International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 10 Issue 5, Sep-Oct 2026 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

In-situ Transesterification of *Chlorella sorokiniana* for Biodiesel Production: Process Optimization and Techno-Economic Feasibility in Libya

Fouad Sadig Rashed[1], Abdulmajed Abduljalil Almagoz[2]

[1]Chemical Engineering Department, College of Engineering, Sabratha University, Sabratha, Libya
[2]Laboratories at Mellitah Oil and Gas Company

ABSTRACT

Microalgae represent a promising feedstock for biodiesel commercialization owing to their high lipid content and productivity. This study investigated the effects of alcohol volume, reaction time, temperature, and catalyst concentration on biodiesel production from the Chlorella sorokiniana algal strain via in-situ transesterification a process that combines lipid extraction and transesterification into a single operational step. The experimental results demonstrated that optimal reaction conditions comprised 100% H_2SO_4 concentration (wt./wt. oil) as catalyst at 65°C for 8 hours, utilizing 15 g of biomass and 80 mL of methanol, achieving a biodiesel yield of 84.7%. A techno-economic analysis with ±10% accuracy was conducted based on an international biodiesel price of US$1,332 per metric ton (MT) and an evaluated algal biomass cost of US$500/MT. The economic viability of a daily 1 MT algal biodiesel production facility was confirmed through favorable profitability indicators for the investigated algal species. Break-even analysis revealed that the maximum permissible algal biomass cost was US$599/MT for the C. sorokiniana strain, based on the established biodiesel selling price of US$1,332/MT. Furthermore, the global market price for algal cake as poultry feed should not decrease below US$589/MT for economic sustainability. The break-even price for algal biodiesel was determined to be US$984/MT (US$157.4/barrel) at an algal biomass cost of US$500/MT. Positive prospects for industrial-scale algal fuel production were identified when Brent Crude Oil Spot prices exceed US$120 per barrel, particularly considering the added value of algal cake as poultry feed.

KEYWORDS: *Techno-economic analysis, algal biodiesel, in-situ transesterification, break-even analysis, poultry feed.*

How to cite this paper: Fouad Sadig Rashed | Abdulmajed Abduljalil Almagoz "In-situ Transesterification of Chlorella sorokiniana for Biodiesel Production: Process Optimization and Techno-Economic Feasibility in Libya" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-10 | Issue-5, October 2026, pp.1134-1140, URL: www.ijtsrd.com/papers/ijtsrd142316.pdf

IJTSRD142316

Copyright © 2026 by author (s) and International Journal of Trend in Scientific Research and Development Journal. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0) (http://creativecommons.org/licenses/by/4.0)

1. INTRODUCTION

Biodiesel constitutes a promising alternative fuel for diesel engines, offering renewable and sustainable characteristics. It is synthesized from diverse feedstocks, including waste frying oils, non-edible oilseeds (e.g., Jatropha curcas oil), microalgae-derived oil, and animal fats. This fuel exhibits biodegradability, non-toxic properties, and negligible sulfur and aromatic content. Furthermore, its utilization results in substantial reductions in exhaust emissions—up to 75% compared to conventional diesel fuels, as documented in previous investigations [1,2]. Biodiesel has been widely recognized by researchers as the preferred green fuel. The term itself was introduced circa 1984 to designate primary esters derived from natural oils. Initially employed sparingly, it achieved broader acceptance by 1991, subsequently experiencing rapid growth in utilization and recognition [3].

Chemically, biodiesel is characterized as mono-alkyl esters derived from long-chain fatty acids obtained from renewable bio-lipids. Biodiesel production typically involves transesterification, wherein triglycerides present in fats or oils react with a short-chain alcohol, such as methanol, in the presence of a homogeneous or heterogeneous catalyst. This reaction

yields methyl esters (biodiesel) and crude glycerol as a byproduct [4].

Following product refining, the obtained biodiesel exhibits properties closely resembling fossil diesel grades and can be blended with petro-diesel. Blends of biodiesel and petro-diesel are designated by BXX, where XX represents the biodiesel proportion in the mixture with diesel fuel. The B20 blend (20% biodiesel, 80% fossil diesel) represents the most widely adopted and cost-effective formulation, requiring no engine modifications. B20 consists predominantly of petroleum diesel and exhibits comparable properties to conventional diesel fuel. B100 properties include high Cetane Index (CI: 47-65), conducive to compression ignition, high lubricity, and reduced sulfur content [5].

This study aims to investigate the optimal conditions for the conversion of Spirulina-platensis microalgae lipids directly to fatty acid methyl esters via in-situ transesterification. The investigation focuses on optimizing process variables affecting the yield and purity of algae biodiesel. The extent of transesterification and side reactions depends upon feedstock type, oil content, catalyst concentration, alcohol-to-oil ratio, and reaction temperature. Additionally, this study examines the feasibility of biodiesel production from microalgae to contribute to cost reduction and waste minimization from biomass.

2. Biodiesel Industry: Origin and Development

Research into utilizing natural oils, including vegetable oils and animal fats, as renewable fuel alternatives to fossil sources dates to the early 1800s. The transesterification process for converting triglycerides in oils was first implemented by Duffy and Patrick in 1853 [6]. In 1893, Rudolf Diesel proposed a revolutionary engine design capable of operating on any fuel ignited by high compression, eliminating the need for spark ignition. This original diesel engine operated on vegetable oil, resulting in a patent in 1893 and successful functional model demonstration by 1897. In 1900, a smaller version of the diesel engine was exhibited at the Paris Exposition, operating on peanut oil and demonstrating the versatility of diesel technology in utilizing different fuel types. By 1911, Rudolf Diesel declared that his engine could function effectively with vegetable oils, emphasizing its potential to support agricultural development in countries adopting such alternative fuels [7]. The term biodiesel was subsequently coined to describe trans-esterified lipids employed as replacements for petro-diesel fuel.

Vegetable oils, both edible and non-edible, were commonly utilized in diesel engines until the 1920s. However, manufacturers began transitioning their engine designs to accommodate the lower viscosity of petro-diesel compared to vegetable oils during this period [8]. The 1973 Arab oil embargo marked a turning point, causing crude oil prices to increase fourfold and rekindling interest in biofuels. A significant challenge emerged: pure vegetable oil proved excessively viscous for modern diesel engines, clogging injection systems and failing to spray evenly into compression cylinders. Two potential solutions were identified—either heating the oil onboard to reduce viscosity (a method now utilized by Greasecars, operating on recycled fryer grease) or chemically modifying the oil to produce smaller molecules. The latter approach led to the development of biodiesel as currently known [9]. Following the energy crisis, Austria initiated early discussions on utilizing alternative biofuels for diesel engines, particularly for agricultural machinery such as tractors. These discussions generated several experiments blending vegetable oils with conventional petro-diesel fuel. Researchers continued optimizing biodiesel to meet engine requirements [10]. In 2003, Dr. Mittelbach conducted laboratory experiments using rapeseed oil to refine biodiesel production processes. Chemically, vegetable oil typically comprises three fatty acid molecules bonded to a glycerol molecule in a triglyceride structure. These experiments facilitated advancements in producing high-quality biodiesel suitable for modern engines [11].

3. Materials

3.1. Microalgae Feedstock and Growth Conditions

Chlorella sorokiniana (green microalgae species) was axenically cultivated on glycerol medium in 250 mL Erlenmeyer flasks containing optimized culture medium (OCM) as described by Heredia-Arroyo, with yeast removed from the medium. Cultures were illuminated with tubular fluorescent lamps (PHILIPS Master TL-D 85 W/840), with light intensity adjusted to 100 µmol photons $m^{-2} \cdot s^{-1}$ under 16:8 h light-dark photoperiods at a constant temperature of 25°C ± 2°C and agitated in an orbital incubator shaker at 120 rpm.

3.2. Alcohol

Methanol is the most commonly employed short-chain alcohol in biodiesel production due to its greater availability and lower cost. It ensures stable final ester yield and facilitates easier extraction of unreacted alcohol following treatment.

3.3. Acid Catalyst

Catalysts play a crucial role in enabling reactions that would otherwise be kinetically infeasible. In the biodiesel industry, commonly utilized acid catalysts include sulfuric acid (H_2SO_4), calcium carbonate

($CaCO_3$), and phosphoric acid (H_3PO_4). These substances are favored due to their affordability, accessibility, and ease of transportation and storage, provided appropriate safety precautions are observed.

4. Production Methodology Using In-Situ Transesterification

This study employs the in-situ transesterification method, regarded as a promising alternative to conventional processes due to its potential to streamline processing units and reduce fuel conversion costs. Key parameters influencing microalgae lipid transesterification were investigated to determine optimal operating conditions: (i) Catalyst concentration (30%, 50%, 100%, and 200% wt./wt. oil): Higher concentrations increase material costs. (ii) Reacting alcohol volume (40.0, 60.0, 80.0, and 100.0 mL): Greater alcohol volumes increase material expenses. (iii) Temperature (27°C, 40°C, 50°C, and 65°C): Higher temperatures elevate energy requirements. (iv) Reaction time (2, 4, 8, and 10 hours): Longer reaction durations may reduce product quantities, negatively impacting profitability. (v) Process stirring: Represents the primary energy demand for agitation during reaction.

The objective was to identify conditions achieving maximum yield while minimizing material and energy inputs to reduce overall process costs. Methanol was selected as the alcohol, and sulfuric acid (H_2SO_4) served as the catalyst.

In-Situ Transesterification Process Summary

Feedstock Preparation: Chlorella sorokiniana microalgae were dried using sun drying beds, ground into powder, and stored below 20°C.

Reactant Calibration: Fixed reactant ratios were employed, except for alcohol volume optimization. Methanol containing sulfuric acid served as the catalyst, with concentrations ranging from 0.0046 to 0.0308 mol. Fresh acid-methanol solutions were stirred for 5 minutes to maintain catalyst activity.

Reactant Mixing: Ground microalgae were blended with the catalyst/alcohol mixture, initiating transesterification wherein the catalyst solution broke down triglycerides to release fatty acid chains. The reaction vessel was heated at specified temperatures for designated durations.

Settling and Separation: The reaction mixture was cooled for 20 minutes, then filtered. Residues were washed in methanol to recover FAME traces. Water facilitated hydrophilic component separation in a 500-mL funnel, left to settle for 4 hours. Hexane extraction separated two layers: a hydrophobic layer (FAME, glycerides) and a hydrophilic layer (water, glycerol, excess methanol).

Layer Observation: Successful reaction was indicated by glycerin settling at the bottom, forming two distinct layers—biodiesel (lighter, slippery) and glycerin (dark, thick).

Alcohol Removal: Excess alcohol in both phases was removed via flash evaporation and recycled.

Glycerol Neutralization: Glycerol phase was heated to 100°C for 10 minutes, then cooled to room temperature and weighed.

Biodiesel Wash: Biodiesel layer was washed with water, filtered, and purified by fractional distillation to remove methanol and hexane. FAME yield was calculated based on the reaction equation and monitored for conversion efficiency. Conversion reached equilibrium with increased purified glycerol weight as unreacted glycerides mixed with biodiesel were minimized.

5. Results and Discussion

5.1. Process Optimization

Optimal conditions for achieving maximum biodiesel yield using Chlorella sorokiniana microalgae as feedstock were investigated for in-situ transesterification. The extent of the in-situ transesterification process depends upon reaction time, microalgae feedstock lipid content, alcohol-to-oil molar ratio, catalyst concentration, stirring efficiency, and reaction temperature. Biodiesel yield was calculated using measured by-product glycerol weight as an indicator of fatty acid methyl esters.

5.2. Effect of Alcohol Volume on In-Situ Transesterification

The molar ratio of alcohol to triglycerides represents one of the most significant variables affecting ester yield. The stoichiometric ratio for transesterification requires three moles of alcohol per mole of triglycerides to yield three moles of fatty acid alkyl esters and one mole of glycerol. However, transesterification is an equilibrium reaction requiring excess alcohol to drive the reaction toward product formation.

Using the average molar mass of Chlorella sorokiniana oil as determined, and accounting for transesterification stoichiometry involving 3 mol of reacting alcohol per mole of oil, the methanol volumes investigated represent a reacting alcohol-to-oil molar ratio range of 1857:1 – 4643:1 (calculated according to methanol density of 0.7918 g/cm^3).

Percentage conversion yield of produced FAME was calculated based on total co-product glycerol obtained, accounting for experimental and analytical error of ±5% for the investigated reacting methanol volumes, using fixed reaction time of 8 hours, temperature of 65°C, fixed acidic catalyst molar

concentration (0.0154 mol sulfuric acid), and constant stirring rate of 650 rpm.

The results (Figure 3) indicate improved microalgae oil conversion to FAME with increasing alcohol volume, with the lowest FAME equilibrium conversions observed at the reacting molar ratio of methanol-to-oil of 1857:1 (methanol volume of 40 mL) for all conditions studied. However, with alcohol volumes exceeding 80 mL (reacting molar ratio of alcohol to microalgae oil greater than 3714:1) for in-situ transesterification of 15 g microalgae biomass, no significant trends were observed for FAME yields.

Table 1: Effect of Alcohol Volume on Biodiesel Yield

Alcohol vol. (ml)	Molar ratio (x:1)	Yield (%)
0	0	0
40	1857.07	71.80
60	2785.61	82.90
80	3714.16	84.2
100	4642.69	84.2

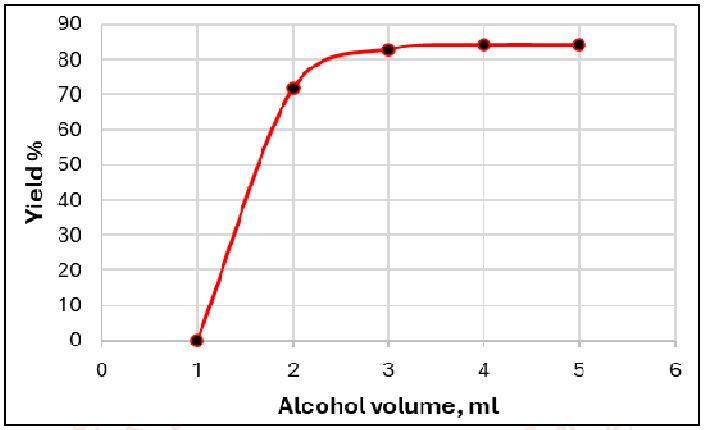


Figure 3: Effect of Alcohol volume on Biodiesel yield
(At 65°C for 8 hr., stirring of 650 rpm and 100% wt. H_2SO_4)

5.3. Effect of Catalyst Concentration on In-Situ Transesterification

The in-situ transesterification process was studied at four catalyst loadings (30%, 50%, 100%, and 200% H_2SO_4 wt./wt. algae oil content). Higher yields of 84.716% (with ±5% analytical error) were achieved with 100% H_2SO_4 (wt./wt. oil) at 65°C for 8 hours using methanol-to-oil molar ratio of 3714:1. Further increases in catalyst concentration did not significantly improve conversion efficiency.

Table 4: Effect of Catalyst Concentration on Biodiesel Yield

H_2SO_4 conc. (%)	H_2SO_4 vol. (ml)	Yield %
30	0.25	55.0
50	0.41	76.3
100	0.82	84.7
200	1.64	84.7

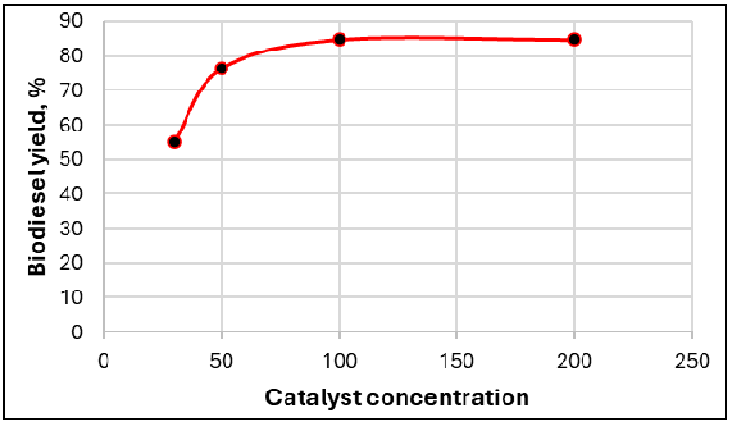


Figure 4: Effect of catalyst concentration on biodiesel yield
(at 65°C for 8 hour using methanol-to-oil molar ratio of 3714:1)

5.4. Effect of Reaction Time and Temperature

Temperature exhibited no detectable effect on ultimate conversion to ester; however, higher temperatures decreased the time required to achieve maximum conversion. The temperature range investigated was 27°C to 65°C. The effect of temperature on transesterification for time intervals from 2 to 10 hours was studied. The optimum temperature was 65°C for 8 hours. At lower temperatures (27°C), the process remained incomplete with no FAMEs observed.

Table 5: Effect of reaction time at different temperatures on FAME yield

Time, hour	Yield %			
	27 °C	40 °C	50 °C	65 °C
2	1.400	25.00	38.20	43.10
4	10.70	46.01	70.49	76.22
8	30.10	62.30	81.60	85.00
10	35.00	62.51	81.59	84.81

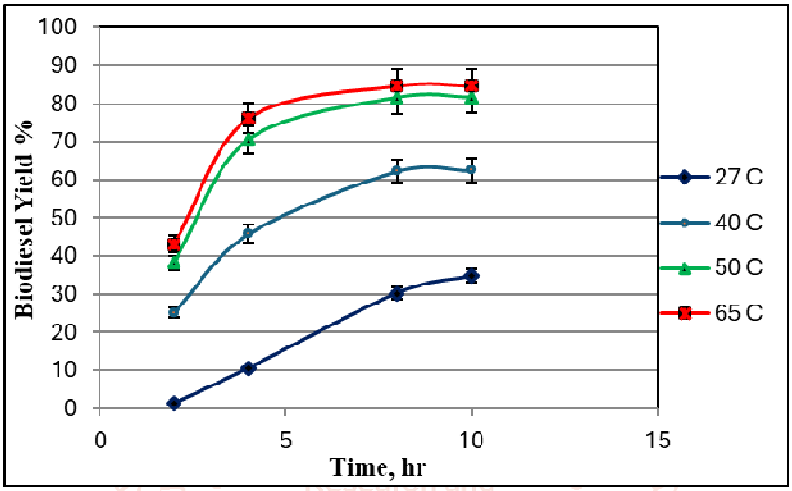


Figure 4: Effect of reaction time at different temperatures on FAME yield

(Using methanol-to-oil molar ratio of 3714:1, 100% wt. H_2SO_4 with stirring rate of 650 rpm)

5.5. Effect of Stirring on In-Situ Transesterification

Stirring intensity appears to be of particular importance for the alcoholysis process. Mass transfer of triglycerides from the microalgae oil phase toward the methanol-oil interface may be a critical step limiting alcoholysis reaction rate due to the heterogeneous nature of the reaction mixture (two immiscible phases). Poor mass transfer between phases in the initial reaction phase results in slow reaction rates, with the reaction being mass transfer controlled. Additionally, low solubility of methanol in Chlorella sorokiniana oil may limit alcoholysis reaction rate during early stages. Therefore, variations in stirring intensity are expected to alter transesterification reaction kinetics.

The effect of stirring on in-situ transesterification was investigated as a potential process performance strategy. When conducted without stirring, no reaction occurred, and zero conversion of microalgae oil content to biodiesel was obtained, compared to continuously stirred samples. This indicates stirring is required to enhance reaction progress, evidently by aiding initial miscibility of reacting species. After 4 hours of reaction under identical conditions, intermittently stirred samples (1 h on and 1 h off) achieved only 58.7% yield, while continuously stirred samples achieved 76.22% yield, confirming the positive influence of stirring during reaction.

Table 6: Effect of stirring on the in situ transesterification of microalgae lipids

(at 65oC for 4 hours with 100% wt. H_2SO_4 conc. using methanol volume of 80 ml)

Stirring treatment	Yield %
No stirring	0
Stirred intermittently (1 h off, 1 h on)	58.7
Stirred continuously	76.22

5.6. Quality Assessment of Produced Biodiesel

Following biodiesel production, a series of tests were conducted to establish properties of the produced microalgae biodiesel. Viscosity, density, flash point, cold flow properties, and cetane number were measured using EN methods. The obtained values were compared to EN 14214 standards.

Table 7: Biodiesel analysis compared to fossil diesel and EN 14214 standards

Property	Unit	Global Petro-diesel	Biodiesel (EN14214)	Algal diesel (B100)
Density at 15°C	g/cm^3	0.8422	0.86-0.90	0.8637
Viscosity at 40°C	cSt	1-7	3.5-5.0	12.4
Acid number	mg KOH/g oil	0.023	<0.5	0.75
Pour point	°C	-3	-	-9
Cloud point	°C	+6	-4	-3
Flash point	°C	82	>101	189
Ash	wt.%	0.009	<0.02	Nil
Calorific value	MJ/kg	45.35	32.9	45.63
Cetane number		50	>51	70
Diesel index		48	-	67

6. Conclusions

The present study demonstrates the prospective utilization of the Chlorella sorokiniana green microalgae species for biodiesel production. Biodiesel was shown to be a clean substitute fuel for petro-diesel requiring no modifications in diesel engines. It is renewable, environmentally friendly, non-toxic, energy-efficient, and biodegradable. The standardized definition of biodiesel as fatty acid methyl esters (FAME) was confirmed, producible from vegetable oils (edible or non-edible), animal fats, or specialized microalgae strains.

Experimental findings established that increasing reacting alcohol volume beyond 80 mL did not produce significant changes in biodiesel yield when using 15 g of Chlorella sorokiniana biomass under constant other variables. Results further demonstrated that 100% H_2SO_4 concentration (wt./wt. oil) as catalyst at 65°C for 8 hours represented optimal reaction conditions using 15 g of biomass and 80 mL of reacting methanol to achieve 84.7% biodiesel yield. The reaction temperature of 65°C proved beneficial considering total energy consumption and operational costs of the overall biodiesel conversion system. The experiments also confirmed the positive influence of stirring during the reaction process, as absence of stirring resulted in no product formation.

The high lipid content yield and substantial proportion of polyunsaturated fatty acids exhibited by the investigated strain provide evidence of its suitability for employment in the biofuel industry. The techno-economic analysis confirmed the viability of algal biodiesel production with favorable economic indicators, establishing break-even parameters for algal biomass cost, algal cake market price, and biodiesel selling price relative to crude oil benchmarks.

7. References

[1] Alsultan, A.G.; Asikin-Mijan, N.; Ibrahim, Z.; Yunus, R.; Razali, S.Z.; Mansir, N.; Islam, A.; Seenivasagam, S.; Taufiq-Yap, Y.H. A Short Review on Catalyst, Feedstock, Modernized Process, Current State and Challenges on Biodiesel Production. Catalysts, 2021, 11(11), 1261.

[2] Pata, U.K. Linking renewable energy, globalization, agriculture, CO_2 emissions and ecological footprint in BRIC countries: A sustainability perspective. Renewable Energy, 2021, 173, 197–208.

[3] Sarin, A. Biodiesel Production and Properties. RSC Publishing, 2012.

[4] Osman, W.N.W.; Rosli, M.H.; Mazli, W.A.; Samsuri, S. Comparative review of biodiesel production and purification. Carbon Capture Science and Technology, 2024, 13.

[5] Huang, D.; Zhou, H.; Lin, L. Biodiesel: An Alternative to Conventional Fuel. Energy Procedia, 2011, 16, 1874–1885.

[6] El Bann, S.; Nour El Deen, O. Biodiesel an Alternative Vehicles Fuels; Analytical View. TESCE, 2004, 30(2), 495–521.

[7] Mollenhauer, K.; Schreiner, K. History and Fundamental Principles of the Diesel Engine. Handbook of Diesel Engine, 2010; pp. 1–28. DOI: 10.1007/978-3-540-89083-6_1.

[8] Knothe, G.; Van Gerpen, J.V.; Krahl, J. The Biodiesel Handbook. AOCS Press, 2005.

[9] Encinar, J.M.; Juan, F.; Gonzalez, J.F.; Rodriguez-Reinares, A. Ethanolysis of used frying oils: Biodiesel preparation and

characterization. *Fuel Processing Technology, 2007, 88(5), 513–522.

[10] Maksum, H.; Ilham; Purwanto, W. Various Types of Biodiesel as Sustainable Fuel Choices: A Review. Jurnal Inovasi Vokasional dan Teknologi, 2023, 23(2), 119–128.د

[11] Vlad, E.; Bildea, C.S.; Pleşu, V.; Marton, G.; Bozga, G. Process Design of Biodiesel Production from Rapeseed Oil. Chemical Engineering Transactions, 2010, 21, 1267–1272.