Optimization of Ethanol-based Reactive Extraction Process for Spent Coffee Grounds Biodiesel production using Duo-function Diazabicycloundecene as catalyst and solvent

Mr. Huan Luong Trien

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Hazardous Substance and Environmental Management
Inter-Department of Environmental Management
GRADUATE SCHOOL
Chulalongkorn University
Academic Year 2021

การเพิ่มประสิทธิภาพกระบวนการสกัดไบโอดีเซลโดยตรงจากกากกาแฟด้วยเอทานอลและ Diazabicycloundecene ที่เป็นทั้งสารเร่งปฏิกิริยาและตัวทำละลาย

นายฮวน ลอง เตรียน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาการจัดการสารอันตรายและสิ่งแวดล้อม สหสาขาวิชาการจัดการสิ่งแวดล้อม
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2564

Thesis Title	Optimization of Ethanol-based Reactive Extraction Process for Spent Coffee Grounds Biodiesel production using Duo-function Diazabicycloundecene as catalyst and solvent
By	Mr. Huan Luong Trien
Field of Study	Hazardous Substance and Environmental Management
Thesis Advisor	Nattapong Tuntiwiwattanapun, Ph.D.

Accepted by the GRADUATE SCHOOL, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

-- Dean of the GRADUATE SCHOOL

(Associate Professor YOOTTHANA CHUPPUNNARAT, Ph.D.)

THESIS COMMITTEE

-- Chairman

(Associate Professor MANASKORN RACHAKARAKIJ, Ph.D.)

-- Thesis Advisor

(Nattapong Tuntiwiwattanapun, Ph.D.)

-- Examiner

(Assistant Professor Vorapot Kanokkantapong, Ph.D.)

-- Examiner

(Vacharaporn Soonsin, Ph.D.)

-- External Examiner

(Associate Professor CHANTRA TONGCUMPOU, Ph.D.)

ฮวน ลอง เตรียน : การเพิ่มประสิทธิภาพกระบวนการสกัดไบโอดีเซลโดยตรงจากกากกาแฟด้วยเอทานอลและ Diazabicycloundecene ที่เป็นทั้งสารเร่งปฏิกิริยาและตัวทำละลาย. (Optimization of Ethanol-based Reactive Extraction Process for Spent Coffee Grounds Biodiesel production using Duo-function Diazabicycloundecene as catalyst and solvent) อ.ที่ปรึกษาหลัก : ณัฐพงศ์ ตันติวิวัฒนพันธ์

วัตถุประสงค์หลักของงานวิจัยชิ้นนี้ คือ การประเมินประสิทธิภาพของสาร 1,8-Diazabicyclo [5.4.0] undec-7-ene (DBU) สำหรับการเพิ่มความสามารถในการสกัดและตัวเร่งปฏิกิริยาในการผลิตไบโอดีเซล (เอทิลเอสเทอร์ของกรดไขมัน; Fatty acid ethyl ester; FAEE) จากกากกาแฟโดยตรงด้วยเทคนิค อินซิตู ทรานส์เอสเตอร์ริฟิเคชั่น (In-situ transesterification; In-situ TE) สภาวะที่เหมาะสมในกระบวนการผลิตดังกล่าวถูกพัฒนาขึ้นจากเทคนิคการวิเคราะห์พื้นผิวตอบสนอง ได้แก่ อุณหภูมิ, เวลา, ปริมาณเอทานอล, ปริมาณ DBU และปริมาณน้ำในเอทานอล ผลการทดสอบแสดงให้เห็นว่าการใช้ DBU สามารถผลิต FAEE จากกากกาแฟ ได้ในอุณหภูมิ 40°C และการเพิ่มอุณหภูมิกลับส่งผลเสียต่อ FAEE yield เนื่องจากการระเหยของ DBU และเอทานอลที่ 70°C สภาวะที่เหมาะสมในการผลิตไบโอดีเซลชนิด FAEE จากกากกาแฟ 5 กรัม คือ 40°C เวลา 30 นาที ที่สัดส่วน เอทานอลต่อ DBU 0.8 และมีปริมาณสารเคมีทั้งหมด 120 มิลลิลิตร ได้ FAEE yield 100% ในการทดสอบผลกระทบของปริมาณน้ำในเอทานอลพบว่า ปริมาณน้ำในเอทานอลส่งผลลบต่อ FAEE yield โดยสามารถลดผลกระทบนี้ได้โดยการลดอุณหภูมิของกระบวนการลง ในสภาวะที่มีปริมาณน้ำในเอทานอลที่ 2.5% ณ อุณหภูมิ 40 °C สามารถผลิต FAEE yield ได้ถึง 80% ซึ่งสูงกว่าการใช้อุณหภูมิ 50 และ 60 °C ที่ให้ FAEE yield ระหว่าง 50-60% การทดสอบการวนใช้สารเคมี เอทานอล-DBU พบว่าประสิทธิภาพในการผลิต FAEE yield ลดลงเหลือเพียง 60% เนื่องจากการสูญเสีย DBU จากการดูดซับบนกากกาแฟของกระบวนการผลิตก่อนหน้า

สาขาวิชา	การจัดการสารอันตรายและสิ่งแวดล้อม	ลายมือชื่อนิสิต ....
ปีการศึกษา	2564	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

6288521320 : MAJOR HAZARDOUS SUBSTANCE AND ENVIRONMENTAL MANAGEMENT

KEYWORD: Spent Coffee Grounds (SCGs); Waste Utilization Biodiesel In-situ transesterification (in-situ TE); 1.8-Diazabicyclo [5.4.0] undec-7-ene (DBU); Eco-friendly process.

Huan Luong Trien : Optimization of Ethanol-based Reactive Extraction Process for Spent Coffee Grounds Biodiesel production using Duo-function Diazabicycloundecene as catalyst and solvent. Advisor: Nattapong Tuntiwiwattanapun, Ph.D.

The intention of this master's thesis was conducted to evaluate the potential of using 1,8-Diazabicyclo [5.4.0] undec-7-ene (DBU) as an eco-friendly solvent and catalyst in the in-situ transesterification (in-situ TE) process for producing fatty acid ethyl ester (FAEE) from spent coffee grounds (SCGs) waste. Several parameters were also optimized the FAEE yield in this process by response surface methodology, including temperature, time, ethanol and DBU loading and water content in ethanol. Results showed that promising FAEE yield could achieved under 40 °C. Moreover, FAEE yield tended to decrease under high temperatures due to DBU and ethanol evaporation at 70 °C. The optimal conditions for 5 g SCGs biodiesel production were recorded at 40 °C, 30 minutes, 0.8 ethanol-DBU ratios (total 120 mL), and no moisture, which the FAEE yield was approximately 100%. The presence of water content significantly reduced the yield. However, declining the operating temperature could partly mitigate the detrimental effects of moisture content on FAEE yield. Under low temperature and high ethanol-DBU loading, the FAEE yield was achieved above 80% when the water content in ethanol was 2.5%. The post-ethanol-DBU solution was continued to reuse for the subsequent batch to reduce extra chemical usage. However, the FAEE yield was dramatically lowered to roughly 60% due to the loss of DBU on extracted SCGs from previous batch.

Field of Study:	Hazardous Substance and Environmental Management	Student's Signature	
Academic Year:	2021	Advisor's Signature	

ACKNOWLEDGEMENTS

I would like to express my most profound appreciation to my supervisor Dr. Nattapong Tuntiwiwattanapun for his enthusiastic guidance during the planning, implementation, discussion, and study performance. I gratefully acknowledge the assistance of Mr. Preechapat Puasuwon, MSc., in helping me during the laboratory work. Many thanks to Miss Chantana Intim - a laboratory researcher, for providing the equipment and tools under which I completed my experiments. I deeply appreciate to International Program of Hazardous Substance and Environmental Management (IP-HSM) for providing the scholarship to participate in the master's course as well as the funding support for this research. Finally, I would like to recognize the assistance from everyone directly or indirectly involved in this work, especially my friends and lab mates, for their support during the course.

Huan Luong Trien

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ABBREVIATIONS AND ACRONYMS

ASTM : American Society for Testing and Materials

ANOVA : Analysis of variance

B-100 : 100% biodiesel

B-20 : 20% biodiesel with 80% petroleum

B-5 : 5% biodiesel with 95% petroleum

BOD5 : Five-day biochemical oxygen demand

CH3NaO : Sodium methoxide

CO2 : Carbon dioxide

DBU : 1,8-Diazabicyclo [5.4.0] undec-7-ene

DCE : 1,2-dichloroethane

DOE : Design of experiments

ELSD : Evaporative light scattering detector

EN : European standards

EPA : Environmental protection agency

ETA : Average S/N ratio

EtOH : Ethanol

FAAE : Fatty acid alkyl esters

FAE	: Fatty acid esters
FAEE	: Fatty acid ethyl esters
FFA	: Free fatty acid
GC	: Gas chromatography
GHGs	: Green-house Gas
H2SO4	: Sulfuric acid
HPLC	: High-performance liquid chromatography
In-situ TE	: Direct transesterification
IPA	: Isopropanol
KOH	: Potassium hydroxide
MeOH	: Methanol
NaOH	: Sodium hydroxide
RES	: Renewable Energy Source
RPM	: Revolution/rotations per minute
RSM	: Response Surface Methodology
SCGs	: Spent coffee grounds

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CHAPTER I: Introduction

1.1. Background

Petroleum was an indispensable source of raw materials to serve humans' demands, including electricity [1] and transportation [2]. The article by Bhagea, R., et al., (2019) predicted that fossil fuels would still provide more than 84% of world energy consumption in 2030 [3]. In fact, the prior study indicated that in 2016, the usage of domestic fossil fuels accounted for 90% of total energy consumption. Notably, China was the country that consumed the most coal for energy production, about half (50.01%) of the world [4]. The growing electricity demand was the reason that partly caused this high fossil fuel consumption annually. In Thailand, energy usage has risen dramatically, from around 70,000 kilotonnes in 2001 to 140,000 kilotonnes in 2019. However, there was a relatively high decrease from 2019 to 2020 due to the Covid epidemic affecting power consumption. On the other hand, when the pandemic situation would under control, electricity consumption will tend to increase, as shown in **Figure 1** [5]. Since the consequences of climate change in recent years and the shortage of crude oil could occur in the future, the global request for renewable energy has expanded.

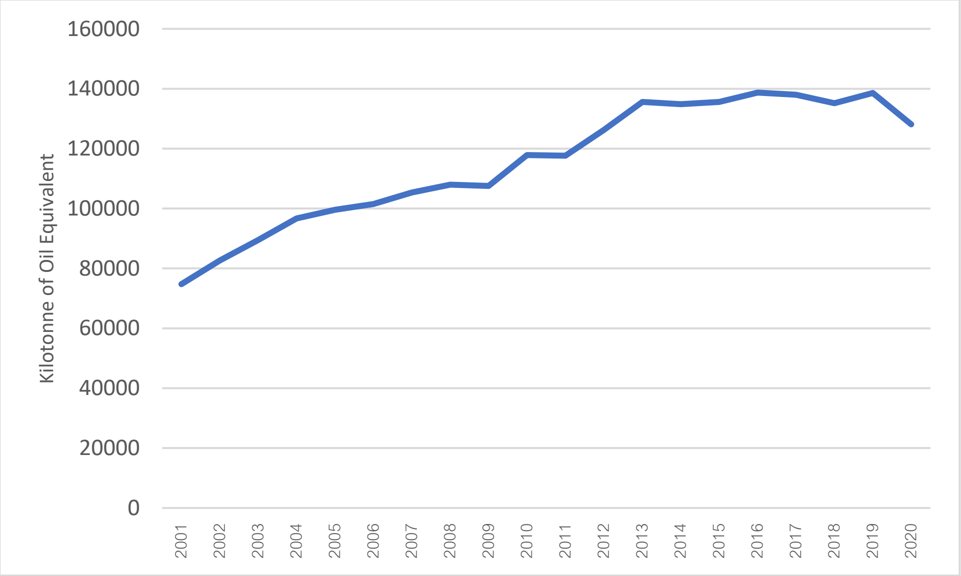


Figure 1. Total energy consumption in Thailand from 2001 to 2020.

Due to the environmental problem caused by greenhouse gas (GHGs) emissions, renewable energy sources (RESs) were presented as the top alternative solution. Numerous RESs have been developed to supply energy for industry activities and reduce environmental impact, including solar, wind power, and hydroelectricity. The article of Somorin, T.O., et al., (2017) presented that a big area is needed for building the mentioned RESs, while biodiesel is occupied a small region for manufacture [6]. Additionally, the majority of RESs derived from nature were regarded as unstable. A typical example from Marchuk, A., et al., (2019) explained that solar and wind energy only produced power efficiency under sunny or windy conditions, which can be understood that it is difficult for widely applied in the world [7]. Thus, the requirement for sustainable renewable sources is crucial in the future. Several studies pointed out that many power plants still used petroleum as a primary raw material [8, 9]. Due to the similar functions of petroleum and biofuel (biodiesel), those plants would continue to utilize instead of dismantling. Moreover, biofuel application was considered eco-friendly to the environment which can serve for both electricity and transportation field [7]. Sharma, Y.C., et al. (2017) attempted to evaluate that biodiesel was a solution to issues with energy security, limiting GHGs emissions, replacing fossil fuels, satisfying the increasing energy needs of the people, contributing to diversification, and creating clean energy [10]. As a result, many countries globally have focused on researching, developing, and promoting biodiesel production [11].

However, there were also some negative points about using biofuels. Although much bioenergy plays a beneficial role in sustainable energy, the dedication of cropland for biofuel production was not feasible [3]. Therefore, selecting an appropriate biofuel feedstock, which does not affect the land used and food production, is essential. Indeed, it is now well established from various studies that waste feedstock has gained more attention as the promising biodiesel feedstock. In this study, spent coffee grounds (SGGs), which are regarded as a large amount of solid waste released annually, are the primary raw material for biodiesel synthesis. The application of these SCGs not only reduces solid waste released into the environment but also utilizes waste sources to create a new product, specifically biodiesel. It contributes a part to the process of designing the life cycle of coffee, which improves sustainability in the future [12].

Surveys that conducted by Efthymiopoulos, I., et al., (2019) and Abomohra, A.E., et al., (2021) had shown that the conventional biodiesel producing process, which is currently applied in an industrial scale, was seen as a complicated method due to it extremely affecting production cost and time. In addition, the traditional processes must go through many stages to create a completed product, including oil extraction, biodiesel synthesis, and biodiesel purification [13, 14]. Moreover, methanol (CH_3OH), sodium hydroxide ($NaOH$), potassium hydroxide (KOH), sodium methoxide (CH_3NaO), sulfuric acid (H_2SO_4), and n-hexane (C_6H_{14}), considered as solvents and catalysts commonly used in oil extraction, were listed as hazardous to health and the environment [15, 16]. Hence, appropriate chemicals and methods were considered to limit environmental hazards and operating time.

Accordingly, this study focuses on developing an alternative SCGs' biodiesel production by the reactive extraction process, named in-situ transesterification (in-situ TE). Research on in-situ TE has been carried out by Go, A.W., et al ., (2016) and El-Enin, S.A., et al., (2013), who showed that this method could save time by merging oil extraction and biodiesel synthesis in a single step [17, 18].

With the characteristic of a switchable-polarity solvent, 1,8-Diazabicyclo [5.4.0] undec-7-ene (DBU) could be used in its low-polarity form as a solvent for oil extraction, then switched back into high-polarity form for oil immiscible, which investigated by Phan, L., et al., (2019) [19]. Combined with EtOH, this mixture was evaluated for the potential to apply in this study based on environmental criteria. To sum up, this study combined using eco-friendly chemicals (DBU and EtOH) with SCGs by applying in-situ TE to reduce the environmental problem, production time, and significant economic efficiency in the whole system. A summary of the biodiesel production process based on the methodology of this study is shown in **Figure 2**.

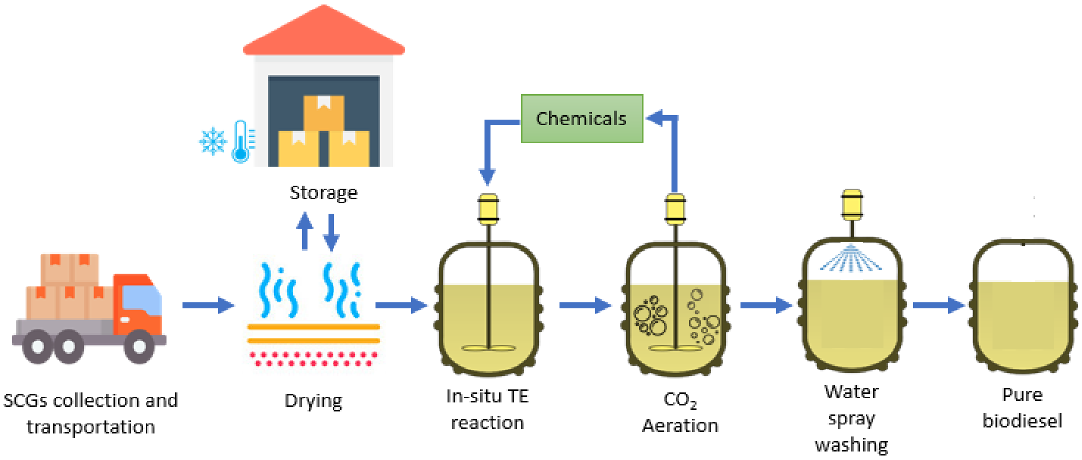


Figure 2. Process chart to produce biodiesel from SCGs

1.2. Objective

a. Maximize EtOH-based in-situ TE process for SCGs biodiesel production using DBU as the switch-able solvent and catalyst by response surface methodology.

b. Evaluating the reusability of DBU in the in-situ TE process.

1.3. Hypothesis

a. Increasing operating temperature could reduce the required amount of DBU and EtOH.

b. The effect of moisture content in EtOH on biodiesel yield could be mitigated by increasing operating temperature.

c. Post EtOH/DBU from the process could be reused and provide the new biodiesel yield for the subsequential batch.

1.4. Scope of the study

The scope of the study was using SCGs in a specific coffee shop to produce biodiesel. Ethanol and DBU was applied for this project using in-situ TE method to perform the experiments. Besides optimizing biodiesel production, this research also evaluated the reusability of post-EtOH-DBU after each batch to save material and limit the negative impact on the environment which can cause climate change in the future. Design of experiments (DOE) and statistical analysis software were implemented in Statistica 8 software. All the experiments will be carried out at the laboratory on the 10th floor of the CU research building. For SCGs in other sources, this experiment needs to be repeated to verify the data due to the differences in ingredients, mix mass percent, and the store's previous brewing conditions. The results of this report are neither applied to other types of SCGs nor other coffee stops

CHAPTER II: Literature Review

2.1. Biofuels and fossil fuels

Fossil fuels were carbon-based energy sources, such as oil, coal, and natural gas. These sources have been created over the millennia from decayed creatures and plants, whereas biofuels are any fuels made from biomass materials. The two most common biofuels are biodiesel and bioethanol. Over these last two decades, the interest has been rising in biofuels to reduce the dependency on fossil fuels and develop RESs, which are environmentally friendly energy [11].

A review of the World Energy Issues Monitor 2019 illustrated that the global energy market had been continuously evolving and shifting towards renewable energy. Global biofuel production has risen around 11.5% over the past decade. The global demand for fuel and energy would continue to grow through 2040. The United States and Brazil typically produced about 70% of the world’s biofuel supply. Biofuels currently comprise approximately 1.4% of the European Union’s total fuel consumption, in which biodiesel accounts for nearly 82% of the EU biofuel market [20].

In Asia, biodiesel production and consumption in China have increased yearly since 2015. Although the country’s biofuel consumption amounted to less than 3% of the entire usage of renewable energy, China contributed to 3.2% of the world’s total biofuel production compared to other countries. Nevertheless, Indonesia, which generated 2.5 million cubic meters of biodiesel in 2017, was considered to surpass China and Thailand in biodiesel production [21].

The status of renewable energy consumption in Thailand was shown in **Figure 3.** It has increased over the years, except in 2012 for bioethanol and 2011 for biodiesel. Moreover, bioethanol production in 2013 rapidly rose to 1,048 million liters from 790 million liters. For biodiesel, the amount also sharply increased from 630 million liters in 2011 to 910 million liters in 2012. Although there were high deviants between biodiesel and bioethanol consumption until 2012, the output of both has increased since 2013. They were estimated to be at almost the same levels of production in 2018 (1,500 million liters for bioethanol and 1,480 million liters for biodiesel). This statistic partly showed the determination to apply renewable energy was on the rise to limit fossil fuel usage in Thailand [22].

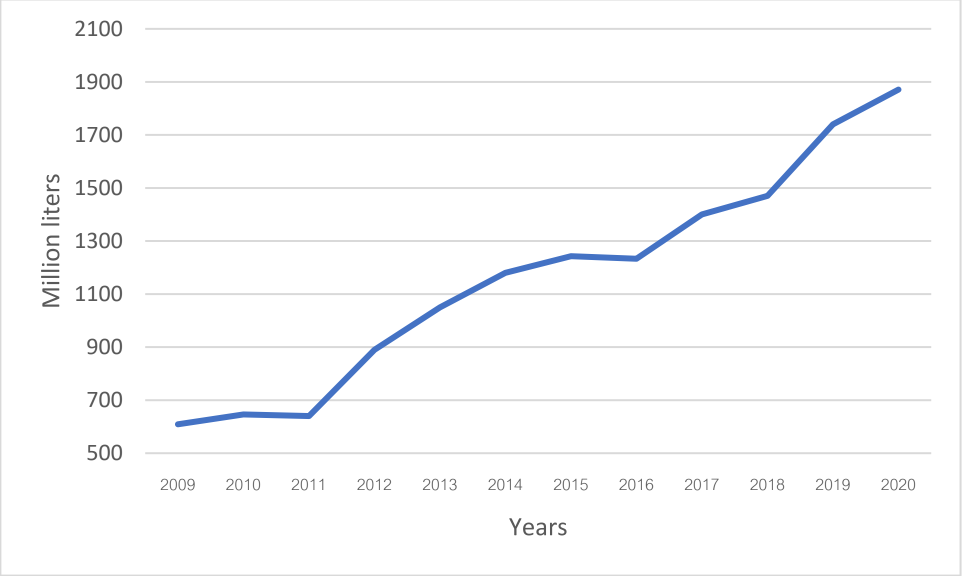


Figure 3. Biodiesel consumption in Thailand [22].

One great promise of biofuels was that they would provide an alternative solution to petroleum fuels. Biofuels can solve environmental problems due to their ability to reduce pollution more than fossil fuels during consumption. The net energy balance was a significant concern about biofuels, which shows that fuel production requires more inputs than outputs. Thus, several new advanced technologies have dramatically improved production efficiency, resulting in a net positive energy balance. A typical example is the use of arable land to grow agricultural products for biodiesel [3].

In the early first period of biofuel was explored, there were some conflicting opinions about using edible feedstock to produce biodiesel, as presented by Chong, C.T., et al., (2021) [20]. Then, a few countries planned to use the cropland to grow rich-oil seeds exclusively for biodiesel production. This period was the second generation of the development of biofuels [23, 24]. Still, the inputs used in the first two generations were edible foods or crops, including soybean, sunflower, corn, palm oil, and rapeseed [25]. This function diversion of edible crops could cause more hunger in the future. Therefore, further improvements were required regarding using raw materials and keeping the quality and quantity of the oil produced.

Many non-edible crops, agricultural waste, or insect biomass were gradually being studied. It can be seen as raw materials that are easy to collect in nature and cost less for purchase. In addition, waste sources were also listed for the inputs to produce biofuel, which contributed to the reduction of the environmental problems caused by solid waste. Some raw materials that have been studied for biodiesel production include waste cooking oil [24], rubber seed oil [26], black soldier fly larvae [27], and soy sauce residue [28]. Moreover, much research has shown feasible technologies to utilize SCGs [12-14, 29-31].

2.2. Spent coffee grounds

The environmental impacts of coffee were enormous, with large quantities of solid and liquid wastes generated globally. In a life cycle analysis of coffee, Salomone (2003) reported cultivation and consumption of coffee as the most significant contributors toward negative environmental impacts [32]. SCGs and processing wastewater represent the two waste streams that most contribute to soil and water contamination. Process water required to obtain clean coffee beans ranges from 5 to 20 L/kg of beans, with effluent BOD_5 levels increasing from 13 to 11,000 mg/L [33]. SCGs and husks also represent a significant soil and water contamination risk through highly degradable components, such as proteins and sugars, and more complex compounds, which may be phytotoxic [34]. SCGs have been evaluated as a compost amendment to soils, alone and in combination with other feedstocks such as rice hulls, with mixed success to increasing rice grain and straw yields (Yankaraddi et al., 2009). In North America, the 1.8 million metric tonnes of SCGs generated are either landfilled or processed at municipal composting facilities with other organic wastes.

Additionally, as a part of the third-generation biofuel feedstock, SCGs were given to make it become a potential material beneficial to the environment and economy. Mongkholrattanasit, R., et al., (2020) reported that around 10.2 million tons of coffee were consumed globally from October 2019 to September 2020 [35]. In Thailand, in 2016, over 56,000 acres of arable land were used for growing coffee beans, in which, Arabica coffee was planted in the northern mountainous areas accounting for 45.2% [36]. Approximately 6 million tons of coffee waste is generated globally annually. Most of it ends up in landfills or surroundings, posing a risk to humans and the environment [37]. Furthermore, these untreated SCGs would lead to a relatively large amount of solid waste in the future and harm the environment [38]. Although the SCGs utilization for biodiesel has just been researched in recent years, some studies showed optimal outcomes in high quality and quantity of oil production of SCGs to generate clean energy and mitigate environmental pollution [39]. One of the most outstanding findings following Blinová, L., et al., (2017) was that SCGs had a high oil content, around 11-20 wt% [38]. Therefore, with the 6 million tons of waste per year, SCGs provides an appealing non-edible feedstock for the biodiesel sector, a maximum of approximately 1.2 million tons of oil per year.

In the commercial coffee industry, there are two important coffee species, including Arabica and Robusta. Arabica has higher commercial value and, in general, more favorable sensory characteristics. Depending on the type of coffee and the roasting operation of each store, the amount of oil extracted will change [40]. This study cannot be synchronized the result with other types of SCGs in other coffee shops due to the different sources.

By analyzing the composition of SCGs, a rich source of bioactive compounds such as antioxidant, anti-tumor, anti-inflammatory, and anti-allergic compounds was found by Lam, M.K., et al., (2019) [41]. Simultaneously there was a large amount of calorific value, containing abundant cellulose, hemicellulose, and lignin between SCGs and defatted SCGs which can be understood as before and after extracting oil from SCGs (**Table 1**). Moreover, the water content of SCGs (about 70%) might decrease the SCGs oil quality owing to hydrolysis during transit and storage. Thus, most wet biomass must be kept at a freezing temperature and/or dried to a 5%

mass percent moisture content to avoid oil hydrolysis, followed by Tuntiwiwattanapun, N., (2016) [42].

Table 1. Nutrient composition and high heating value of SCGs and defatted SCGs [43].

Nutrient composition (%)	SCGs	Defatted SCGs
Crude protein	15.4	18.2
Crude lipid	16.2	0.3
Neutral detergent fiber (hemicellulose, cellulose and lignin)	45.2	58.9
Acid detergent fiber (cellulose and lignin)	29.8	40.2
Ash	1.8	2.4
High heating value (MJ/kg)	23.4	20.1

In addition to manufacturing oil, the post-reaction product can also be utilized in other fields. For example, glycerol would be used in cosmetics. After the oil extraction, the defatted SCGs could be used as biochar, fertilizer, bioethanol, and material composite (Vardon., et al., 2013, Kwon., et al., 2013, Rocha., et al., 2014) [43-45]. This contributes to the sustainability of coffee bean usage. A coffee life cycle study would be ideal for conducting in the future **(Figure 4).** Therefore, the selection of SCGs was highly reasonable and meaningful to help create oil and utilize waste instead of discarding it.

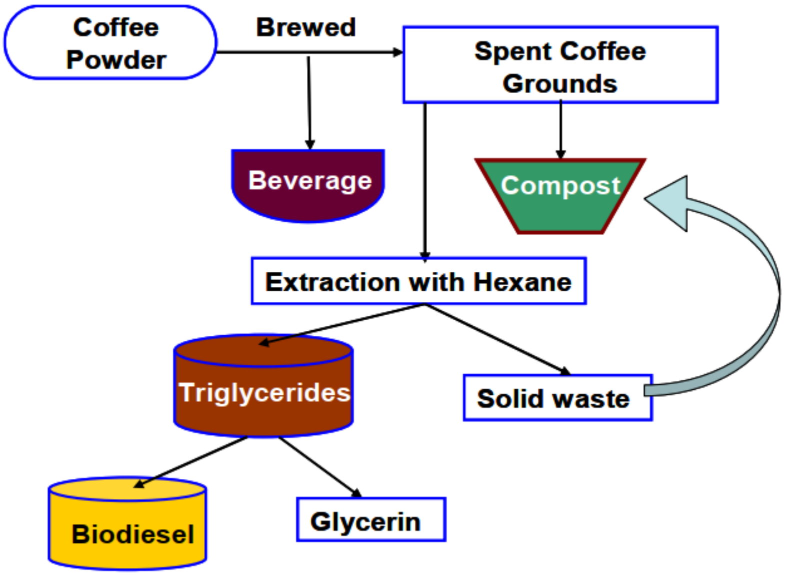


Figure 4. Schematic representation of the biodiesel production process from SCGs

2.3. Biodiesel

2.3.1. Definition

Biodiesel, a friendly-environmentally alternative fuel, can be considered a use-value equivalent to conventional or fossil diesel [46]. Biodiesel was a fuel composed of long-chain fatty acids from mono-alkyl esters derived from plant-seed oils [25], animal fats, and cooking oil… [47]. Based on cleaner characteristics after the combustion, biodiesel reduces greenhouse gases and smog and lowers particulate [48]. Biodiesel is composed of fatty acid alkyl esters FAAE including fatty acid methyl ester (FAME) and fatty acid ethyl ester (FAEE). In general, FAME and FAEE are produced through transesterification reaction between alcohol and triacylglycerol [49].

Biodiesel can be used as heating oil in domestic and commercial boilers, which can be used for electricity generation [1]. Recently, biodiesel had reported that it was attracting enormous attention from the transport industry all over the world as an alternative fuel for diesel engines because of its renewability. Biodiesel was used in standard diesel engines at low blends with conventional diesel (almost 100% pure biodiesel) and in modified diesel engines at higher blends, including neat fuel up to 20% blend. For example, B-5 was 5% biodiesel with 95 percent petroleum, B-20 was 20% biodiesel with 80% petroleum, or B-100 was 100% biodiesel without any petroleum content [2].

The advantages of using biodiesel fall into three broad categories, including environmental impact, energy security, and economic impact. Concerning environmental impact, compared to fossil diesel, biodiesel made from vegetable oil causes a 57% reduction in GHGs, while cooking oil biodiesel had an 86% diminution in GHGs. In addition, some harmful exhaust emissions were measured that showed around 47% of particulate matter was reduced in biodiesel [1, 2]. Therefore, it is possible to conclude that biodiesel was renewable because of using raw natural materials in production and minimizing pollutants during use.

2.3.2. Biodiesel standard

To be certified as biodiesel, the fatty acid alkyl ester must pass the international or national biodiesel standards such as ASTM 6751 and EN 14214. These guidelines provide criteria for evaluating biodiesel and specify practical limitations for several physical and chemical characteristics of the engine fuel. For more detail, **Table 2** shows the comparison of the three types of standards, which were updated in 2018. Thailand’s biodiesel standards had progressively adjusted according to EN 14214 [50, 51]. Current biodiesel is produced based upon differential standards. European standard

(EN 14214) and US standard (ASTM D 6751) are widely used as reference standards. The EN 14214 is based on biodiesel produced from rapeseed and combination of oils that together provide similar characteristics to rapeseed oil, while the US ASTM D 6751 biodiesel standard was developed to address biodiesel produced predominantly from soybean and waste cooking oils. The quality of biodiesel produced depends on several factors such as climate, feedstock, and vehicle type. The difference in those factors may require slightly different biodiesel specifications to fulfill market acceptance [52].

Biodiesel quality was impacted by various factors such as feedstock composition, the oil extraction method, biodiesel synthesis technique, refining procedures, and the impact of experimental conditions. Critical criteria have been researched and developed for assessing the quality of biodiesel.

However, the oil quality analysis based on the ASTM standard will not be carried out in this research due to the actual amount of oil obtained on the laboratory scale is not enough to conduct the estimation

Table 2. Comparison of biodiesel standards between the United States (ASTM D6751: 2011), Europe (EN 14214: 2003) and Thailand (DOEB B-100 Spec., B.E. 2552: 2009) [44,45].

Property specification	**Units**	**ASTM D6751: 2011**			**EN 14214: 2003**			**DOEB B100 Spec., B.E. 2552 (2009)**		
		Test Method	**Min**	**Max**	**Test Method**	**Min**	**Max**	**Test Method**	**Min**	**Max**
Ester content	%mass	-	-	-	14103	96.5	-		96.5	-
Flash point	°C	D93	130	-	ISO 3679	101	-		101	-
Cloud point	°C	D2500	3	12	-	-	-		-	-
Pour point	°C	D97	15	16	-	-	-		-	-
Cetane number		D613	47		ISO 5165	51	-		51	-
Density at 15 °C	Kg/m^3	D1298	880	880	ISO 3675/ 12185	860	900		860	900
Kinematic viscosity at 40 °C	mm^2/s	D445	1.9	6.0	ISO 3104	3.5	5.0		3.5	5.0
Iodine number	g I_2/100 g	-	-	-	14111	-	-		-	-
Acid number	mg KOH/g	D664	-	0.5	14104	-	0.5		-	0.5
Cold filter plugging point	°C	D6371	-	5	14214	-	-		-	-
Oxidation stability	hours	-	-	-	14112	3	-		3	-
Carbon residue	%m/m	D4530	-	0.05	ISO 10370	-	0.3		-	0.3
Copper corrosion		D130	-	No. 3	ISO 2160	Class 1	Class 1		Class 1	Class 1
Distillation temperature	°C	D1160	360	360	-	-	-		-	-
Lubricity (HFRR)	m	D6079	-	520	-	-	-		-	-
Sulphated ash content	%mass	ASTM	-	0.002	ISO 3987	-	0.02		-	0.02
Ash content	%mass	-	-	-	-	-	-		-	-

Table 2. (Continuous)

Water and sediment	%mass	D2709	-	0.005 vol%	ISO 12937	500 mg/kg	500 mg/kg	500 mg/kg	500 mg/kg
Monoglycerides	%mass	-	-	-	14105	-	0.8	-	0.8
Diglycerides	%mass	-	-	-	14105	-	0.2	-	0.2
Triglycerides	%mass	-	-	-	14106	-	0.2	-	0.2
Free glycerine	%mass	D6584	-	0.02	1405/ 14016	-	0.02	-	0.02
Total glycerine	%mass	D6548	0.24	0.24	14105	0.25	0.25	0.25	0.25
Phosphorus	%mass	D4951	-	0.001	14107	-	0.001	-	0.001
Sulphur (S 10 grade)	ppm	-	-	-	-	-	-	-	-
Sulphur (S 15 grade)	ppm	D5453	-	150	-	-	-	-	-
Sulphur (S 50 grade)	ppm	-	-	-	-	-	-	-	-
Sulphur (S 500 grade)	ppm	D5453	-	500	-	-	-	-	-
Carbon	wt%	PS121	77	77	-	-	-	-	-
Hydrogen	wt%	PS121	12	12	-	-	-	-	-
Oxyygen	wt%	PS121	11	11	-	-	-	-	-
BOCLE scuff	g	PS121	7000	-	-	-	-	-	-
Conductivity at ambient temperature	pS/m	-	-	-	-	-	-	-	-
Total contamination	mg/kg	D5452	24	24	12662	24	24	24	24
Boiling point	°C	D7398	100	615	-	-	-	-	-
Saponification value	mg KOH/g	D5558-95	-	370	-	-	-	-	-

2.4. Biodiesel production

2.4.1. Overview of technologies affecting biodiesel production

The TE reaction was a reversible process in which raw oils (from animal fat, vegetable oil and so forth) were converted to biodiesel between triacylglycerol and alcohol in the presence of base or acid catalyst. In other words, it can be understood as separating the fatty acids from their glycerol into fatty acid esters (FAE) and free glycerol. This reaction occurs stepwise, with monoglycerides and diglycerides as intermediate products. A simple molecular representation of the reaction is shown below in **Figure 5**. The catalyst here played a role of increases the experiment’s speed. An excess of alcohol must be used as a solvent to achieve a high conversion factor **[53]**.

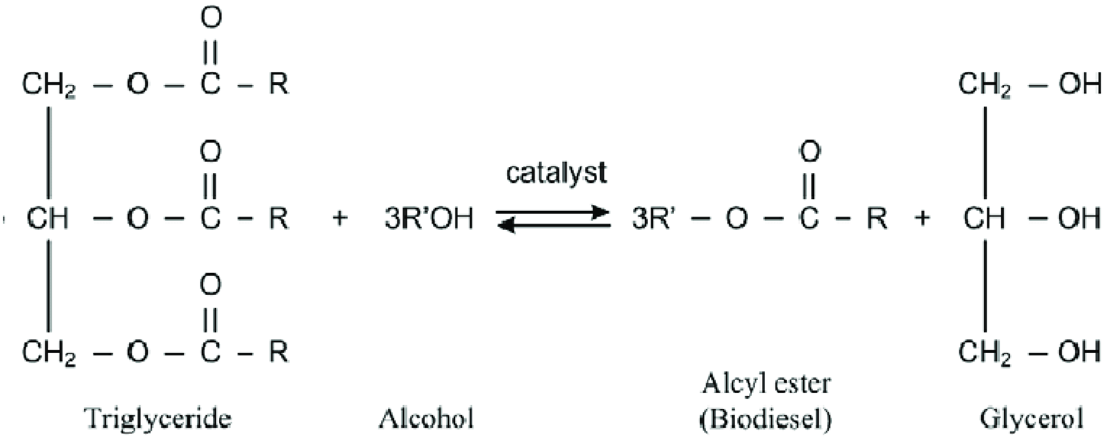


Figure 5. Biodiesel synthesis by transesterification reaction

In that reaction, the fatty-content materials (triacylglycerol) were reacted in the presence of a catalyst with an alcohol solvent (usually MeOH) to give the corresponding alkyl esters of the fatty acid and glycerol.

As shown in **Table 3**, several studies on biodiesel production using SCGs by various methods have been carried out. The in-situ method was still the most promising based on biodiesel yield. Although ultrasonic and 1-2 TE methods produce high yields, it still has some limitations that need to be improved for more feasibility. In the 1-2 step TE methodology, the operating process took a long time and included many stages [54]. While the ultrasonic method spent less reaction time, it was still quite complicated in operation [45]. Both of these methods, in general, it has not yet seen optimization in the production process, compared with the in-situ TE method, which can solve the above problems.

Table 3. Summary of studies of SCGs biodiesel methods.

Methodology	Reaction			Yield (%)	Ref
	Condition	Temp	Time		
Ultrasonic	Sonication: MeOH-KOH, 40 Hz, 160W, Esterification: 40 Hz, 160W	Sonication: - Esterification: 60 °C	Sonication: 30 min Esterification: 60 min	97	[45]
1-2 step TE	One-step: 9 mole MeOH-KOH/mole FFA Two-step: Esterification: 0.1 Ml H_2SO_4 and n-hexane/100 Ml oil, 10–20 Ml MeOH/100 Ml oil TE: 1.5 wt% KOH, 6 mole MeOH/mole oil	Step 1: 65 °C Step 2: Esterification: - TE: 60 °C	Step 1: 4 h Step 2: Esterification: 4 h TE: 6 h	One-step: 85.5 Two-step: 99	[54]

In-situ TE	**MeOH: 29.87 mole/oil** **Acid – H_2SO_4 20 wt%**	**70 °C**	**12 h**	**98.61**	**[55]**
In-situ TE	MeOH: 400 mole /mole oil Alkaline NaOH 0.2 mole /L	333 °K	1.5 h	96	[56]
In-situ TE	MeOH 250 Ml/30 g SCGs Alkaline – NaOH 2.25 g/30 g SCGs	50 °C	3 h	81.8 ± 1.6%	[31]

Although the use of an alkaline catalyst reduces yield slightly, this approach was still favored due to if feasibility of other factors. Most experiments using alkaline catalysts usually react at low temperature. In addition, time was also an indispensable factor in expanding production on an industrial scale. These conditions were considered to avoid energy in the production process. Moreover, previous research has not mentioned much about the reuse of chemicals to limit using extra substances.

2.4.2. In-situ TE

A simultaneous biodiesel synthesis and solvent extraction method that employs catalyst and the extraction solvent as both the biodiesel reactant **(Figure 6)** was called in-situ TE. It uses a reactive extraction technique that converts fatty-content materials into biodiesel directly. This technique had two advantages that can be considered, including eliminating toxic n-hexane from oil extraction and reducing the complexity of the manufacturing system [42].

Therefore, based on its ease of implementation, the advantages of the in-situ TE method can be applied in both areas. The in-situ TE process can be set up close to the agricultural site (i.e., biodiesel feedstock area), allowing locals to produce and use their biodiesel for farm machinery and irrigation pumps. Moreover, biodiesel could be used for power generators, especially in remote areas where the national power grid was not yet connected. On the industry scale, the in-situ TE technique enables businesses to set up on-site biodiesel production using their waste as a biodiesel feedstock, leading to a better waste management strategy. More information about the co-benefits of biodiesel may be found in “Benefits of Biodiesel.”

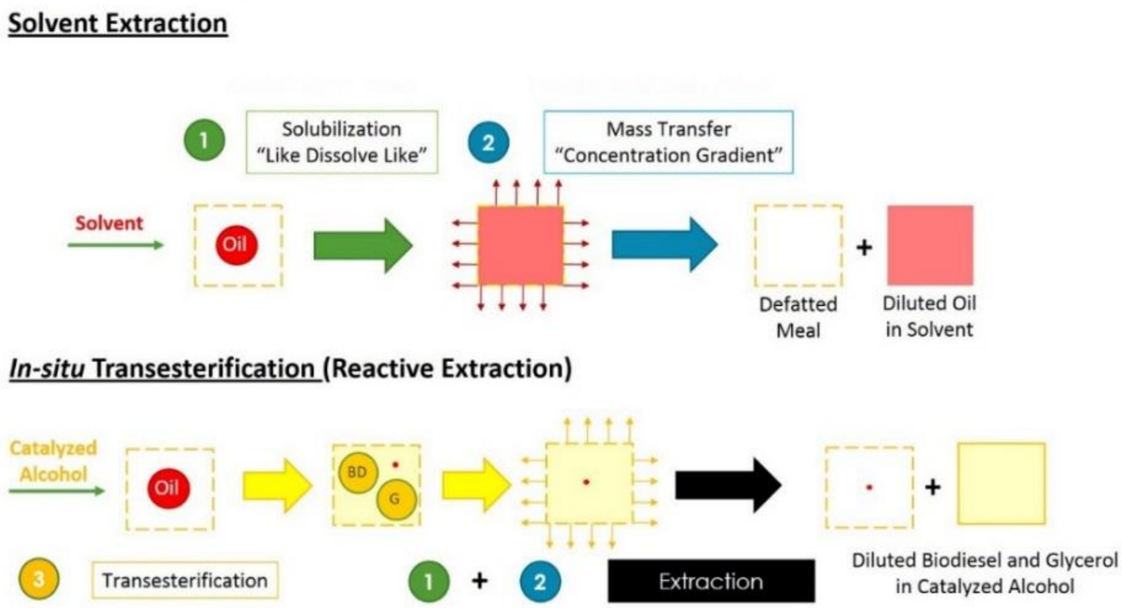


Figure 6. Diagram of solvent oil extraction (A) and the in-situ TE process (B) [42]. Where BD was biodiesel, and G was glycerol.

The in-situ TE had been considered a method that produces biodiesel with high efficiency, which could improve factors in previous studies (lipid-alcohol ratio, temperature, catalyst-solvent usage, reaction time). However, this method stumbled the primary restriction in the commercialization, which required a significant amount of solvent [41]. According to Haas and Wagner (2011), it was easy to see that in-situ TE had improved over TE in **Table 4** [57].

Table 4. Comparison between TE and in-situ TE

	TE	In-situ TE

Heating value	Low	High
Product yield	Low	High
Implementation the process:		
● **Time**	Slow	Fast
● **Level**	Complex	Simple
Prodigality	Lipid loss during the process	Avoided potential lipid loss
Emissions	Wastewater incurred	Reduce wastewater pollutants
Cost	High	Low (Absence of harvesting and dewatering lowers the cost)

2.5. Impact of in-situ TE biodiesel production factors

Solvent

According to **Table 5**, which shows some studies related to applying the in-situ TE method, a more significant amount of yield was obtained when using acid catalysts. However, the requirement of other conditions such as temperature (Rice bran) [58] and reaction time (Jatropha seeds) [59] was also high. Unlike acid catalysts, using alkaline catalysts showed the optimal time was lower to reach the highest yield. The most prominent finding is that SCGs were a waste product; nevertheless, the obtained results oscillated in 80-100% range. Therefore, the advantage of using SCGs to create renewable energy sources should be considered.

Table 5. Research on biodiesel production by in-situ TE

Feedstock (F)	Solvent (S)	Catalyst I	Condition			Yield (%)	Ref
			Ratio (F : C : S)	Temp	Time		
Jatropha seeds	MeOH/ hexane	Acid – H_2SO_4	1 g : 7.5 Ml : 15 wt%	60 °C	24 h	99.8	[59]
Jatropha seeds	MeOH /hexane	Alkaline – KOH	1 : 6 : 0.075 mole/l MeOH	60 °C	4 h	87	[60]
Rice bran	EtOH (99.1%)	Acid – H_2SO_4	50 g : 200 Ml : 7.5 Ml	78 °C	1 h	76.4	[58]
Rice bran	IPA	Acid – H_2SO_4	50 g : 200 Ml : 7.5 Ml	82 °C	1 h	69.7	[58]

Soybean	MeOH	Alkaline – NaOH	1 : 543 : 2.0 N	23 °C	8 h	84	[61]
Soybean oil	IPA	Alkaline – NaOH	1 g : 2 cm^3 (1 l : 0.45 g)	70 °C	1.5 h	85	[62]
Rapeseed	MeOH	Alkaline – NaOH	1 : 475 : 0.1 mole/l MeOH	60 °C	1 h	88.8 ± 0.1	[63]
SCGs	MeOH	Alkaline – NaOH	**30 g : 250 Ml : 2.25 g**	50 °C	3 h	81.8 ± 1.6%	[31]
SCGs	MeOH	Acid – H_2SO_4	**1 mole : 28.87 mole :** 20 wt%	70 °C	12 h	98.61%	[55]
SCGs	MeOH – DBU	DBU	**1 g : 6.25 Ml : 14.46 Ml**	60.2 °C	28.65 m	97.18% ± 1.30%	[12]
SCGs	MeOH – DBU	DBU	**1 g : 10 Ml : 20 Ml**	130 °C	1h	96.13%	[30]

* Whereas: g: gram Ml: milliliter wt%: weight percentage mole: molecular N: Normality

2.5.1. Reaction time

According to I. A. Daniyan, et al., (2015), the research was implemented to compare the reaction time factor on both homogeneous catalysts (alkaline and acid) with MeOH when using the TE process. The amount of biodiesel was recorded using acid catalyst, reached a peak at the optimal time (8 hours), and reduced progressively afterward. It was similar to an alkaline catalyst with a shorter optimization time (2 hours), and the quantity of biodiesel production remained fixed after the optimal time was attained. In this process, the obtained oil was 80%, while 60% was observed by using an acid catalyst. Moreover, the reversible TE process leads to biodiesel yield loss and increases soap production for a longer time. This also can be seen as a possible explanation for the biodiesel yield decrease [64].

2.5.2. Temperature

Several systematic reviews of temperature influence in TE reaction have been undertaken. To enhance biodiesel yield, Mathiyazhagan, M., et al., (2011) indicated that a higher temperature could speed the reaction rate as the oil viscosity was reduced [65]. However, except reaching the optimal point, the more temperature rises, the less biodiesel yield obtained because the saponification reaction occurs and evaporation of MeOH while using an alkaline catalyst, as shown in the study of Eevera, T., et al., (2009) [66]. Depending on the feedstock, the optimum range might vary between 40 °C and 60 °C, which was close to the boiling point of EtOH [67]. Therefore, the selected temperature in this study will change from 50-90 °C to estimate the optimal temperature range for biodiesel production.

2.5.3. Water content

Water content was also a determining factor in biodiesel production. Park, J., et al., (2016) indicated that the more amount of water content, the more biodiesel yield decreased [68]. A possible explanation for this might be that the presence of water in biomass could lead to saponification reaction, which generated soap formation and reducing biodiesel yield while using alkaline catalysts. It also explained that several research had applied the acid catalyst and hydrophobic co-solvent to mitigate the water in biomass. Hence, Tuntiwiwattanapun, N., et al., (2017) applied MeOH washing as the pretreatment step for water and FFA removal from wet SCGs before in-situ

TE. More than 90% of biodiesel yield was achieved [31]. However, original SCGs obtained from the coffee industry or a coffee shop always have a high water content (> 70%) [31]. Therefore, other alkaline catalysts have been studied to limit the adverse effects of water on the system.

2.5.4. Alcohol

The first fatty acid esters introduced for biodiesel were fatty acid methyl esters (FAME), formed by enzyme-mediated esterification of MeOH with fatty acids. However, the use of fatty acid ethyl esters (FAEE), which were esterified by EtOH, in biodiesel is gaining popularity. Methanol is the most often used alcohol for biodiesel production due to its low cost and mild reaction conditions. Today, methanol is mainly obtained from a petroleum source, natural gas. Thus, bio methanol cannot be termed as entirely renewable. Moreover, MeOH was hazardous to people and can lead to blindness and severe nausea [69].

EtOH had higher solvency qualities and less danger than MeOH. It could use as a solvent by extracting oils and chemicals from vegetables [70]. While petroleum mined MeOH, EtOH was typically collected from farming sources, such as sugar cane and corn [69]. Also, MeOH was relatively inefficient at separating the liquid fraction layer from the oil [19]. This proves EtOH was considered a necessary chemical and green material for biodiesel production and used for this study.

2.5.5. Catalyst

Besides applying the in-situ TE method used on SCGs, the chemical factor used in this reaction was equally important. MeOH, sodium methylate, sulfuric acid, and n-hexane raise an environmental concern due to toxic chemicals being the key substances to run the in-situ TE process. Respond to this concern, previous studies had developed an in-situ TE method involving the use of a chemical as a green solvent and an enzyme as a biocatalyst for biodiesel production [71]. Therefore, research on the application of DBU in the in-situ TE process for biodiesel production was carried out.

2.5.5.1. Homogeneous catalysts

Acid catalysts, especially sulfuric acids, can be used to catalyze TE. These catalysts produce high ratings compared to alkyl esters. However, such reactions were sluggish, usually needing temperature above 100 °C and over 3 hours for the conversion to complete [72]. For example, most studies on biodiesel production on granules by in-situ TE had extremely high yields, about over 80%. The highest in which Jatropha seeds accounted for 99.8% of the yield with the condition of 60 °C within 24 hours. The ratio of 1 g : 7.5 Ml : 15 wt% corresponds to the raw materials : MeOH catalyst : H_2SO_4 solvent, respectively [59]. Although the temperature was 70 °C, extracting time in biodiesel oil using SCGs was only 12 hours and achieved the second yield in **Table 5**, 98.61% [55]. Still, the catalysts include significant energy consumption (acid catalysts) and wastewater treatment due to unreactive substances (both catalysts). Acid catalysts were only applied when there was a large content of FFAs (> 40%) to avoid saponification occurring while using alkaline [73].

Another disadvantage of using base-catalytic, even with non-water content in the reaction between vegetable oils and alcohol, was the amount of water appearing when reacting between NaOH and alcohol as the solution of the RONa. This reaction leads to the process of saponification. The sodium metal application gives superior biodiesel production compared to sodium hydroxide because it did not produce water when alcoholic salt was generated, as indicated in **Figure 7.**

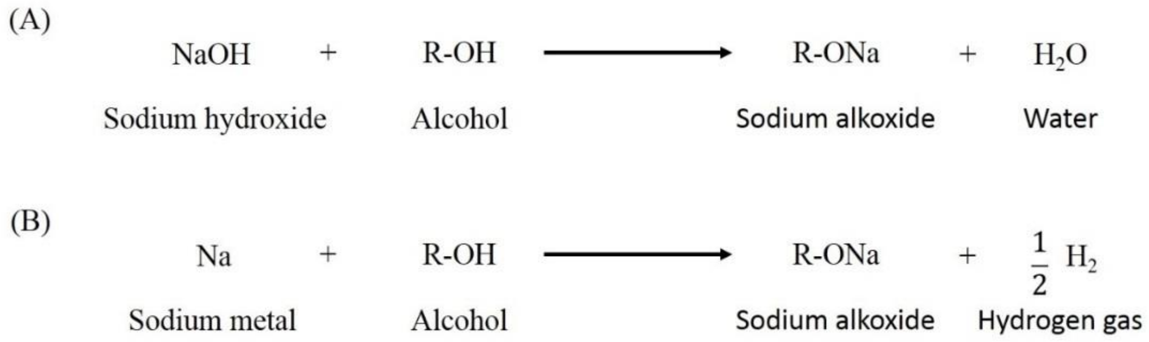


Figure 7. Illustrated equation of sodium hydroxide (A) and sodium metal (B) reacting with alcohol.

2.5.5.2. Heterogeneous catalysts

A heterogeneous catalyst, including BeO, MgO, CaO, SrO, BaO, and RaO [74], which were considered eco and cheap substances, eliminates additional costs of in-situ TE processing as well as the reduction in pollutant production [75]. It can separate from the reaction, recovery, reusability easily [76]. In contrast, leaching occurs in some catalysts, particularly CaO, which made harm impact on the reaction. Most essential, not more than 90% of the biodiesel yield was produced [77].

2.5.5.3. Biocatalyst

The advantages of biocatalysts such as reduced energy consumption, and ecologically friendly with a high biodiesel yield obtained from in-situ TE, were similar or even better than homogeneous catalysts. Nevertheless, the cost of biodiesel synthesis remains high as the enzymes were costly and reusable. Some biocatalyst was commonly known as free lipase, traditional immobilized lipase, or lipase immobilized on magnetic nanoparticles [77].

2.6. Duo function of DBU as a switchable solvent and biodiesel catalyst

With the environmental priorities, it is vital to find a substance that has multifunctional to limit extra chemicals usage and is environmentally friendly. The easily recyclable and cheaper CO_2 switchable solvent system first reported by Phan, L., et al., (2009) [19]. l,8-Diazabicyclo [5.4.0] undec-7-ene (DBU), as shown in **Figure 8.** is a sterically hindered amidine base. It is a liquid with a boiling point of 261 °C. It is one of the strongest organic neutral bases. It is a non-nucleophilic base, and due to this, it has been found to be useful in reactions where side reactions due to the inherent nucleophilicity of basic nitrogen pose a problem. It is advantageous to use DBU in organic reactions as it is cheap, commercially available, homogenous, and most importantly recoverable. In recent years, DBU has been used as a catalyst, complexing ligand, and a nucleophilic/non- nucleophilic base in organic reactions [78].

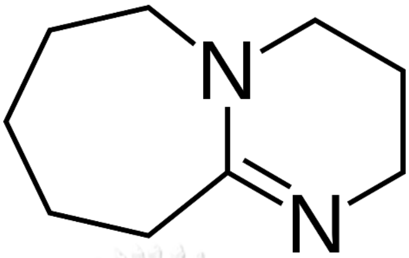


Figure 8. DBU molecular formula

A switchable solvent that was introduced can easily transform itself from hydrophobic by reacting with CO_2 to hydrophilic when bubbling with N_2 or heating. Thus, it can be recovered after the reaction and implemented for the next experiment. The in-situ TE reaction using DBU is depicted in **Figure 9**. This equation is the nonderivative reaction between DBU and alcohol. As a result, the addition of CO_2 will create a mixture that was considered a solvent to easily dissolve the oil in the SCGs [79].

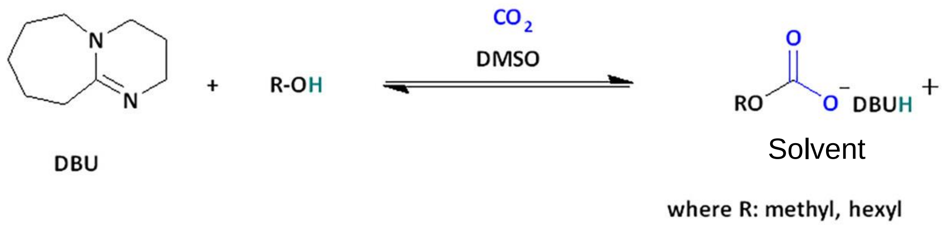


Figure 9. Transesterification reaction using DBU

Although it was an acutely harmful substance to humans health, such as irritant, corrosive, and other reproductive diseases through basic types of contact, DBU had been approved for TE and in-situ TE as both eco-friendly polarity-switchable solvents and biodiesel catalysts due to the strong alkaline properties it contains [80]. With low toxicity and high conversion of biodiesel, refining and extracting biodiesel from DBU has been noted as an ecologically benign alternative to organic solvent [12].

The benefit of DBU over conventional alkaline catalysts was the reaction selectivity. The presence of water in biodiesel production can cause a serious problem due to soap formation (saponification reaction). Both NaOH and KOH were consumed during soap formation with vegetable oil and FFA. On the other hand, DBU does not illustrate such a chemical reaction.

The DBU possesses strong alkaline characteristics and increases oil solubility in alcohol. Studies have shown that combining DBU with alcohol creates and effectively extracts biomass oil even by biodiesel in forming a DBU-alcohol combination. The CO_2-induced combination, which helps to separate the oil from the alcohol-DBU-mixed, can be more polar. In the condition of reaction scenarios of 110 °C, 16:1 DBU/biomass (Ml/g), 8:1 MeOH/biomass (Ml/g) and 60-min were reached following the study of Nguyen, H.C., et al., (2019) [27].

Moreover, n-hexane can be replaced by DBU. As a result, Phan, L., et al., (2009) assumed that the use of n-hexane would not be necessary since DBU had the same function as n-hexane. Usually, the obtained biodiesel had separated after in-situ TE was further washed with water and n-hexane solution at a ratio of 1: 1, then heated at 75 °C overnight [62]. This work aims to remove the solvent, water, residual n-hexane and thoroughly obtain biodiesel in the oil-glycerin mixture and glassware adhering. However, the amount of oil recovered was not about 70%, which was lower than n-hexane [19].

After the literature review, a combination of all the information from the articles was carried out in this study. Applying the in-situ TE method on SCGs using a dual function of DBU to produce biodiesel. This research will improve the efficiency of biodiesel production, optimize the manufacturing conditions, and recover chemicals. In addition, this research is also towards waste utilization, using environmentally beneficial chemicals, and aims for sustainable development. In addition, this research also strives for waste utilization, environmentally beneficial chemicals, and sustainable development.

CHAPTER III: Methodology

Based on the research hypothesis and objectives, the primary method for this study was sampling, experiment, data collection, and data analysis. Its framework was also established and described below in Figure 10.

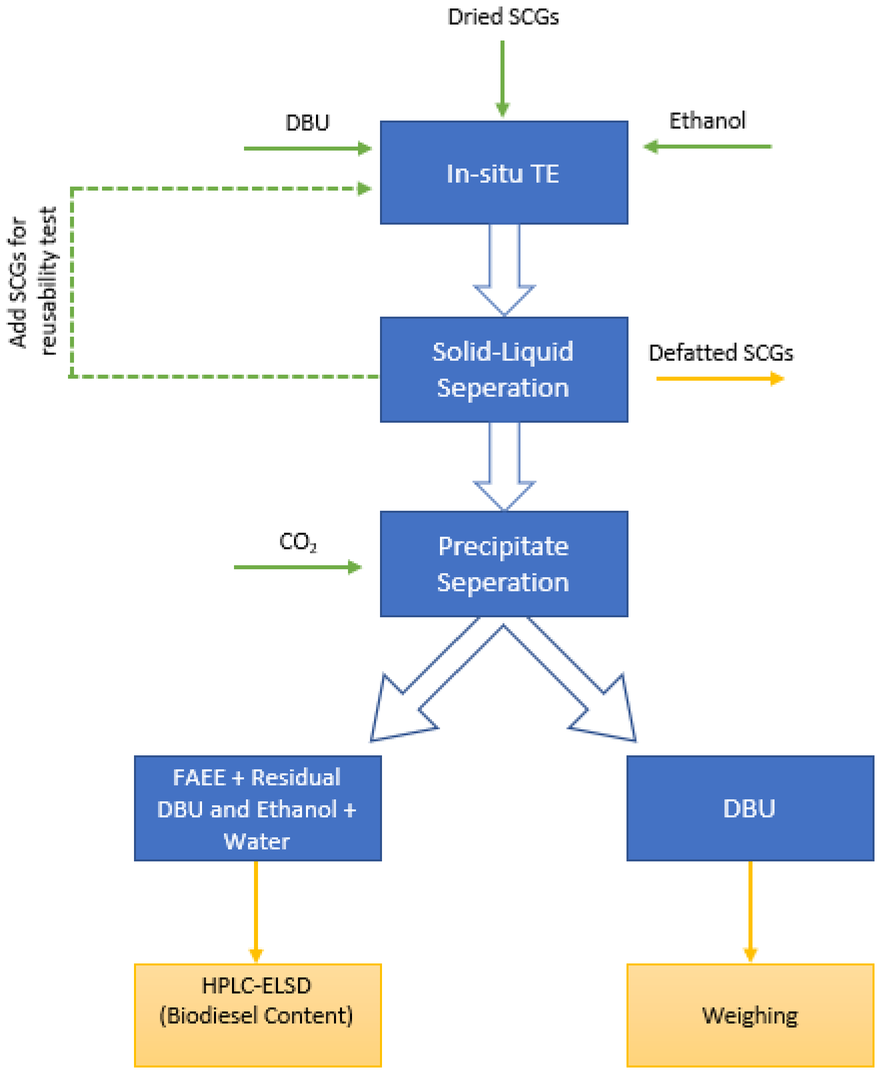


Figure 10. Diagram of experimental summarize process

3.1. Materials and reagents

Wet SCGs was mainly taken from the "Café Amazon" coffee shop. It was first thermal dried in a hot air oven at 105 °C overnight. This step aims to remove the water content and avoids mold during storage [31, 42]. After that, the dried SCGs was stored in zipper storage bag and kept in 4 °C conditions for further use.

In addition, Ethanol (99%), DBU (99%) were used for the in-situ TE process. N-hexane was used in soxhlet oil extraction for total oil content analysis. Analytical grades such as Methanol and Isopropanol were supported for HPLC-ELSD. All mentioned chemicals were purchased from the Sigma-Aldrich Corporation (St. Louis, MO, USA). HPLC-ELSD were acquired from Agilent, LC, USA.

3.2. Statistical analysis

The statistical methods used in this study include the Taguchi (Taguchi Orthogonal Array Design) and Box-Behnken (Central Composited Design) methods included in the Statistica 8 software. The Taguchi quality control approach was a statistical instrument that used a table design system to remove flaws and mistakes by employing a particular set of arrays called orthogonal arrays, including the level combinations of the independent components for each experiment. This set can, thus, minimize the number of trials yet give all the elements influencing the reaction [81]. The primary influence of the independent factors on response, however, was isolated in this DOE. Thus, there were no interactions between the independent factors. The data was analyzed using the Taguchi technique, which uses the signal-to-noise ratio (S/N ratio). There were many S/N ratio criteria dependent on applications, such as "Smaller-the-Better" for defect minimization, "Larger-the-Better" for yield maximization, and "Nominal-the-Best" for selecting a certain number. The efficiency of each independent component on the response will be determined after data analysis based on S/N ratio circumstances.

In fact, if the experimental design table is not applied, the number of experiments that need to be done is very large. It is not only waste of time as well as material for the experiment. According to Yyimaz., (1999), based on the number of factors and level of factors, a reasonable number of experiments will be designed as **Table 6**.

Table 6. Table of orthogonal arrays for different factor levels

Orthogonal Array (OA)	Number of factors	Level of factors	Number of experiments for OA	Number of experiment for full factorial design
L4 (2^3)	3	2	4	8
L8 (2^7)	7	2	8	128
L9 (3^4)	4	3	9	81
L12 (2^{11})	11	2	12	2048
L16 (2^{15})	15	2	16	32768
L16 (4^5)	5	4	16	1024
L18 (2^1 x 3^7)	1	2	18	4374
	7	3		

Similarly, the Box-Behnken design was a sort of response surface methodology (RSM) that offers detailed data on space, which was comprised of the first-order model (2N; min (-1) and max (+1) value) augmented with central (mean (0) value) and axial (+α value) points in which α can be calculated using the equation $\propto$ = ⟦Number of Factor⟧ ^(1/4) [42]. Due to the difficulties of identifying a large number of independent factors, two or three most significant elements were accounted for in Box-Behnken for process optimization after utilizing Taguchi in this study [82].

3.3. In-situ Transesterification (In-situ TE)

1 g of SCGs was vigorously mixed with a composite of 2-6 mL/g EtOH and 5-15 mL/g DBU in a water bath with heating (50-90 °C) and shaking (130 RPM) to perform an in-situ TE process. However, the amount of EtOH and DBU would be reacted with 5 g SCGs with the same ratio mentioned above in an erlenmeyer flask to get the proper volume for stirring when using water bath. The mixture, collected after finishing the in-situ TE, would be separated into liquid fractions and defatted SCGs. 50 mL/minute CO_2 in 30 minutes was then added to the solution and continued centrifuge for 10 minutes when it

reached 6000 RPM to classify DBU [42]. Each experiment will be repeated in triplicates to ensure to validate empirical data and the observed results

3.4. FAEE content analysis by HPLC-ELSD

3.4.1. Preparation of biodiesel standard

The soxhlet extraction and n-hexane were used to obtain SCGs crude oil. The oil yield here showed that for every 10 g of SCGs, 1.5 g of oil was obtained, which means the efficiency of SCGs in this study is up to 15%. From here, the crude SCGs oil was reacted at the ratio of 1 g oil : 5 mL of MeOH at 30 °C for 2 hours to reduce the high acid value present in crude SCGs oil.

Based on the National Biodiesel Board, the biodiesel standard was completed following these recipes. By dissolved 2.5 g KOH as a catalyst and 72 g EtOH in stirring and slight heating conditions, the mixture was added to 250 g oil, stirring strenuously for 2 hours at room temperature. The solution would be completely separated in a separatory funnel overnight. Later, the liquid fraction (glycerol) in the lower layer was removed. The oil, which was the upper part, would come to the biodiesel washing process. The water would be spayed slightly on top of a tall column of biodiesel carefully. It was stored for 1 or 2 days until the water had settled and the solution completely separated into 2 layers again. Then, the solution below was removed, and the oil was transferred from separatory funnels to glass beakers without being capped in a drying oven at 60 °C overnight to evaporate the residual water inside the oil. This standard would next be analyzed through HPLC-ELSD to get data for comparison.

3.4.2. Quantification of FAEE by HPLC-ELSD

The liquid fraction after the in-situ TE process was directly injected into the HPLC-ELSD instrument to determine the FAEE concentration. The Inertsil ODS-3 C18 5um 4.5 x 250 mm column was applied for the HPLC-ELSD test under conditions at 70 °C. The mobile phase consisted of MeOH and IPA (gradient elution: descending MeOH : IPA from 100% MeOH to 15% MeOH during the first 30 minutes, then return to 100% MeOH and maintain for 10 minutes for the next batch preparation). The injection volume and flow rate at 0.2 cm^3 and 1 cm^3/min, respectively. The ELSD detection condition was fixed at 40 °C for the temperature and kept in range from 210-220 kPa for the pressure.

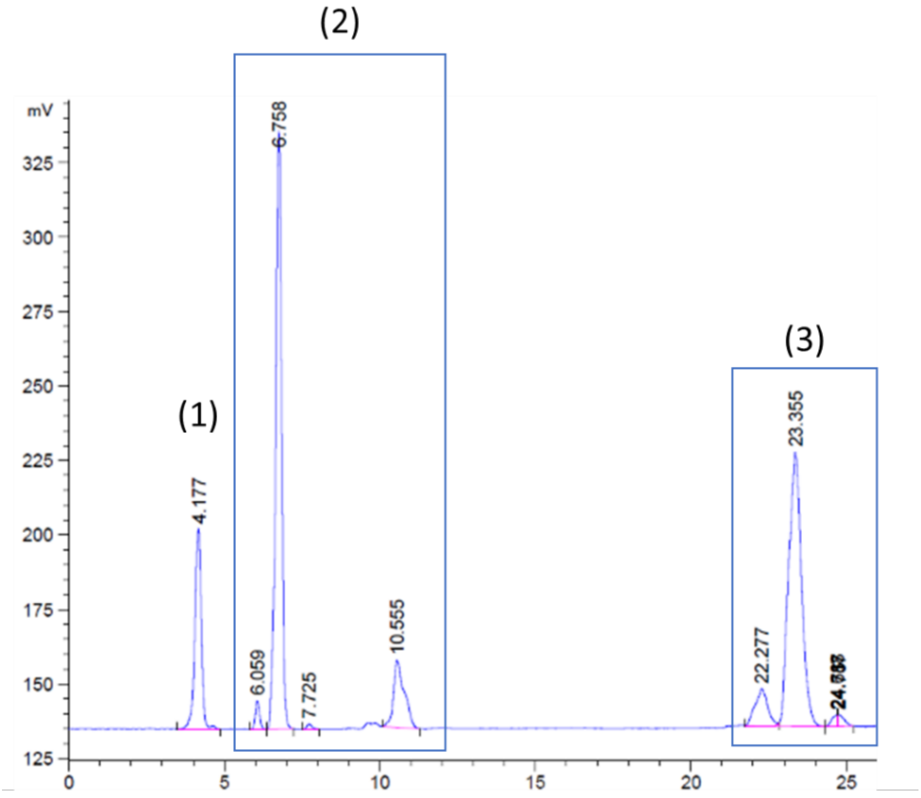


Figure 11. Graph showing the signal of each substance present in the liquid fraction. (1)-DBU; (2)-FAAE; (3)- triacylglycerol.

It can be seen from the data in Figure 11 that three signals appear during the HPLC-ELSD run, which are DBU, FAAE, and triacylglycerol. The determination of each substance is based on actual testing to determine the time to appear in the graph. In sequential, the DBU signal will appear between the 3rd and the 5th minutes. From the 5th minute onwards, it will be FAAE. Finally, the resulting signal was determined to be the triacylglycerol present in the liquid fraction. Therefore, the data of the second will be collected for calculating the FAEE as well as the biodiesel yield in Equation 1.

$$FAEE\ (\%) = \frac{FAEE_{extract}}{FAEE_{total}} \times 100 \quad (1)$$

where $FAEE_{extract}$ (g) was calculated by multiplying FAEE content (g) with a volume of liquid fraction from the *in-situ* TE process, and $FAEE_{total}$ (g) was the theoretical weight of FAEE which can be derived from the SCGs oil in 5 g of dried SCGs sample and multiplying a convert the mass of the oil, which was set at 1.0 (FAEE) determined from the stoichiometric relationship [83].

3.5. Determination of DBU recovery

Based on the obtained results in the previous steps, the conditions for performing this experiment include 40 °C, 30 minutes for reaction time, in no moisture environment with 0.8 ratios of EtOH-DBU. The experiment will be divided into 2 parts which are the 1st solution and 2nd solution. The order of experiments will be done through Figure 12 to analyze the possibility of reusing DBU in the mixture after doing in-situ TE between the first and the second batch. The results of this experiment are similarly based on the oil yield obtained after each experiment by HPLC-ELSD.

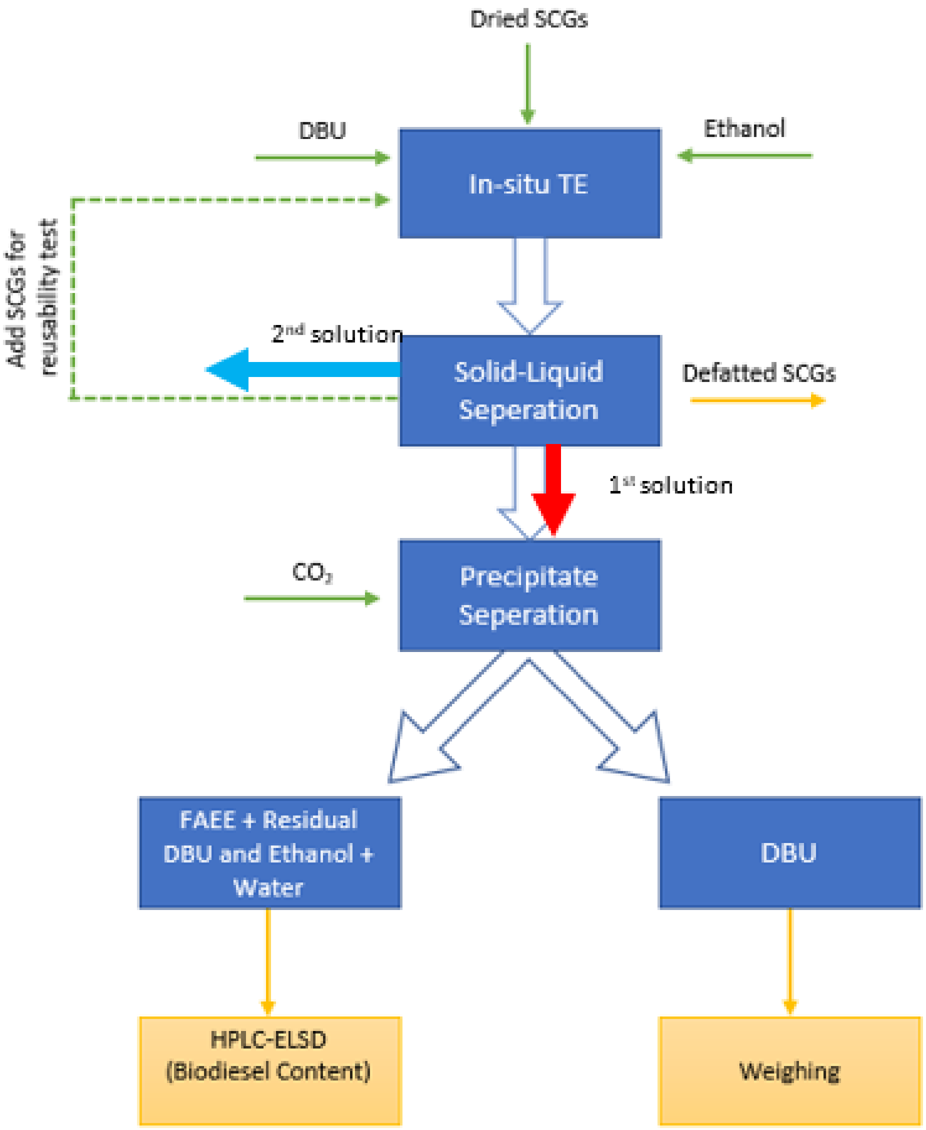


Figure 12. The experiment direction of 1st solution and 2nd solution in the process

For 1st solution, 5g SCGs were included in the experiment under the conditions mentioned above. After the completion of the in-situ TE stage, the SCGs were filtered out of the liquid fraction. The liquid fraction was divided into 2 parts, one part was analyzed by HPLC-ELSD, and the rest was further reacted with the corresponding amount of SCGs to create 2nd solution.

CHAPTER IV: Result and Discussion

4.1. Screening important factors of FAEE production using the in-situ TE process.

Taguchi orthogonal arrays method was applied to screen the important factors that governed the FAEE yield of EtOH in-situ TE using DBU, including temperature, time, DBU loading, and EtOH loading. Each experimental condition was repeated three times to enable the estimation of experimental error, which can increase the precision. Table 7 shows the average FAEE yields under different ratios of conditions (temperature: time: EtOH : DBU). From the Table 7, the FAEE ranged from 39.5 ± 5.85% (90:120:30:25) to 98.06 ± 5.21% (50:120:30:75). It is encouraging to compare the highest figure with those found by Nguyen, H.C., et al., (2019, 2020), who found that when using the same method but a different solvent (MeOH), the FAEE yields were 96.13% and 97.18%, respectively [12, 30].

Based on the TE reaction, it can be seen that FAEE production would stop when EtOH and DBU reacted completely with triacylglycerol. It can be understood that the FAEE yield decreased when the amount of extracted triacylglycerol was low. However, when the EtOH-DBU load is low, the amount of reacted triacylglycerol will be insufficient, leading to incomplete reaction and excess triacylglycerol. There are three lowest FAEE yields that were illustrated for this finding, with minuscule portions of EtOH (15 ml in experiment 5 and 30 ml in experiment 9), along with a small amount of DBU as catalyst (25 ml in experiment 5 and 25 ml in experiment 9)

Table 7. FAEE yield was obtained according to the Taguchi DOE for 5 g SCGs

No	Temperature (°C)	Time (mins)	Ethanol (Ml)	DBU (Ml)	FAEE Yield (%)
1	50	30	15	25	67.81 ± 1.72
2	50	60	30	50	62.51 ± 5.34
3	**50**	**120**	**45**	**75**	**98.06 ± 5.21**
4	70	30	30	75	85.21 ± 1.16
5	70	60	45	25	53.14 ± 4.93
6	70	120	15	50	55.19 ± 2.62
7	90	30	45	50	71.41 ± 3.98
8	90	60	15	75	72.8 ± 2.36
9	90	120	30	25	39.5 ± 3.85

In addition, as shown in Table 8, the one-way analysis of variance (ANOVA) analysis revealed that DBU had the highest and most significant value (SS=67.75259, $p<0.05$), twice as high as that of time (SS=30.99858, $p<0.05$). Also, temperature and EtOH had smaller values, with SS values were 30.99858 ($p<0.05$) and 21.12381 ($p<0.05$), respectively. It means that DBU was the most significant factor affecting the FAEE yield of the reaction, followed by the remaining factors. This finding is consistent with that of Nguyen, H.C., et al., (2020), who confirmed that DBU's effect is more significant than temperature [12].

Table 8. ANOVA analysis of Taguchi L-9

Effect	SS	df	MS	F	P
Temp (°C)	23.13095	2	11.56548	8.43934	0.002597
Time (mins)	30.99858	2	15.49929	11.30985	0.000659
Ethanol (Ml)	21.12381	2	10.56190	7.70703	0.003820
DBU (Ml)	**67.75259**	2	33.87629	**24.71956**	0.000007
Residual	24.66764	18	1.37043		

Furthermore, to optimize the experiments, the “Larger-to-Better” tool was applied to identify the optimal conditions through the average of the signal-to-noise (S/N) ratio within observations across variables, including DBU, time reaction, temperature, and EtOH, as shown in Figure 13. The figure illustrates that the maximum amount of FAEE yield would be obtained at 50 °C, 30 minutes, 45 Ml EtOH and 75 Ml DBU for 5 g SCGs.

The most striking result to emerge from the figure is that the FAEE yield decreased when temperature and reaction time increased, while it would increase when the amount of DBU and EtOH increased. There are some explanations for the result as follows. First, in accordance with the present results, previous studies (Pisarello, M.L., et al., 2018 and Nguyen, H.C., et al., 2020) have demonstrated that the more X(OH) and DBU are added, the more acceleration in the yield of biodiesel [12, 84]. Second, based on the theory of EtOH and DBU, which respective boiling points at 78.37 °C and 80 – 83 °C, would be evaporated when the operation temperature increased. Thus, higher temperature would diminish the productivity of the reaction.

In addition, FAEE yield would reach the highest value at an optimal time of 30 minutes (ETA=37.6), then it dropped when the reaction time was longer and remained unchanged from the 60th minute onwards (ETA=35.4). This finding is consistent with that of Encinar, J., et al., (2002), who used the same method on Ethyl esters and showed that the production of this type of biodiesel tended to decrease after reaching the optimal time of 10 minutes [85].

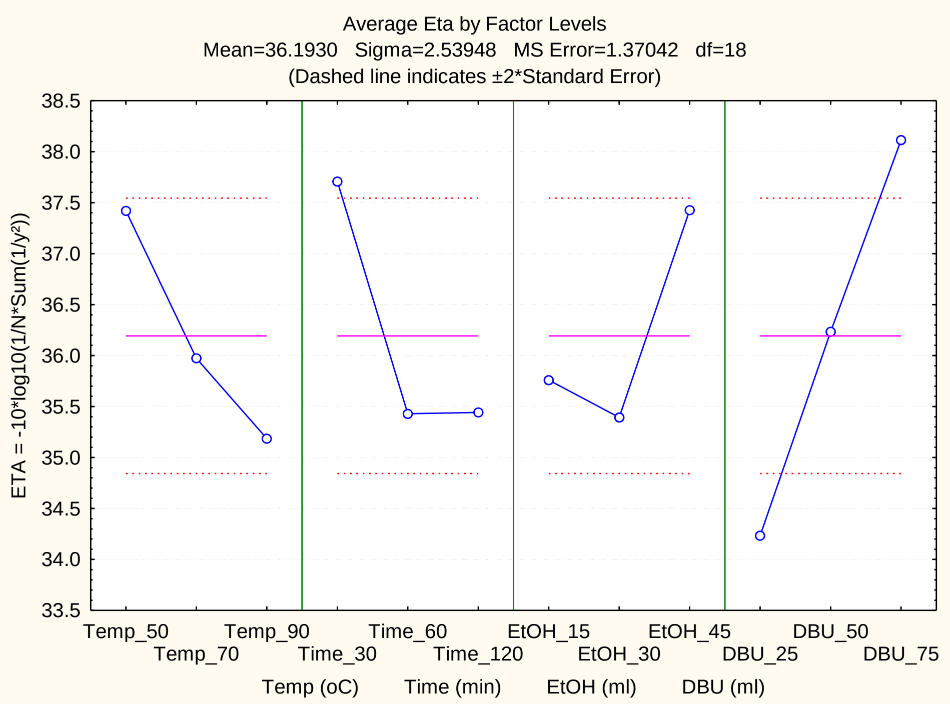


Figure 13. Average S/N ratio (ETA) using the Larger-to-Better for maximum the SCGs FAEE yield by three factor levels of temperature, time, EtOH and DBU loading.

Indeed, after conducting the experiment under the above optimal conditions, the amount of obtained FAEE was up to nearly 100%. Compared with most studies that used in-situ TE with alkaline chemicals as a catalyst (Table 7), this study showed a marked improvement when the optimum conditions for oil production reached high efficiency (100%) at low temperature (50 °C) and short reaction time (0.5 hour).

For this experiment, the results indicated that the highest oil yield in a short time and at low temperatures. Therefore, this research was considered as an economically efficient to helps limit energy use to generate heat for the reaction process. In addition, the production time to obtain the biodiesel depended not only on the in-situ TE method but also on the reaction time.

4.2. Effect of moisture content in EtOH on FAEE yield of EtOH-DBU ratio in-situ TE process.

In fact, the water content of the oil is a negative factor that reduces the yield as well as the oil quality. In addition, the water content available in SCGs is very large (over 70%) [31]. In addition, EtOH was hygroscopic, so it was difficult to obtain moisture-free ethanol. The price of 99.5% ethanol was 3-4 times higher than 95%. In order to make our developing process economically feasible, 95% ethanol should be used instead of 99.5%. The presence of water in such a process reduces both biodiesel yield and quality. Thus, the moisture factor is added to EtOH aim to investigate the effect of temperature and DBU to Ethanol ratio on mitigating the negative impact of water content.

A total of 45 experiments (15 x 3 repeated times) were conducted separately to get the experimental response of FAEE yield based on Box–Behnken design, which was shown in Table 9. The data table reported amount of biodiesel yield, which fluctuated above 80-100%, was obtained at the moisture condition of 0%. This shows the significant influence of moisture on biodiesel yield. Although biodiesel yield reduced gradually with higher moisture content, the amount of biodiesel obtained was still quite feasible for experiments 5th (72.84%) and 7th (88.6%). In these two experiments, the results showed that lower temperature could partly mitigate the moisture content in the system. Simultaneously, the increased ratio of EtOH-DBU also contributed to improving the efficiency of the reaction under the presence of water in the system. An unexpected finding was that during the experiment, some samples obtained results higher than 100%. An explanation for this result is based on Wolfs, J., et al., (2021) that DBU has a dual role in this system requiring no additional catalyst for successful acetylation of cellulose with both being part of the solubilization process and acting as a transesterification catalyst. Therefore, it is more efficient to extract oil from SCGs using DBU while the ability of n-hexane is limited to extracting seed oil [86].

Table 9. Result of Box-Behnken design

No	Temperature (°C)	Moisture content (%)	EtOH-DBU ratio	FAEE Yield (%)
1	40	0	0.6	97.95 ± 2.13
2	60	0	0.6	103.27 ± 4.37
3	40	5	0.6	47.14 ± 1.75
4	60	5	0.6	56.01 ± 4.14
5	40	2.5	0.4	72.84 ± 4.97
6	60	2.5	0.4	22.36 ± 1.77
7	40	2.5	0.8	88.6 ± 2.09
8	60	2.5	0.8	68.78 ± 3.87
9	50	0	0.4	80.87 ± 1.53
10	50	5	0.4	40.57 ± 3.78
11	50	0	0.8	114.07 ± 2.4
12	50	5	0.8	43.94 ± 3.42
13	50	2.5	0.6	47.02 ± 3.45

14	50	2.5	0.6	47.5 ± 0.47
15	50	2.5	0.6	46.43 ± 1.6

After that, three independent variables were used for multiple regression analysis, including temperature, moisture content, and EtOH-to-DBU ratio. In other words, observed FAEE yields from experiments were put into the Statistica software to run ANOVA and generate a multiple regression polynomial. As a result, the functional relationship equation between FAEE yield and reaction factors is shown below:

$$\text{FAEE Yield (\%)} = 509.975 - 14.309X_1 + 0.112X_1^2 - 17.321X_2 + 2.859X_2^2$$
$$-238.519X_3 + 120.771X_3^2 + 0.035X_1X_2 + 3.859X_1X_3 - 15.27X_2X_3 \quad (2)$$

Where X_1: Temperature value (°C)

X_2: moisture content value (%)

X_3: EtOH-DBU ratio value.

In addition, from Equation 2, the predicted and actual FAEE yield (%) were scatter plotted in Figure 14. It can be seen that the actual value and predicted value do not change muc. There is a high correlation between them, with an R-squared value of 0.9022 ($p<0.05$).

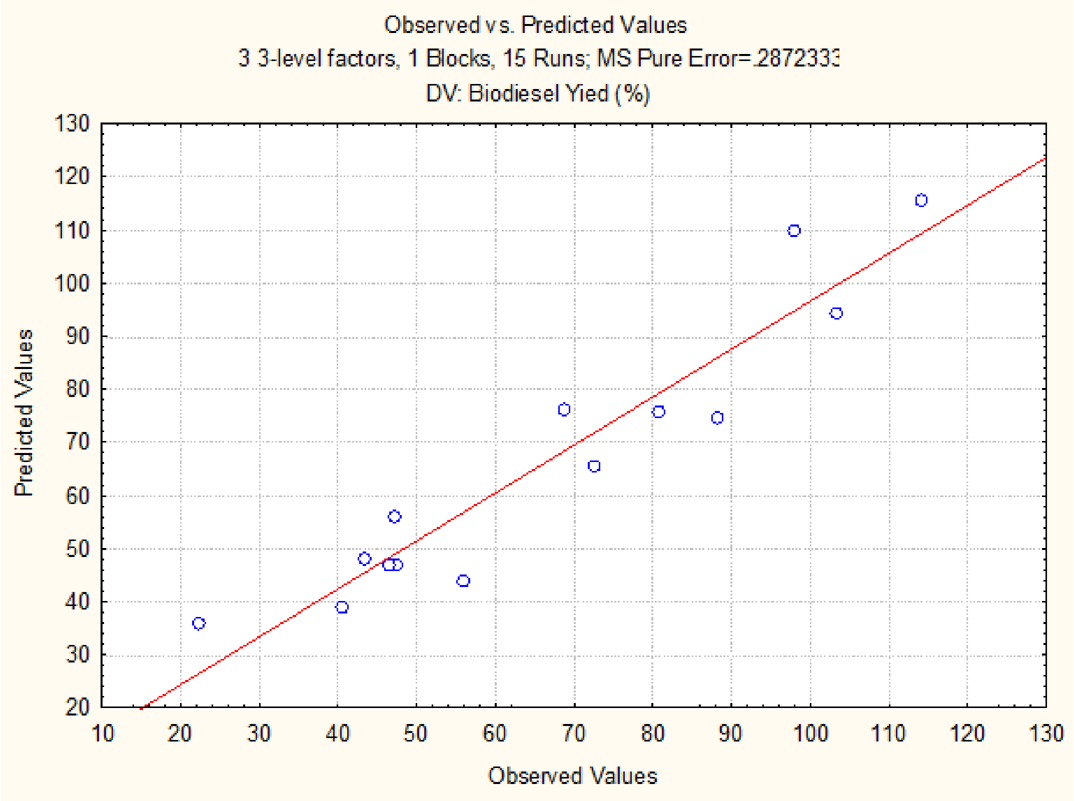


Figure 14. Correlation graph between actual and predicted FAEE yield

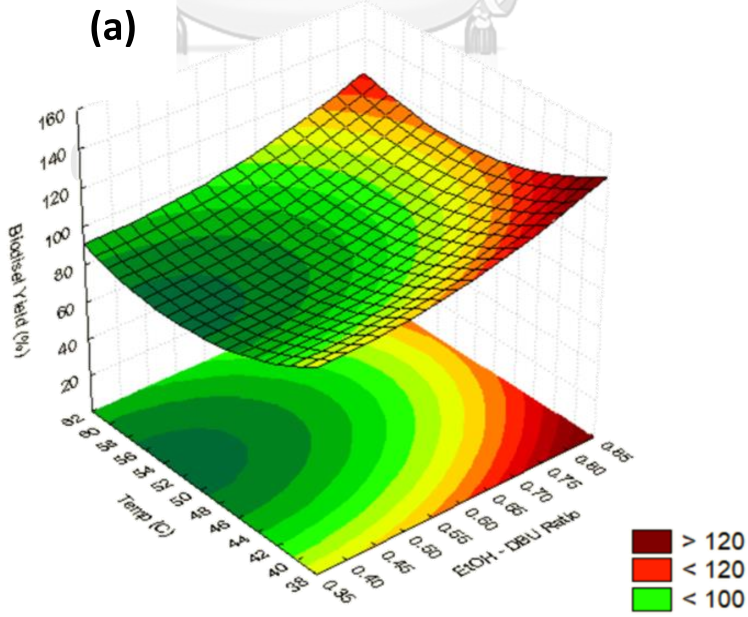
Furthermore, the ANOVA analysis supports the above findings, as shown in Table 10. From the table, it is evident that the most significant factor reported was moisture content with SS=6651.35. Second, as can be seen, the correlation between temperature and EtOH-DBU ratio (SS=1283.48) was lower than that of moisture content. With a positive coefficient, it can be concluded that the correlation between these two factors is proportional. It is possible to hypothesize that these conditions are likely to occur in the ideal FAEE yield also will be obtain at high temperature and high EtOH-DBU ratio, which can be seen as lower the amount of DBU usage.

Table 10. Anova analysis of Box-Behnken Design

Factor	**ANOVA; Var.:FAEE Yied (%); R-sqr=.9022; Adj:.72617 (box-behken) 3 3-level factors, 1 Blocks, 15 Runs; MS Pure Error=.2872333 DV: FAEE Yied (%)**				
	SS	df	MS	F	p
(1)Temp © L+Q	849.30	2	424.649	1478.41	0.000676
(2)Moisture Content (%) L+Q	6651.35	2	3325.675	11578.30	0.000086
(3)EtOH-DBU Ratio L+Q	1283.48	2	641.742	2234.22	0.000447
1*2	2.98	1	2.976	10.36	0.084477
1*3	238.24	1	238.239	829.43	0.001203
2*3	233.17	1	233.173	811.79	0.001230
Lack of fit	985.41	3	328.470	1143.56	0.000874
Pure Error	0.57	2	0.287		
Total SS	10081.91	14			

Following the above results, Figure 15 shows the observed FAEE yield in three distinct moisture levels in SCGs, comprising 0%, 2.5%, and 5%. It can be illustrated that the highest FAEE yield values were obtained at the EtOH:

DBU ratio of 0.8 at all moisture levels, compared to lower ratios. Furthermore, the less moisture inside the oil, the higher the FAEE yield, indicating the negative impact of moisture on the reaction. In fact, the FAEE productions ranged from 100% at the temperature of 60 °C to around 120% at the temperature of 40 °C for the level of 0% (Figure 15.a), followed by 80% (60 °C) to around 90% (40 °C) for 2.5% (Figure 15.b), and from around 70% (60 °C) to almost 80% (40 °C) for 5% (Figure 15.c). This finding corresponds with Nguyen, H.C., et al., (2020) and Park, J., et al., (2016), who reported there was a negative effect of moisture on the reaction because it caused reversed reaction where biodiesel was hydrolyzed back to FFA and EtOH [12, 68].



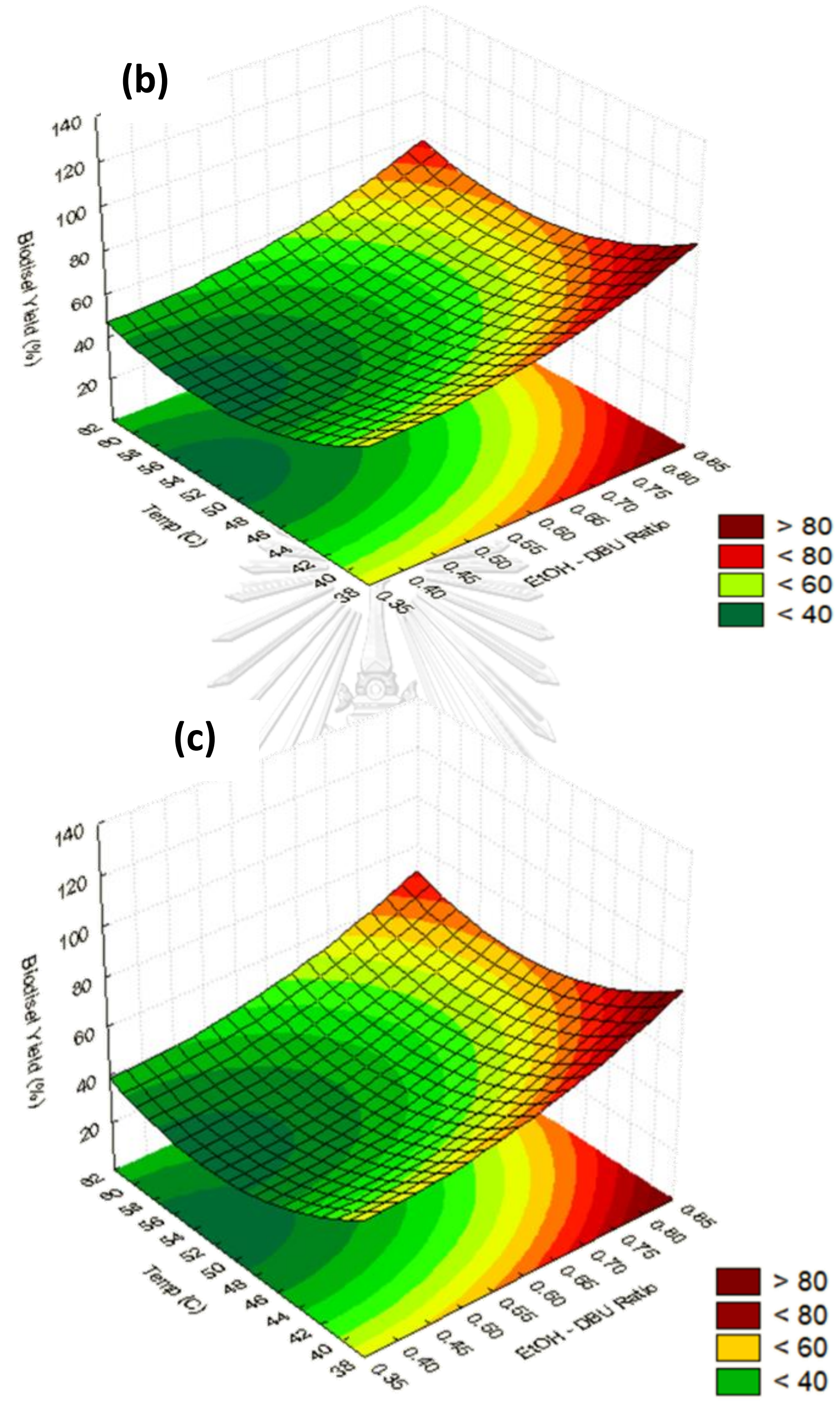


Figure 15. The FAEE yield obtained in moisture content (a): 0%; (b): 2.5%; (c): 5%

Regarding the reaction temperature factor, the most interesting finding was that the FAEE yields were high at the low temperature of 40 °C with 0.8 EtOH-DBU ratio used (Figure 16). This result supports the earlier research's finding that with the significant effect of DBU acting as an alkaline catalyst in the reaction, there was no need to raise reaction temperature in the condition of 0% water content. However, the efficiency decreased slightly at around 50 °C and then increased again.

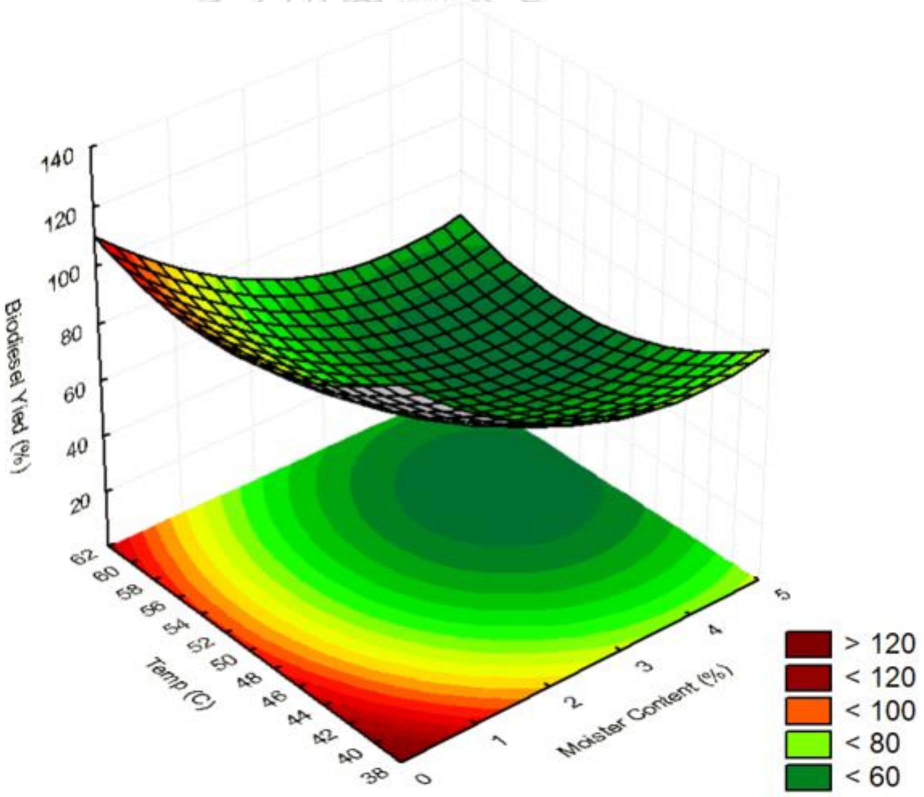


Figure 16. The FAEE yield obtained in ratio of EtOH-DBU 0.8

With the same EtOH-DBU (0.8) loading ratio as shown in Figure 16, the relationship between the operating temperature and moisture content was evaluated. The lowest yields can be determined at 5% water content and around 54 °C, which was approximately 60%. However, several studies such as Al-Hamamre, Z., et al., (2012), Tuntiwiwattanapun, N., et al., (2017) Nguyen, H.C., et al., (2020) indicated that the ideal biodiesel production could be ranged from 80-100%. The collected yield was above 80% when the moisture content in the system reached 2.5%, indicating the intriguing result that lower temperature can reduce the moisture content that influenced the system [12, 31, 54].

In conclusion, the optimal conditions for the in-situ TE experiment to extract FAEE from SCGs were determined at 40 °C operation temperature, 0% moisture content, and 0.8 EtOH-DBU, which FAEE yield reached around 100%.

These experimental results highlight the serious influence of the process water content on the duration of the reaction. The removal of water from the very beginning is necessary to improve production efficiency. However, water removal is resource intensive including time and energy for heating and freezing for storage. Nevertheless, the effect of water content can be minimized when large amounts of EtOH and DBU are used. Combined with low-temperature factors and short reaction times, this study demonstrates the potential for oil production for industrial applications.

4.3. Reusing DBU

One unanticipated finding was that there was no way to recognize the amount of FAEE separately after the in-situ TE experiment due to the overlayed of substances' signals, as shown in Figure 17. It is mainly because there was still a large amount of residue DBU inside the solution with a significantly high signal. This explanation was supported by Nguyen, H.C., et al., (2020), who reported that there is an amount of DBU that can be recovered up to at least ten times without a significant reduction [12]. Also, Ostonen, A., et al., (2014) indicated that the residual triacylglycerol and the temperature generated when aeration of CO_2 in DBU also partly reduced the efficiency of precipitation formation of the reaction [87].

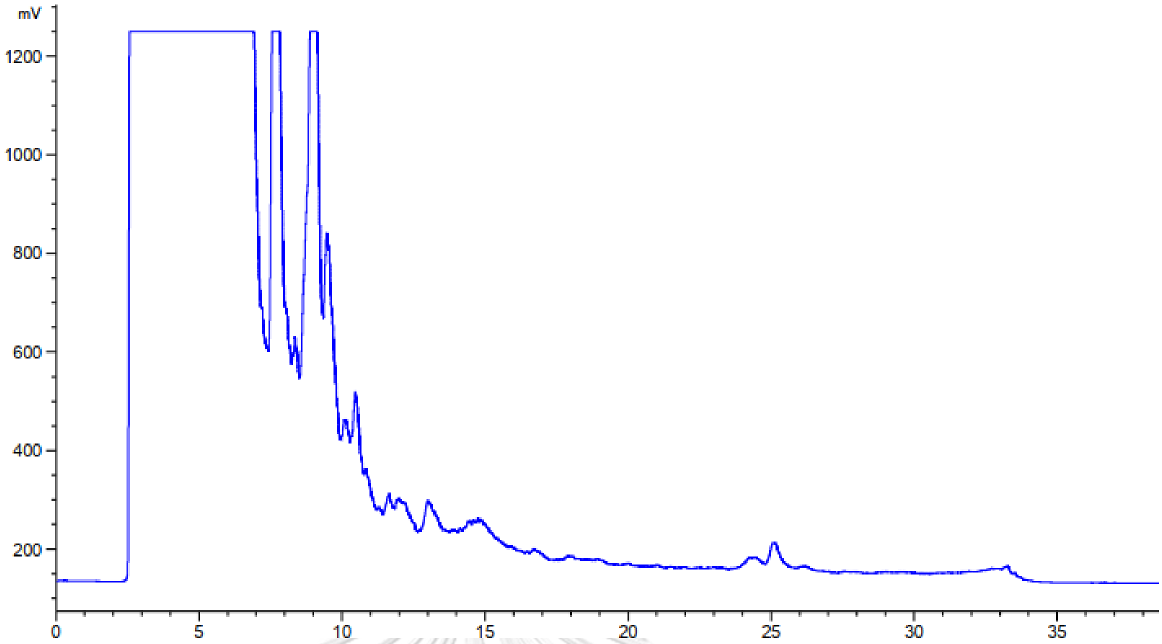


Figure 17. Signals of substances recorded after in-situ TE experiment

Therefore, the following purpose of this study was to identify the residue of DBU and its possibility of reuse. After the in-situ TE, the obtained solution was divided into two distinct batches. In contrast, the first batch was used to identify the portion of residue DBU and FAEE yield in the solution by HPLC-ELSD. The other one was for further reaction with a suitable amount of SCGs to find the efficiency of FAEE extraction with reused DBU. **Table 11** shows the amount of residue DBU and FAEE yield in each solution.

Table 11. Comparison of FAEE yield and DBU in 2 batches.

1st solution

Concentration of DBU in EtOH-DBU solution (%)	44.95 **± 1.77**
FAEE Yield (%)	**99.27 ± 0.97**
2nd solution	
Concentration of DBU in EtOH-DBU solution (%)	25.75 **± 0.48**
FAEE Yield (%) -	**62.22 ± 1.54**

It is evident that there was a similarity in compositions between the two different solutions divided after the in-situ TE reaction, including EtOH-DBU. From Table 11, the FAEE yield in the second solution, which used post-EtOH-DBU, was reduced to 62.22%, lower than that with fresh EtOH-DBU solution of around 40%. However, the concentration of DBU in EtOH-DBU solution was reduced from 44.95% to 25.75%. DBU absorption in the SCGs to extract the oil is one explanation for DBU loss. Additionally, some were partially released during the reaction. For the FAEE yield, based on the TE (Figure 4), it can be seen that lack of DBU, as well as EtOH, might have caused a reversed reaction, which remade triacylglycerol and produced less FAEE.

Overall, this experiment indicates that the reuse efficiency of this study is not high. One solution to this problem is to change the method of reuse by separating each individual substance in the mixture to create a new medium for the next batch of experiments.

CHAPTER V: Conclusion and Recommendation

In biodiesel production, feedstock selection strongly affects the commercialization of the biodiesel production process because the feedstock accounts for 70–80% of biodiesel cost (Tuntiwiwattanapun et al., 2017). Due to this concern, the use of non-edible/waste feedstocks, especially SCGs for biodiesel production, has gained much interest. Consequently, the amount of this waste is sufficient for large-scale production.

After the first experiment of screening the important factors, the amount of FAEE obtained was up to nearly 98.06% using 5 g SCGs at 50 °C temperature reaction, 30 minutes with 45 mL EtOH and 75 mL DBU. In addition, DBU loading was the most significant factor for FAEE production using the in-situ TE reaction, followed by reaction time, temperature, and EtOH loading. Based on that finding, it is possible to comprehend that a low-temperature value (40 °C) generated high FAEE yields. While the FAEE yield drops as temperature rises due to a phenomenon of the evaporation of EtOH and DBU. In addition, the available moisture in environment could induce the reverse reaction, which hydrolyzes the biodiesel back to free fatty acid and EtOH, thus lowering the biodiesel yield.

However, the FAEE yields were still dependent on the moisture available in the process. Therefore, the second experiment to assess the impact of moisture was implemented and showed that the optimal conditions to obtain the highest FAEE yields of 99.93% were 40 °C, 30 minutes, 0.8 EtOH-DBU ratio, and 0% moisture content during the reaction. Accordingly, the ideal range of FAEE yield (>80%), the yield could be obtained in the conditions of 2.5 % moisture content, which was still maintained at that low temperature and high ratio of EtOH-to-DBU. Therefore, lower temperature can mitigate the negative effect of moisture content in the system.

Furthermore, with the FAEE liquid obtained after the in-situ TE experiment, another experiment to reuse DBU was done and showed that the average efficiency of FAEE using the post-EtOH-DBU was reduced to 60% due to the lack of DBU, which caused a reversed reaction of transesterification. A hypothesis is that the liquid mixture during the reaction can affect the amount of oil produced in the next batch. To improve this problem, another study on the separation of EtOH and DBU in the liquid fraction needs to be conducted in the future. The purpose of this research is to collect the raw DBU and EtOH and reuse them for the next sample as new input.

Although the study results show that it is economically viable when the temperature and time required for each batch are feasible. This method is not yet applied on an industrial scale for the extended production of oil production from SCGs. The reduction in oil yield in the liquid fraction reusability process is an important factor in scaling up the process. In addition, an analysis of oil quality has not been performed yet, so further studies are needed to ensure oil quality which meet the EU standard for biodiesel after production.

APPENDIXES

Appendix A: Photos of the experiments

Figure 18. SCGs was contained in Erlenmeyer flask

Figure 19. The in-situ TE process was performed in a water bath

Figure 20. The process of filtering SCGs with a funnel

Figure 21.DBU conversion by CO_2 aeration process

Figure 22. Separation DBU and liquid fraction after centrifugation

Appendix B: Design of experiments

1. Screening the important parameters of ethanolic-DBU in-situ TE

Throughout the literature review and available conditions, the data for each factor were assigned and presented in the experimental design table. It is separated between list of code of each factor (Table 13) and the experiment runs (Table 14) based on the Taguchi orthogonal array.

Table 12. Code conventions of factors by Taguchi L-9 for 5 g SCGs

Factors	Symbols	Levels		
		1	2	3
Reaction Temperature (°C)	A_1	50	70	90
Reaction Time (minutes)	A_2	30	60	120
Ethanol (mL)	A_3	15	30	45
DBU (mL)	A_4	25	50	75

Table 13. Taguchi orthogonal for running in-situ TE experiment

Variables

Experiment no.	A_1	A_2	A_3	A_4
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

2. Mitigating the effect of water content in EtOH on FAEE yield

Presence of water in such a process reduces both biodiesel yield and quality. Thus, the main objective of this part was to investigate the effect of temperature and EtOH to DBU ratio on mitigating the negative impact of water content. The application of Box-Behnken in the following factors aims to minimize the influence of water content in EtOH on biodiesel yield.

Table 14. Code conventions of factors by the Box-Behnken design

Factors	Symbols	Levels		
		-1	0	1
Temperature (°C)	X_1	40	50	60
Moisture content (%)	X_2	0.0	2.5	5.0
Ethanol-DBU Ratio	X_3	0.4	0.6	0.8

Table 15. Box–Behnken design for mitigating the moisture content in the biodiesel production process

Experiment no.	Variable		
	X_1	X_2	X_3
1	-1	-1	0
2	1	-1	0
3	-1	1	0

4	1	1	0
5	-1	0	-1
6	1	0	-1
7	-1	0	1
8	1	0	1
9	0	-1	-1
10	0	1	-1
11	0	-1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

The code conversion quantities of 3 reaction factors, such as the temperature value (X_1), moisture content value (X_2), and EtOH-DBU ratio value (X_3) are listed in (Table 15). To mitigate the moisture content in the biodiesel production process, an experiment design (Table 16) was carried out. The correlation between the reaction parameters and the calculated biodiesel yield was then developed using the second quadratic model following the Equation:

$$\text{Biodiesel Yield (\%)} = \beta_0 + \beta_1 X_1 + \beta_{1a} X_1^2 + \beta_2 X_2 + \beta_{2a} X_2^2 + \beta_3 X_3 + \beta_{3a} X_3^2 + \beta_{1-2} X_1 X_2 + \beta_{1-3} X_1 X_3 + \beta_{2-3} X_2 X_3$$

Where: β_0, β_1, β_2, … are regression coefficient through analysis in the Statistica software.

X_1: Temperature value (°C)

X_2: moisture content value (%)

X_3: EtOH-DBU ratio value

Appendix C: Result of the Taguchi design

No	Temperature (˚C)	Reaction time (minutes)	Ethanol (mL)	DBU (mL)	FAEE Yield (%)
1.1	50	30	15	25	66.14
1.2	50	30	15	25	67.74
1.3	50	30	15	25	69.52
2.1	50	60	30	50	58.45
2.2	50	60	30	50	60.5
2.3	50	60	30	50	68.55
3.1	50	120	45	75	92.06
3.2	50	120	45	75	100.8
3.3	50	120	45	75	101.32
4.1	70	30	30	75	85.17
4.2	70	30	30	75	84.07
4.3	70	30	30	75	86.37
5.1	70	60	45	25	56.4
5.2	70	60	45	25	55.54
5.3	70	60	45	25	47.47
6.1	70	120	15	50	52.24
6.2	70	120	15	50	57.23
6.3	70	120	15	50	56.1
7.1	90	30	45	50	72.63

7.2	90	30	45	50	71.61
7.3	90	30	45	50	70.11
8.1	90	60	15	75	71.13
8.2	90	60	15	75	71.81
8.3	90	60	15	75	75.25
9.1	90	120	30	25	43.18
9.2	90	120	30	25	39.04
9.3	90	120	30	25	36.29

Appendix D: Result of the Box-behnken design

No	Temperature (°C)	Moisture content (%)	EtOH-DBU ratio	FAEE Yield (%)
1.1	40	0	0.6	95.89
1.2	40	0	0.6	97.8
1.3	40	0	0.6	100.15
2.1	60	0	0.6	98.38
2.2	60	0	0.6	104.64
2.3	60	0	0.6	106.79
3.1	40	5	0.6	48.98
3.2	40	5	0.6	45.49
3.3	40	5	0.6	46.95
4.1	60	5	0.6	59.68
4.2	60	5	0.6	51.52
4.3	60	5	0.6	56.82
5.1	40	2.5	0.4	67.74
5.2	40	2.5	0.4	77.67
5.3	40	2.5	0.4	73.12
6.1	60	2.5	0.4	24.19
6.2	60	2.5	0.4	20.64
6.3	60	2.5	0.4	22.26
7.1	40	2.5	0.8	90.23
7.2	40	2.5	0.8	86.24

7.3	40	2.5	0.8	89.32
8.1	60	2.5	0.8	72.08
8.2	60	2.5	0.8	64.52
8.3	60	2.5	0.8	69.74
9.1	50	0	0.4	79.12
9.2	50	0	0.4	81.55
9.3	50	0	0.4	81.93
10.1	50	5	0.4	38.65
10.2	50	5	0.4	44.92
10.3	50	5	0.4	38.13
11.1	50	0	0.8	116.33
11.2	50	0	0.8	114.34
11.3	50	0	0.8	111.55
12.1	50	5	0.8	40.99
12.2	50	5	0.8	43.13
12.3	50	5	0.8	47.7
13.1	50	2.5	0.6	45.14
13.2	50	2.5	0.6	44.92
13.3	50	2.5	0.6	51.01
14.1	50	2.5	0.6	47.96
14.2	50	2.5	0.6	47.02
14.3	50	2.5	0.6	47.53
15.1	50	2.5	0.6	48.22

15.2	50	2.5	0.6	45.95
15.3	50	2.5	0.6	45.13

Appendix E: Result of the reusing DBU experiments

Column (C)	**Repeated experiments**	**1^{st}**	**2^{nd}**	**3^{rd}**
	Temperature (°C) : moisture content (%): EtOH-DBU ratio	(40 : 0 : 0.8)	(40 : 0 : 0.8)	(40 : 0 : 0.8)
I	**1^{st} solution**			
1	Removed DBU (g)	4.9356	5.0244	4.8673
2	Removed DBU (mL) (C_2*D_{DBU})	4.8483	4.9356	4.7812
3	DBU remaining (mL)	1.7837	1.6417	1.7712
4	Total DBU recovered (mL) (C_2+C_3)	6.6320	6.5773	6.5524
5	**FAEE Yield (%)**	**101.46**	**98.18**	**99.59**
II	**2^{nd} solution**			
6	Removed DBU (g)	22.53	22.08	21.48
7	Removed DBU (mL) (C_2*D_{DBU})	22,1333	21.6972	21.1095
8	DBU remaining (mL)	2.4453	2.3632	2.5951
9	Total DBU recovered (mL) (C_7+C_8)	24.5786	24.0605	23.7046
10	**FAEE Yield (%)**	**62.2**	**60.7**	**63.77**
D_{DBU}=1.018 g/mL				

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America/Caribbean), Asia Pacific/South Asia), Emily Melton (Special, L.F.R.M. Advisor to the Secretary General), Africa), Einari Kisel (Regional, E. Manager, Andrea Arrobo (Regional Manager, Latin America and the Caribbean), Angela, M.Z.D. Wilkinson (Senior Director), Sophie Rose (Senior Manager), Talita Covre, P.B.M. (Manager), Maria Vinograd (Manager), Jorge Romeo (Manager) Joshua, and M.R.I. Oyinlola (Coordinator), Alexandre Tourbah (Intern). , *World Energy Issues Monitor*. 2019, The World Energy Council 2019.

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VITA

NAME	Huan Luong Trien
DATE OF BIRTH	23 July 1995
PLACE OF BIRTH	Ho Chi Minh city, Vietnam
HOME ADDRESS	171 Pho Co Dieu Street, Ward 6 District 11, 72616, Ho Chi Minh city, Vietnam