory stock. The gas chromatograph-mass spectrometer (GC–MS) was type 7890 (Agilent Technologies, Wilmington, DE). The vortex mixer was of type 37,600 (Thermo Fisher Scientific, Waltham, MA).

Methods

Overall *Spirulina* Biodiesel Yield Determination

The overall *Spirulina* biodiesel yield was determined by oil extraction using the Bligh and Dyer method [13], followed by alkali-catalyzed transesterification. Microalgal oil

extraction was conducted in accordance with Bligh and Dyer's description: 0.5 g dry microalgal biomass was placed in a 50 ml conical polypropylene tube. A mixture of 4 ml water, 10 ml methanol and 5 ml chloroform was introduced to the tube, and stirred for 2 min to obtain a monophasic system on a vortex mixer. A 5 ml volume of chloroform was added a second time and stirred for 30 s on the vortex mixer. Finally, 5 ml water was added and stirred for 30 s on the vortex mixer. The homogenate was then centrifuged at 2,000 rpm in a table-top centrifuge for 10 min at room temperature to form a two-phase system (methanol and water on top, chloroform at the bottom). The chloroform layer was recovered and placed into another 50 ml conical polypropylene tube with a pipette. The recovered volume of the chloroform layer in the tube was measured. The microalgal oil was collected after drying under a nitrogen stream.

The microalgal oil was converted to biodiesel through alkali-catalyzed transesterification. Methanol (1 ml) containing 0.0485 g potassium hydroxide was added to dried lipids (around 0.045 g) in a tube. The tube was then immersed in a water bath and stirred using a magnetic stirring bar at 50°C for 30 min. Then, 0.0878 g hydrochloric acid (36%, w/w) was added to stop the reaction, and 1 ml hexane was added and stirred for 1 min to transfer biodiesel into the hexane phase. The hexane phase was thereby recovered with a pipette for biodiesel yield determination by GC–MS. The determination experiment was repeated five times. It is worth noting that the minimum dose of methanol needed to transesterify 0.045 g oil was 14.4 µl. However, since the molar ratio of oil to methanol should be kept the same, both in OBY determination and in situ transesterification experiments, 1 ml methanol was used in order to avoid the co-solvent from diluting the methanol concentration by too much. Moreover, the use of 14.4 µl methanol could easily introduce systematic error to the experiments.

Spirulina Biodiesel Production Through In Situ Transesterification

The solvents investigated for in situ transesterification were selected by the criteria proposed in this paper. A 0.5 g quantity of dry microalgal biomass was placed in a 50 ml conical polypropylene tube; 15 ml of the chosen co-solvent and 1 ml methanol with 0.0485 g of potassium hydroxide were added to the conical polypropylene tube, with continuous stirring for 1 min to homogenize the system. The tube was then kept at room temperature (24°C) without stirring and two layers (solvents on the top, microalgal biomass at the bottom) formed in the tube. After an hour, 0.0878 g hydrochloric acid was added to stop the reaction, and 200 µl of the liquid from the tube was sampled with a pipette for GC–MS analysis. This procedure was repeated three times.

For a second transesterification, the top liquid layer was recovered from the system with a pipette, whereas the microalgae were retained. Fresh co-solvent (5 ml), 1 ml methanol, and 0.0485 g potassium hydroxide were added to the microalgae meal, and stirred for 1 min to homogenize the reaction system. The tube was then kept at room temperature without stirring and two layers formed in the tube. After 1 h, 0.0878 g hydrochloric acid was added to stop the reaction, and 200 µl liquid from the tube was sampled with a pipette for GC–MS analysis. This procedure was repeated three times.

GC–MS Analysis

Spirulina fatty acid profile and biodiesel yield were determined by a GC–MS type 7890 (Agilent Technologies). For FAME analysis, a 30 m capillary column, with an inner diameter of 250 µm and film thickness of 0.25 µm, was used (Agilent, 19091 s-433).

A FAME sample of methyl eicosanoate ($C_{21}H_{42}O_2$), dissolved in hexane (20 mg/ml), was used as an external standard. For a run of GC–MS analysis, 200 µl sample was dissolved in 290 µl hexane with 10 µl external standard (20 mg/ml). Then, 0.2 µl was injected using the on-column technique. The carrier gas was Helium flowing at a flow rate of 0.7 ml/min. The inlet pressure was 7.82 psi. During injection, the oven temperature was 130°C for 1 min, then ramped to 230°C at a heating rate of 5°C/min.

Results and Discussion

GC–MS Characteristics of *Spirulina* Biodiesel

Figure 3 and Table 1 illustrate the GC–MS characteristics of the *Spirulina* biodiesel, which are methyl esters derived

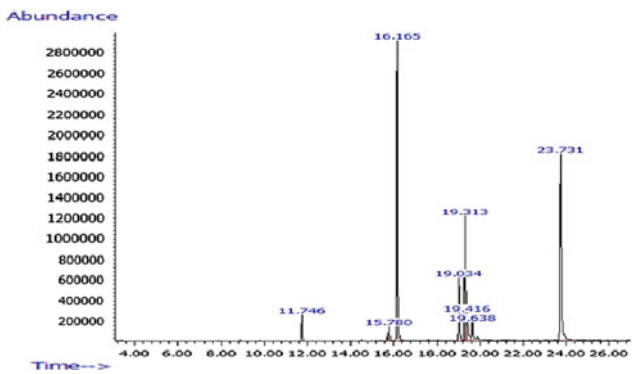

Fig. 3 Gas chromatography-mass spectrometry (GC-MS) profile of *Spirulina* biodiesel. Retention times (min): *15.780* C17:1 methyl ester, *16.165* C17:0 methyl ester, *19.034* C19:3 methyl ester, *19.313* C19:2 methyl ester, *19.416* C19:0 methyl ester, *23.731* C21:0 methyl ester

Table 1 Fatty acid profile of *Spirulina*

Common name	Molecular formula	Molar mass (g/mol)	% of dry biomass
Sapienic acid	$C_{16}H_{30}O_2$	254.42	2.36
Palmitic acid	$C_{16}H_{32}O_2$	256.42	49.31
Alpha-linolenic acid	$C_{18}H_{30}O_2$	278.45	13.06
Linoleic acid	$C_{18}H_{32}O_2$	280.45	26.46
Oleic acid	$C_{18}H_{34}O_2$	282.46	8.81

from sapienic acid (C16:1), palmitic acid (C16:0), alpha-linolenic acid (C18:3), linoleic acid (C18:2) and oleic acid (C18:0) through transesterification. For transesterification, since a 6:1 M ratio of methanol to oil is adopted in industrial production [16], the mean molar mass of triglyceride calculated from Table 1 as 841.71 g/mol is used to determine the dose of methanol in the reaction. To convert 1 g *Spirulina* lipid, a minimum dose of 0.2885 ml methanol and 0.014 g potassium hydroxide (1% hydroxy of oil weight, w/w) is required.

Criteria of Co-solvents for In Situ Transesterification

Criteria for co-solvents are proposed and summarized in Table 2. Since each criterion is a necessary condition for qualified co-solvents, they are all of equal relative importance. The qualified co-solvent is key to the success of in situ transesterification. This co-solvent system should serve as an extraction agent on the one hand, and form a homogenous system spontaneously with microalgal oil, methanol, and alkali on the other. Generally, stirring and heating are required to facilitate the transesterification process but co-solvent assisted in situ transesterification is expected to be carried out under conditions of least energetic demand, namely, without heating and stirring.

Based on the stated principles for the criteria of co-solvents, neither the classical chloroform/methanol system, which is widely used in laboratory-scale lipid extractions, nor the hexane system, which is popularly adopted in industrial-scale edible oil production, qualify as co-solvent systems desirable for in situ transesterification microalgal biodiesel production, because (1) chloroform is decomposed by alkali as described by the following reaction: $CHCl_3 \xrightarrow{KOH} CCl_3^- \xrightarrow{-Cl^-} :CCl_2$ [17], and (2) hexane is poorly miscible with methanol. In addition, THF, which is used by the BIOX Corporation, is not regarded as a co-solvent applicable to microalgal biodiesel production according to the criteria applied in this paper because THF is miscible with water.

Screening of Co-solvents

Co-solvents were selected by screening the general organic solvent pool in Table 3. According to criteria 1 and 2, toluene, benzene, diethyl ether, methyl *t*-butyl ether, ethyl acetate, chloroform and dichloromethane are water-immiscible, lipid-miscible and methanol-miscible. However, ethyl acetate and chloroform are not inert to alkali

Table 2 Criteria for co-solvents for in situ transesterification

Criterion	Description	Objectives
1	"Water"-immiscible	The presence of water in the transesterification reaction significantly decreases the yield of biodiesel. Water-immiscibility of the co-solvent provides a water-free environment for in situ transesterification. It potentially reduces the requirements and costs of microalgal dewatering
2	"Triglyceride and methanol"-miscible	Triglyceride-miscibility allows the co-solvent to extract target oil from microalgae; methanol-miscibility allows in situ transesterification to occur in a homogeneous environment where no agitation is required
3	Chemical inertness during in situ transesterification	Chemical inertness of the co-solvent ensures the normal progress of in situ transesterification, the purity of end products, and the facility for recycling the co-solvent
4	Different density from microalgae	Difference in density ensures microalgae not to suspend in the reaction system but to be separated from the co-solvent by floating or subsiding spontaneously. Thus, no centrifugation is required for the recovery of co-solvent and biodiesel. This greatly saves on operational costs
5	Low toxicity	Low toxicity benefits worker health and the environment, with cumulative effect since consumption of co-solvent required for large-scale microalgal biodiesel production is huge (millions of tons)

Table 3 Properties of organic solvents (sourced from the web page of Division of Organic Chemistry, ACS, http://www2.onu.edu/~b-myers/organic_solvents.html)

Solvent	Formula	Boiling point (°C)	Density (g/ml)	Solubility in H_2O (g/100 g)	Miscible with methanol	Relative polarity
Hexane	C_6H_{14}	69	0.655	0.0014	No	0.009
Toluene	C_7H_8	110.6	0.867	0.05	Yes	0.099
Benzene	C_6H_6	80.1	0.879	0.18	Yes	0.111
Diethyl ether	$C_4H_{10}O$	34.6	0.713	7.5	Yes	0.117
Methyl *t*-butyl ether	$C_5H_{12}O$	55.2	0.741	4.8	Yes	0.124
Ethyl acetate	$C_4H_8O_2$	77	0.894	8.7	Yes	0.228
Chloroform	$CHCl_3$	61.2	1.498	0.8	Yes	0.259
Dichloromethane	CH_2Cl_2	39.8	1.326	1.32	Yes	0.309
Methanol	CH_4O	64.6	0.791	Miscible	Yes	0.762
Water	H_2O	100	0.998	Miscible	Yes	1.000

solution. Benzene and methyl *t*-butyl ether can be discarded due to relatively high toxicity. Of the remaining co-solvents, *Spirulina* sedimentation experiments shows that microalgae initially suspended in the co-solvent settle at the bottom after 15 min (data not shown). As a result, three co-solvents, toluene, diethyl ether and dichloromethane, were selected for further investigation. Other solvents, such as petroleum ether, were also tested for in situ transesterification experiments in order to broaden the co-solvent pool.

Performance of Single Co-solvent for Biodiesel Production Through In Situ Transesterification

The biodiesel yield refers to the biodiesel percentage produced from dry algal biomass. The OBY of *Spirulina* is 8.9%, which is set as the benchmark throughout this paper to evaluate the performance of the selected co-solvents. For example, the biodiesel yield with toluene through in situ transesterification is 3.8% (based on dry biomass), so the performance of toluene is 42.6% of OBY.

Figure 4 compares the performance of the three co-solvents tested. Toluene demonstrated the best result (42.6%), giving significantly better yield than dichloromethane and diethyl ether (30.3 and 27.9%, respectively). This proves the feasibility of the in situ transesterification technique using a co-solvent under mild conditions. During the 1-h reaction, potassium hydroxide not only acts as the catalyst but may also be helpful in cell disruption [18]. Microalgal lipid extraction and transesterification were conducted in the same system, without stirring or heating, demonstrating that significant improvement could be achieved by using this energy-efficient process for industrial production. Of the three co-solvents tested, toluene is likely to have the greatest affinity with non-polar triglycerides. This is consistent with the principle of "like dissolves like", because their polarities are, from small to large as described in Table 3, in the order toluene < diethyl ether < dichloromethane. However, the best result with toluene was less than half of the OBY. One reason for this may be that the non-polar solvent only weakly disrupts associations between triglycerides in *Spirulina* cells with cellular constituents, leading to the unsatisfactory performance of these three non-polar co-solvents.

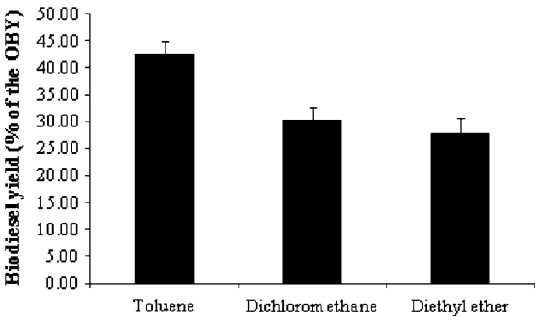

Fig. 4 Biodiesel yields from *Spirulina* through in situ transesterification with three different single co-solvent systems

Performance of Binary Co-solvents with Petroleum Ether for Biodiesel Production Through In Situ Transesterification

Since the use of a single co-solvent proved unsatisfactory, a combination of solvents was designed. Toluene was chosen without hesitation, due to its highest performance among the three co-solvents. Petroleum ether [19], which is commonly adopted as the non-polar lipid extraction agent in most laboratories, is considered a combinative solvent. It is methanol-immiscible but can be methanol-miscible in the presence of a third co-solvent such as toluene. This solvent combination of petroleum ether/toluene is thought to achieve higher biodiesel yield than that obtained by a single solvent. Figure 5 shows the performance of the combined co-solvent system. Single petroleum ether gave similar results to the control of single toluene in terms of in situ transesterification. Highest biodiesel yield (45.5%) was achieved with petroleum ether/toluene at a 1/1 (v/v) ratio.

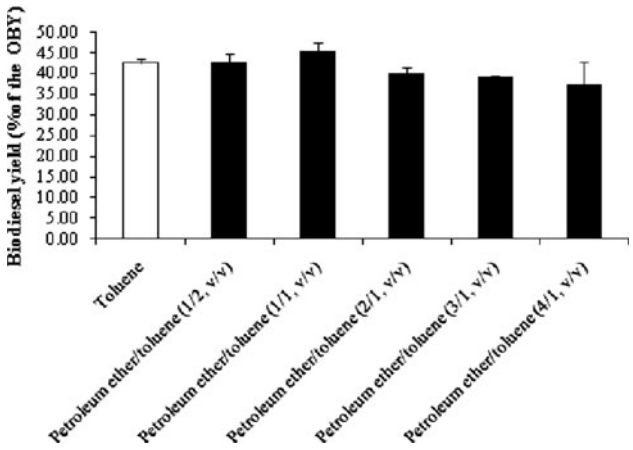

Fig. 5 Biodiesel yield of *Spirulina* through in situ transesterification with a petroleum ether/toluene system

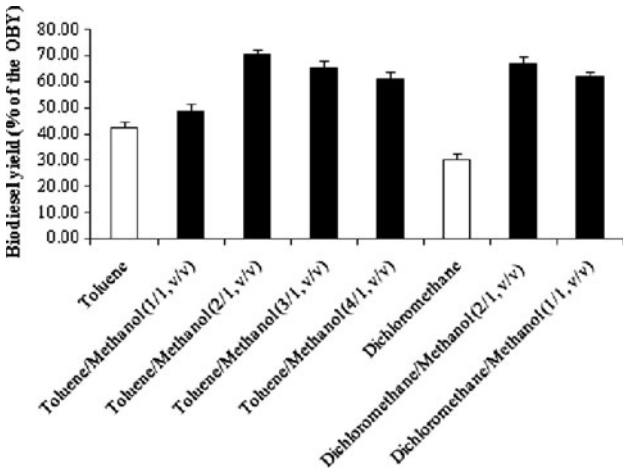

Fig. 6 Biodiesel yield of *Spirulina* through in situ transesterification with co-solvent/methanol system

However, the combined effect is not great, because petroleum ether and toluene are both non-polar and close in polarity, and therefore are less able to disassociate triglycerides from cellular constituents. The combined effect with a polar solvent is expected to be much better. This idea was borrowed from the chloroform/methanol system described by Bligh and Dyer [15].

Performance of Binary Co-solvents with Methanol for Biodiesel Production Through In Situ Transesterification

The collective effect on biomass lipid extraction of a solvent combination consisting of non-polar and polar organic solvents is expressed by the Bligh and Dyer method [15]. Methanol plays an important role throughout the extraction process. In the first step of this method, the system, which is comprised of chloroform/methanol/water (0.5/1/0.4), is relatively polar. This is helpful in dissolving polar lipids and destroying the association between lipids and cell constituents [20]. Figure 6 shows that, with the assistance of methanol, both the toluene system and the dichloromethane system performed much better, increasing from 42.6 to 70.3% and from 33.8 to 67.1%, respectively. In the toluene/methanol system, a volume ratio of 2/1 resulted in 70.3% of the OBY, which was better than the volume ratios of 1/1, 3/1, and 4/1, revealing a tradeoff between the polar and non-polar fractions. A less polar system, which means that the methanol fraction is small, results in a high extraction capacity for non-polar lipids that dissolve in toluene, but may be relatively weak in dissociating lipids from cell constituents. Furthermore, in the co-solvent/methanol system, an obvious advantage is that the addition of methanol shifts the transesterification equilibrium to the biodiesel side.

Dose and Efficiency of Toluene/Methanol System for In Situ Transesterification

As stated above, toluene/methanol (2/1, v/v) is the best candidate co-solvent system found so far for *Spirulina* biodiesel production through in situ transesterification. The solid-to-liquid ratio adopted in our experiments was 0.5 g *Spirulina* to 15 ml co-solvent. A more economical process is expected by reducing the dose of the co-solvent. Figure 7 shows that toluene/methanol (2/1, v/v) system performs slightly better with a dose of 5 ml (76.0%) rather than 15 ml (70.3%). Considering that the same amount of potassium hydroxide is added (1% hydroxy of oil weight, w/w), the concentration of potassium hydroxide is higher in the 5 ml system than in the 15 ml system, which may result in a more drastic cell disruption and catalysis in the small volume system [18]. In the 5 ml system, Fig. 7 shows that the first in situ transesterification cycle produces a biodiesel yield of 76.0% of the OBY, and the second in situ

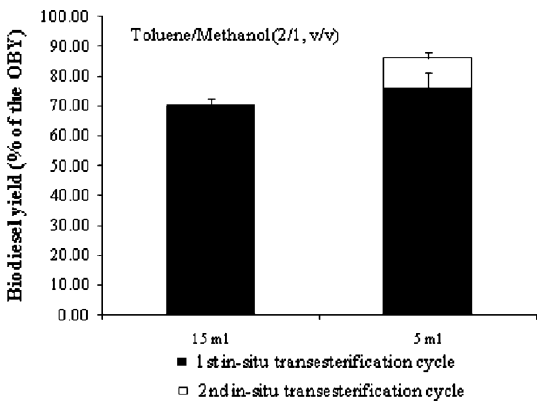

Fig. 7 Biodiesel yield of *Spirulina* through in situ transesterification with a 5 and 15 ml toluene/methanol system

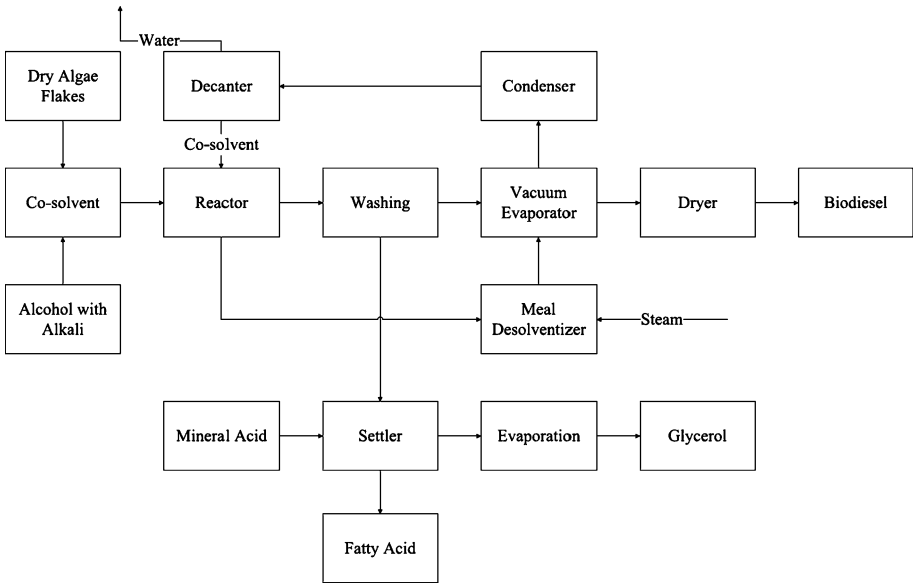

Fig. 8 Simplified process for microalgal biodiesel production through in situ transesterification with co-solvents

transesterification cycle produces a biodiesel yield of 10.1% of the OBY. In total, this amounts to 86.0% of the OBY. This clearly demonstrates that toluene/methanol (2/1, v/v) is an efficient co-solvent system for in situ transesterification. Although the total biodiesel yield is less than the OBY obtained from an extraction-transesterification process with the Bligh and Dyer protocol, co-solvent assisted in situ transesterification is expected to be much less energy consuming. This laboratory-scale method can be easily scaled up to industrial production, whereas the Bligh and Dyer method cannot.

Perspective

The co-solvent plays a crucial role in this novel technical process. Among the co-solvents selected in this study, the toluene/methanol system performed best in terms of *Spirulina* biofuel production. However, it may not be suitable for other species of algae. The best solvent system will differ for different algal species. Lee et al. reported that the dichloroethane/methanol (ethanol) system, which was recommended for lipid extraction from the green alga *Cladofora*, was not effective for the green alga *B. braunii* [21]. We therefore suggest that the appropriate co-solvent be selected by screening a large solvent pool for each different microalgal species.

When applying the co-solvent, the conventional two-step process for crop-based biodiesel production (Fig. 2) can be integrated into a brief flow chart as shown in Fig. 8. Dried microalgal biomass is fed directly into the reactor, which is loaded with an adequate amount of co-solvent, alcohol and alkali. After a short agitation, to thoroughly mix the microalgae feedstock and reaction solvents, microalgal lipid is extracted continuously by the co-solvent and in situ transesterified in the meantime at room temperature without stirring. During the reaction, microalgal biomass settles downward to the bottom of the reactor. After the reaction is complete, the co-solvent is transferred to a vacuum evaporator. Biodiesel is recovered and the co-solvent recycled. The microalgal meal left in the reactor, after the first preceding cycle, can either undergo a second in situ transesterification cycle with fresh co-solvent, or be desolventized and disposed. In addition to this method, a better way to deal with the post-transesterified microalgae meal is to convert this otherwise wasted biomass to energy-rich methane (CH_4) [22, 23]. This additional energy obtained from the microalgal meal can therefore be further used to drive some of the processes (i.e., evaporation) as described in Fig. 8.

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