

Sustainable biodiesel production from microalgae cultivated in wastewater treatment ponds

A Thesis

*Submitted in fulfilment of the requirements for the award of
the degree of*

DOCTOR OF PHILOSOPHY
In
ENVIRONMENTAL ENGINEERING

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Candidate's declaration

I hereby certify that the work which is being presented in the thesis entitled “**Sustainable biodiesel production from microalgae cultivated in wastewater treatment ponds**” in fulfilment of the requirements for the award of the degree of Doctor of Philosophy and submitted to the Department of Energy and Environment, Thapar Institute of Engineering and Technology, Patiala, India is an authentic record of my work carried out during a period from July 2017 to December 2022 under the supervision of Dr. Amit Dhir, Professor, Department of Energy and Environment, TIET, Patiala, India and Dr. Banu Örmeci, Professor, Jarislowsky Chair in Water & Global Health, Department of Civil & Environmental Engineering, Carleton University, Ottawa, Canada.

The matter presented in the thesis has not been submitted in parts or full to any other institute for the award of any other degree in any other university or institute.

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Certificate

This is to certify that this thesis titled “**Sustainable biodiesel production from microalgae cultivated in wastewater treatment ponds**” being submitted by Prabhdeep Kaur Brar, in the fulfilment for the award of Ph.D. in the Department of Energy and Environment, Thapar Institute of Engineering and Technology (Deemed to be University), Patiala, India, for the award of the degree of ‘ **Doctor of Philosophy** ’ is a record of bonafide research work carried out by her under our supervision and has fulfilled all the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard.

The results incorporated in this thesis have not been submitted to any other University or Institute for the award of any degree or diploma.



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Abstract

Microalgae-based biodiesel production has emerged as a promising avenue for renewable and sustainable energy sources. This thesis presents a comprehensive analysis of the technical feasibility and sustainability of microalgae-derived biodiesel production, focusing on cultivating indigenous mixed species in wastewater collected from rural oxidation ponds. Furthermore, a bi-functional KOH/rice bran-derived activated heterogeneous carbon catalyst was employed for the in-situ transesterification process and process parameters were utilized using statistical regression analysis by central composite design (CCD).

The study includes microalgae cultivation sourced from a rural pond, providing a lab-scale demonstration of logistic growth patterns over two weeks. This approach showcases the potential to utilize wastewater as a growth medium, eliminating the need for additional nutrient supplementation and reducing freshwater consumption. Chlorophyll-A in wastewater confirms the presence of microalgae, setting the stage for a sustainable biomass source.

A pivotal aspect of the research involves identifying and characterizing indigenous microalgae species. Morphological observations under the microscope provide valuable visual clues, while molecular techniques, including 16S rRNA gene sequencing and BLASTN searches, confirm the genetic identity of the strains as *Desertifilum salkalinema* and *Chlorella vulgaris*.

Developing a novel bi-functional catalyst consisting of KOH impregnated on rice bran-derived activated carbon support material represents a significant milestone. Characterized through scanning electron microscopy (SEM) and X-ray diffraction (XRD), this catalyst enhanced the efficiency of in situ transesterification by effectively filling porous structures.

Exploration of solvent combinations for direct transesterification with synthesized catalyst was carried out. In-situ transesterification with H₂SO₄ was carried out to find the most suitable

combination. The successful identification of methanol and hexane as the optimal solvent duo maximized the efficiency of the direct transesterification process with the synthesized catalyst.

Optimization of process parameters was achieved through statistical regression analysis based on the central composite design (CCD). The model generated from this analysis predicts a biodiesel yield of 81.8%, with a high coefficient of determination ($R^2 = 0.94$), ensuring the model's reliability. The optimum conditions include a 10:1 solvent and co-solvent to biomass molar ratio, 5 wt% catalyst loading, a temperature of 70°C, and a 1.5-hour reaction time. In practice, these conditions yielded an observed maximum biodiesel yield of 80.9%, with a prediction error below 0.9%. Furthermore, characterization of the obtained biodiesel through gas chromatography–mass spectrometry (GC–MS) analysis revealed a composition containing 53.82% unsaturated fatty acids, making it suitable for extreme weather conditions. Additionally, all biodiesel properties met American Society for Testing and Materials (ASTM) standards, ensuring compliance with industry-scale quality requirements.

In conclusion, this research advances the field of microalgae-based biodiesel production by optimizing the production process, utilizing indigenous mixed microalgae species, and developing an innovative bi-functional catalyst. The findings underscore microalgae-derived biodiesel's technical feasibility and sustainability, positioning it as a cost-effective, green, and renewable energy source with significant potential in the renewable energy sector.

Abbreviations

List of Abbreviations

AC Activated Carbon.....	47
ANOVA Analysis of Variance	53
APHA American Public Health Association	44
ASTM American Society for Testing Materials.....	54
BG-11 Blue-Green	43
BOD Biochemical Oxygen Demand.....	28
CCD Central Composite Design.....	51
COD Chemical Oxygen Demand.....	43
DAF Dissolved Air Flotation.....	33
DIAF Dispersed Air Flotation	33
DO Dissolved Oxygen	43
EDTA Ethylene Diamine Tetraacetic Acid	43
EPS Extracellular Polimeric Substances	33
FAME Fatty Acid Methyl Ester.....	19
FFA Free Fatty Acid	36
GC -MS Gas Chromatography- Mass Spectrometer	53
GHG Green House Gases	31
LED Light Emitting Diode	44
ODF Ozone Dispersed Flotation.....	33
RPM Rotation Per Minute	49
RSM Response Surface Methology	51
SEM Scanning Electron Microscopy.....	48
XRD X-Ray Diffraction.....	48

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Chapter 1

Introduction

Fossil fuels have been the backbone of the global energy industry for decades, but their harmful environmental impact is increasingly evident. As the world's population grows and industrialization accelerates, the energy demand is mounting alarmingly without considering its non-renewability and detrimental ecological effects. Therefore, it is imperative to consider alternative environment-friendly renewable fuels to address the global energy crisis. Biodiesel, biohydrogen, and biogas have emerged as substitutes for non-renewable fuels. The present study explores the potential of microalgae for biodiesel production. This chapter will discuss the background and current state of the research study, followed by stating the research problem and aim of the present study.

1.1 Background of research study

Over the past few decades, rising concerns over fossil fuel depletion prompted the search for alternative fuels (Sooch et al., 2016; Uyumaz et al., 2020). New-generation fuels aim to meet the global energy demand and other criteria such as sustainability, gas emissions, and ethical safety of raw materials used during fuel extraction. Some of the investigated alternative fuels/biofuels are various oils in their pure form or as blends with conventional fuels, ethanol, methane, biodiesel, hydrogen, and other synthetic fuels (Stančin et al., 2020). Many researchers have investigated these alternative fuels, and a detailed review has been done, as summarized in **Table 1. 1**.

All the alternative fuels are divided into different generation groups based on utilizing other raw materials and their processing. **Figure 1. 1** briefly describes all three different generations of biofuels and their feedstocks biofuels. Under the umbrella term of conventional biofuels, including vegetable oils, biodiesel from edible crops, and bioethanol.

They have been associated with sustainability problems because of competition with the global food market and negative environmental impact (Naik et al., 2014). Second-generation biofuels depend on non-edible or existing raw materials for generating fuel (Amin et al., 2016; Hirani et al., 2018; Solmaz and Halis, 2017) Biodiesel from used cooking oils or animal fats, ethanol from lignocellulose, and methane from manure, straw, or residual waste (Jamil et al., 2023). Moreover, synthetic fuels come under the second-generation biofuels that encompass all the fuels produced using the Fischer-Tropsch process (synthetic gas reaction with carbonaceous raw material)(de Blasio, 2019a; Mahmoudi et al., 2017) fuels are also called as Fisher-Tropsch fuels. Although this category of biofuels has overcome the hiccups of the 1st generation of biofuels, it still needs to catch up in commercialization due to the limited stock of feedstock and high-cost intensive methodologies for fuel processing (Chowdhury and Loganathan, 2019).

Table 1. 1 Recent review articles on various alternative fuels

Types of alternative fuels	Content	References
Vegetable oils	<ul style="list-style-type: none"> • Intensive review on using straight vegetable oils in an engine globally, specifically in India. 	(Misra and Murthy, 2010)
	<ul style="list-style-type: none"> • Global overview of utilizing natural vegetable oil in IC engine and review of the limitations and solutions. 	(Mondal et al., 2008)
	<ul style="list-style-type: none"> • Review of the production and characterization of vegetable oil for IC engines. 	(Saikia et al., 2019)
Ethanol	<ul style="list-style-type: none"> • Review of ethanol production and its utilization as a biofuel 	(Mendiburu et al., 2022)
	<ul style="list-style-type: none"> • Highlight recent advances in the production of lignocellulosic ethanol, focusing on sustainability. 	(Viikari et al., 2012)
	<ul style="list-style-type: none"> • Studied the sustainability of ethanol and biodiesel in developing countries 	(Canabarro et al., 2023)

Methane	<ul style="list-style-type: none"> • Review of pretreatment methods for the production of highly efficient bio-methanol. (Yadav et al., 2022) • Review on yield of bio-methane utilizing different agricultural substrates (Garcia et al., 2019)
Biodiesel	<ul style="list-style-type: none"> • Historical review of algae biodiesel to give a global view from past to future research. (Gao et al., 2022) • Review of utilizing algae for wastewater treatment along with biodiesel production (Ghaffar et al., 2023)
Hydrogen	<ul style="list-style-type: none"> • Review presenting the advantages and disadvantages of hydrogen as a fuel source. (Athayde Neto et al., 2022) • Review current advancements in hydrogen biofuel production, storage, and transportation. (Taipabu et al., 2022)
Other Synthetic fuels / Fisher-Tropsch fuels	<ul style="list-style-type: none"> • Review recent innovations in the fisher-tropsch process. (Konarova et al., 2022) • Review of recent technologies for conversion of power to liquid routes for producing methanol, dimethyl ether, and fisher-tropsch fuels. (Dieterich et al., 2020) • Review on biomass-based fisher-tropsch fuels (van den Oever et al., 2022)

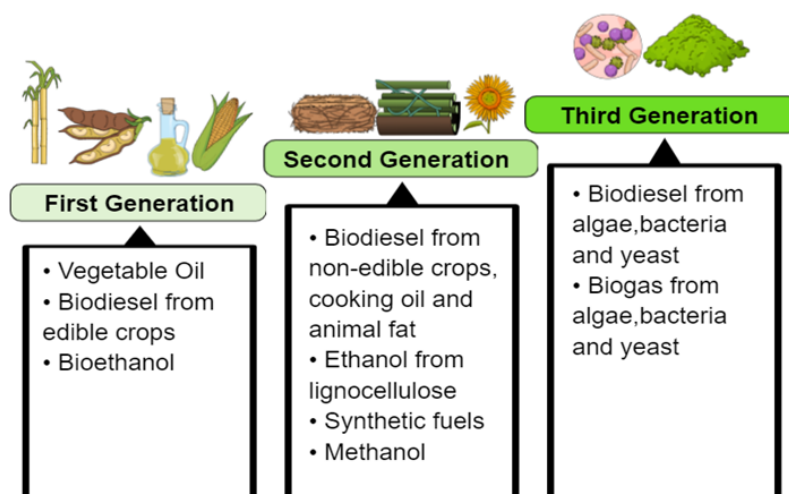


Figure 1. 1 Classification of three generations of biofuels

The third group incorporates the biofuels obtained from biological resources. Biodiesel and biogas from algae, bacteria, and yeast come under this category. This generation has become the most favourable of all the biofuel generations as it counteracts all the disadvantages of previous generation biofuels (Inamuddin Abdullahm, 2020). Algae, among the other biological feedstocks, have the potential to produce alternative fuels while avoiding the need for fresh water and large arable land. Also, algae, especially microalgae, have the advantage of high lipid content, ease of cultivation, and rapid growth rate over other feedstocks (Singh et al., 2020). Furthermore, in terms of practical implementation and worldwide commercialization, only biodiesel and bioethanol have been utilized as transportation fuels and other industrial applications using blends with gasoline/diesel. Bio-ethanol production is not considered a sustainable process where more than 50% of feedstock carbon is lost during conversion. On the other hand, 90% of algal lipids' carbon is conserved during conversion into algal biodiesel. Therefore, it is apparent that the production of micro-algal biodiesel is preferred over bio-ethanol in terms of sustainability and energy conservation from feedstock (Young et al., 2022). Still, the commercialization of micro-algal biodiesel has not yet been recognized because of energy-intensive cultivation, pre-treatment, and conversion processes. However,

various researchers in the past have emerged with different cultivation methods, advanced technology, and affordable processing methods to make it efficient and economical to proceed further in the micro-algal biodiesel industry (Amani et al., 2022; Hossain et al., 2020; Mofijur et al., 2019). Considering all the past studies for viable alternative fuels, micro-algae biodiesel on a broader spectrum offers a credible solution to the search for sustainable biofuel.

1.2 Overview of micro-algae biodiesel production

Intensive research has been done on biodiesel production from micro-algae. Upstream processes include microalgae cultivation and harvesting; downstream processes include extraction and conversion. A brief description of the downstream processes is presented in **Figure 1. 2**. In the first step, oil or lipids are extracted from the microalgae. The extracted oil is further processed by different methods for conversion into biodiesel. Also, biomass can be directly converted into biodiesel by a direct-transesterification process, followed by biodiesel separation and purification for the final finished product.

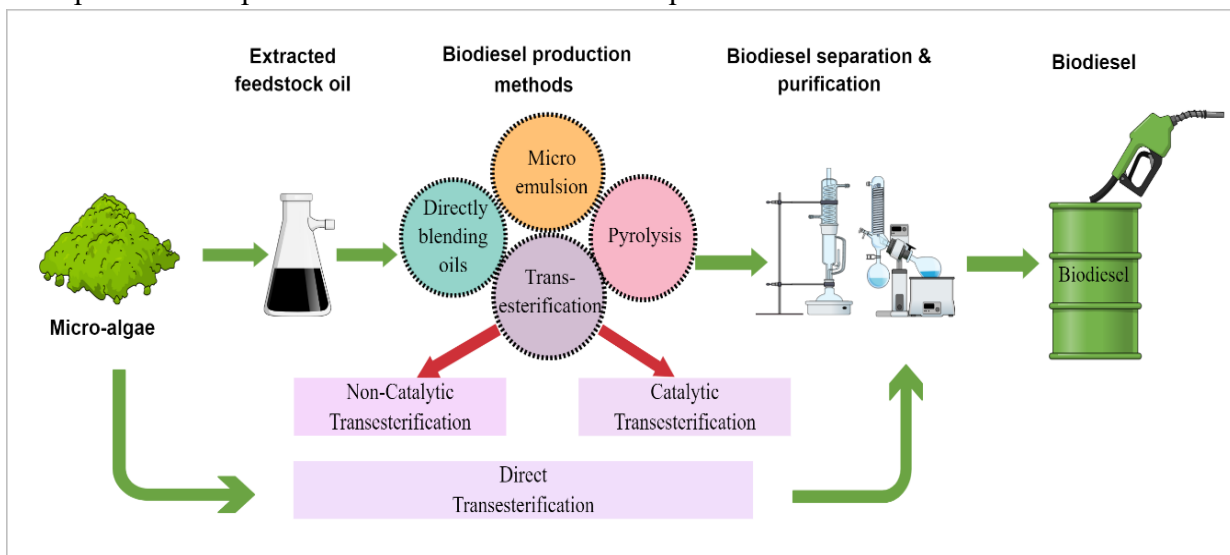


Figure 1. 2 Graphical flow-chart of downstream processes in biodiesel production

1.2.1 Production technology for micro-algae biodiesel

At the commencement of biodiesel research, researchers decoded the utilization of direct lipids in compression engines. However, the direct use of lipids had many inherent failings due to

their highly viscous and non-volatile nature leading to problems like carbon deposition and clogging of the fuel lines (Tabatabaei et al., 2019). To address such issues, some chemical modifications were required to make them compatible with the engines. Four primary methodologies came up to process the lipids into biodiesel – Blending with diesel, micro-emulsion, pyrolysis, and transesterification. Out of these, transesterification has proved to be the most efficient and standard method for microalgae biodiesel production.

1.2.2 Transesterification process

Conventional micro-algae biodiesel production involves two steps - lipid extraction and transesterification reaction. The first step includes the extraction of lipids from the algal biomass feedstock through mechanical or chemical methods followed by the transesterification reaction. Triglycerides (lipids) and alcohol, in the presence of a catalyst, convert into biodiesel or mono-alkyl esters of long-chain fatty acids or fatty acid methyl ester during the transesterification reaction. **Figure 1.3** describes the transesterification reaction for biodiesel production. Numerous factors like feedstock, catalyst type, agitation speed, temperature, reaction time, solvent type, and solvent-to-biomass ratio are essential for the optimum yield of micro-algae biodiesel via the transesterification process. However, this 2-step transesterification is not economically feasible as more than half of the generated energy gets lost in the lipid extraction process (Goh et al., 2019). Therefore, to overcome the drawback of

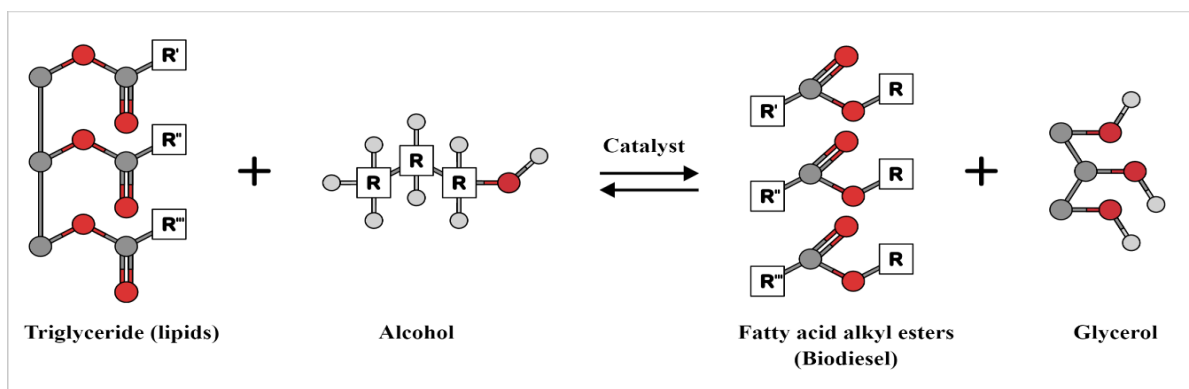


Figure 1. 3 Transesterification reaction for biodiesel production

the 2-step transesterification process, direct or in-situ transesterification is introduced as a “one-pot” process.

1.2.3 Direct-transesterification process

Direct or in-situ transesterification modifies the entire process of extraction and transesterification reaction into a single step in which the solvent used in lipid extraction likewise acts as a reagent in the conversion to fatty acids methyl esters (FAME). Sustainable biodiesel production with reduced time and the cost is the advantage of the direct transesterification process (Chamola, 2019). **Figure 1. 4** shows the comparison between the conventional and direct transesterification processes.

1.3 Current status of micro-algae biodiesel production

1.3.1 Global scenario

Research work on the utilization of microalgal biodiesel started many decades ago. The latest global algae biofuel market report estimates a growth from US\$5.022 billion in 2020 to US\$9.033 billion in 2027 (Research and Markets, 2022). Higher biodiesel output of micro-algae compared to all the conventional feedstocks has extended pathways for many industrial sectors to utilize micro-algae-based biodiesel commercially. However, algae-based biodiesel still needs to improve in commercialization on global levels due to a need for more developments in cultivation that restrict manufacturers from investing in this industry. Challenges in strain isolation, production management, water temperature, and nutrient sourcing have also confined the micro-algae biofuel industry to a limited state (Rao et al., 2021; Syafiuddin et al., 2020).

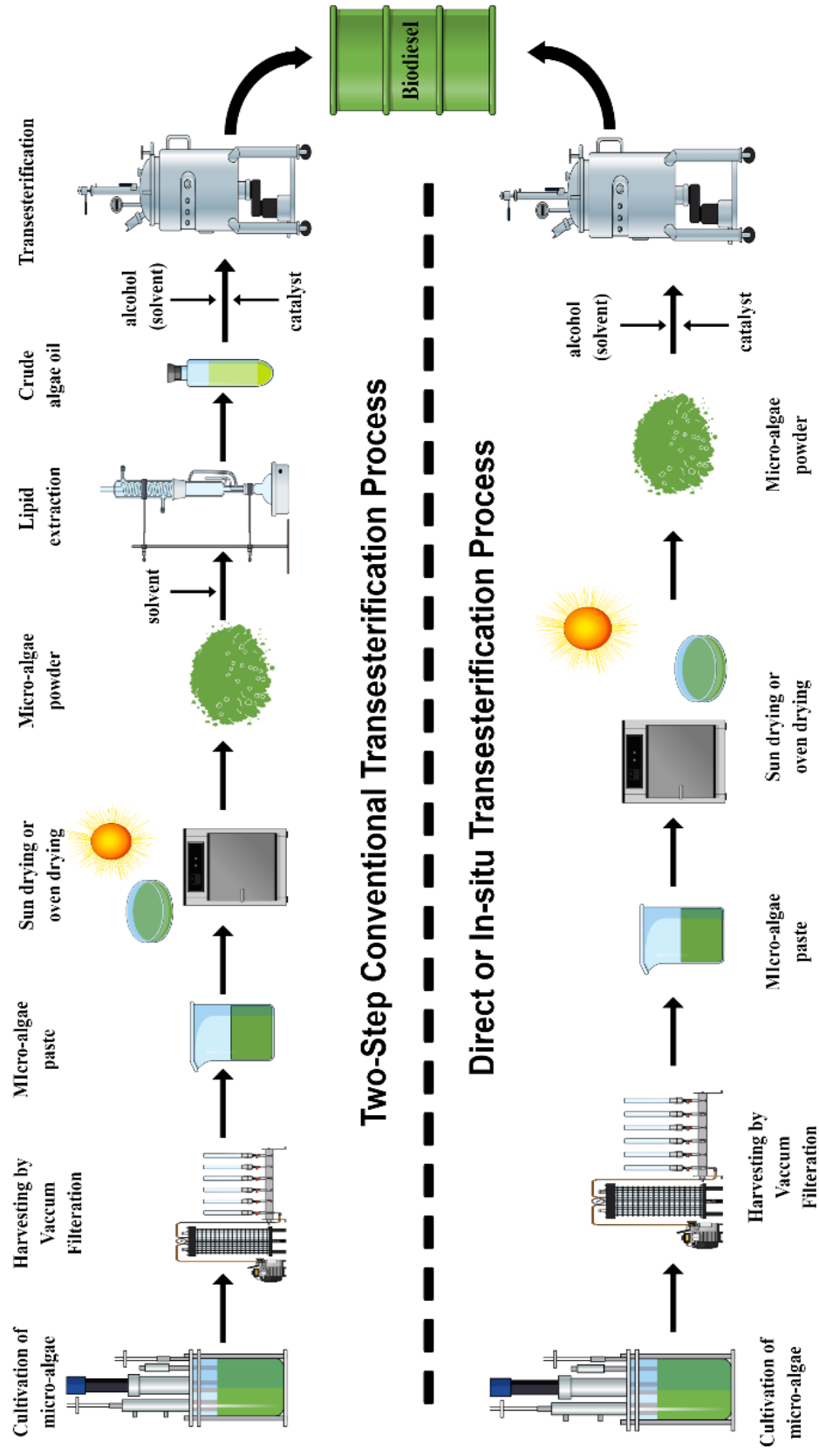


Figure 1. 4 Comparison between the 2-step conventional transesterification process and direct transesterification process for micro-algae-based biodiesel production

1.3.2 Indian Scenario

Several government and private groups work in biofuel production and distribution in India. According to the latest report, palm oil is used in the Indian biodiesel industry. Oil-producing companies have procured 104 million litres of biodiesel for biodiesel blending (Ministry of & Natural Gas, 2020). Researchers in the country have also looked into the potential of micro-algae biodiesel as a possible solution to fulfil energy demands. The drawback of its insufficiency to commercialization lies in its high production cost, dependence on the environment, and geographical location (Sooch S S and Gautam A, 2013). Although climatic conditions in India favor algae growth, it still needs to improve in utilization for biodiesel production (Chandra and Singh, 2017; Dewangan et al., 2018; Syafiuddin et al., 2020).

1.4 Research Problem

Several factors contribute to the high cost of algae biodiesel, such as high expenditure in large-scale cultivation, harvesting, and extraction costs, low oil yield, lack of proper infrastructure, and competition from other industries. All these factors bring a question – “How can the cost-effectiveness of algae biodiesel be improved while ensuring its sustainability?”

1.5 Aim of the proposed study

Based on the research problem, the cost-effectiveness of algae biodiesel needs to be improved. This was achieved by addressing the factors contributing to its high cost. The following were considered to enhance the cost-effectiveness of algae biodiesel production:

- Utilization of mixed algae species found in rural wastewater ponds as an alternative to pure algae cultures. This helped reduce the cost of algae cultivation, which was a significant contributor to the high price of algae biodiesel.
- Utilization of algae grown in wastewater eliminated the need for fresh water, nutrient supplements for growth and other high-cost technologies.

- Direct-transesterification process was employed to eliminate the extraction step in biodiesel production, which reduced the energy requirements for the production process.
- Optimized the direct transesterification process, led to improved efficiency.
- Synthesized catalyst from waste material was considered an eco-friendly approach. This eliminated the need for expensive catalysts and reduced the environmental impact of the production process.

1.6 Thesis organization

Chapter 1 of the thesis briefly introduces micro-algal-based biodiesel and its production technologies. This section also discusses the current status of microalgal biodiesel production, both in India and worldwide. Finally, the research problem and the present study's focus are described.

Chapter 2 reviews available literature on analytical and experimental studies related to micro-algal biodiesel production. The objective of the present study is also mentioned in this chapter.

Chapter 3 presents the step-by-step methodology adapted for direct or in-situ transesterification of mixed indigenous microalgae by commercial and synthesized catalysts. This chapter also explains all the different setups and instrumentation for biodiesel production.

Chapter 4 deals with all the outcomes of the research study, and discussions on the results are presented.

Chapter 5 deals with the conclusion derived from the study made in chapter 4. The final remarks give an overall vision of the effectiveness of the direct transesterification process of mixed indigenous microalgae derived from rural ponds through the synthesized catalyst.

Chapter 2

Literature Review

2.1 Overview

Fossil fuels have been a significant energy source globally and have a prominent impression on the country's economy. Moreover, the call for fossil fuels is mounting continuously to meet the ever-increasing demand for various sectors such as power generation, industrial, transportation, and agriculture. Therefore, other renewable alternative energy sources have been explored to address the global energy crisis posing economic and security threats to all countries. Renewable energy sources such as wind, solar, hydro, and waste/biomass have been explored globally to confine the utilization of fossil fuels. It has been forecasted that renewable combustible sources like biodiesel, hydrogen, and biogas will play a vital role in substituting renewable energy resources (Chamola et al., 2019).

On the one hand, biodiesel has aroused considerable interest from various researchers and local and international traders. It has been recognized as the most favorable renewable and environmentally friendly biofuel. Biodiesel is a biodegradable, non-toxic fuel that emits fewer sulfur and carbon monoxide volumes than other fossil fuels when burned in the CI engine (Sharma and Dhar, 2018). Rapeseed, soya bean, palm oil, canola, corn, coconut, sunflower, and other edible crops emanated as first-generation biodiesel feedstock as these were the introductory crops for biodiesel processing. However, many issues were provoked by this feedstock due to its effect on global food markets and food security. To counteract the hiccups linked to first-generation feedstock, non-edible crops such as tobacco seed, jatropha, mahua, waste cooking oil, jojoba oil, salmon oil, and other non-edible crops came to light. These non-edible feedstocks were second-generation biodiesel feedstock (Verma et al., 2017). Although second-generation feedstock did not directly affect the food supply chain and could be cultivated in waste-barren lands, they may not be enough to replace most transportation fuels.

For this reason, second-generation feedstock was not utilized primarily for biodiesel production (Malode et al., 2022).

Microalgae are adjudged as third-generation biodiesel feedstock. It has become the most favorable alternative source of biodiesel production due to its high photosynthetic efficiency in producing biomass and its higher productivity compared to other feedstock (Halder and Azad, 2019; Nautiyal et al., 2019). They are easily reproducible and are easier to grow than various other types of feedstocks. In contrast to different raw materials, microalgae can be cultivated in freshwater, saltwater, and wastewater and can be grown at any time of the year (Halder and Azad, 2019). They have a short harvesting life and can produce multiple and persistent harvests of biomass throughout the year, unlike oilseeds (Choo et al., 2019; Rangabhashiyam et al., 2017). Microalgae reproduce through photosynthesis to transform solar energy into chemical energy, resetting the whole growth cycle every few days. Also, they can grow almost anywhere and require sunlight and a few pure nutrients that enhance growth with adequate aeration (Chhandama et al., 2021; Kumar et al., 2020). **Table 2. 1** shows that microalgae reproduce shortly, resulting in higher biomass productivity and sterling yield than other oil crops. Studies revealed the benefits of utilizing microalgae for biodiesel generation compared to conventional feedstock (Liu et al., 2013; Saad et al., 2019).

2.2 Microalgae-based biodiesel production

Microalgae is reported as one of the most promising feedstocks for biodiesel production (Chen et al., 2018; Sander and Murthy, 2010). Biodiesel with microalgae has gained significant interest in recent years due to its short harvesting life, high lipids, and carbohydrates inside the cell body for biodiesel production (Ogbeide Ebhodaghe et al., 2021). Several reports seemingly showed the efficiency of microalgae as a better and more potential source for oil extraction than the traditional edible and non-edible crops (Adeniyi et al., 2018; Adetunji et al., 2021). Various researchers explored that biodiesel production from microalgae is economically and

environmentally appropriate (Rawat et al., 2013). It can produce ten times more biodiesel per unit area than typical oil-bearing land plants (Rosenberg et al., 2008; Schenk et al., 2008). This microalgae potential could completely replace petroleum-based transportation fuels without compromising the argument of "food for fuel" (Shuba and Kifle, 2018).

Table 2. 1 Comparison of different feedstock for biodiesel production (Al-Samarae et al., 2017; Kumar and Sidharth, 2018)

Feedstock	Oil or Fats	Oil Content (% oil by wt. biomass)	Land Use (m ² year/kg biodiesel)	Biodiesel yield (kg/m ² year)
Edible	Canola	41	12	0.09
	Soybean	18-22	18	0.06
	Sunflower	25-35	11	0.09
	Palm	30-60	2	0.59
	Peanut	42-52	-	0.14
	Corn	48	66	0.02
	Coconut	63-65	5	0.24
Non-Edible	Cottonseed	18-25	-	0.03
	Jatropha	30-40	15	0.07
	Castor	48	9	0.12
	Microalgae (Chaetoceros sp.)	70	0.1-0.2	2.44-13.69
Neem	30	-	-	

2.3 Process design of microalgae-based biodiesel production

In the view of biodiesel production, the whole process can be separated into two major sections as shown in **Figure 2. 1**. The first is an upstream process that includes the cultivation of microalgae, considering nutrient supply, CO₂ feed, and sunlight absorption. The second section includes the downstream process, including harvesting, extraction of lipids from harvested microalgae, and finally the production of biodiesel from lipids by transesterification process utilizing catalysts (Faried et al., 2017).

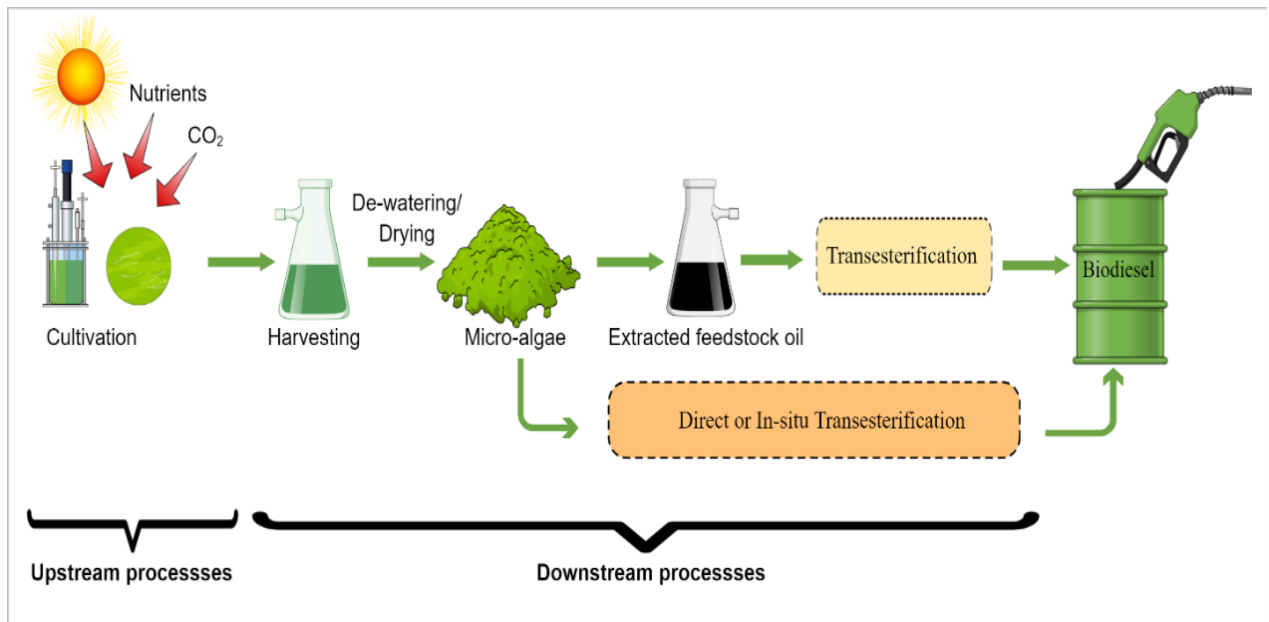


Figure 2. 1 Schematic description of the biodiesel production process

2.3.1 Upstream processes

2.3.1.1 Microalgae cultivation

Microalgae cultivation systems are divided into open and closed systems. For cultivation, different organic or inorganic carbon sources are used, such as glucose, sucrose, glycerol, CO₂, and bicarbonates. The carbon sources are either added to the system or already present if wastewater is the source of cultivation (Kumar et al., 2021). Other cultivation requirements include supplementary nutrients such as phosphorus and nitrogen, light intensity, temperature, and pH. Biomass yield is dependent on the supplements and cultivation conditions and can be increased or decreased by the optimization of the process parameters (Whangchai et al., 2021).

2.3.1.2 Cultivation techniques

There are several techniques to grow microalgae in water/wastewater. A proficient and cost-effective large-scale culture is essential for accomplishing microalgae biomass as a decent supplement to renewable energies. Microalgae culture technologies include wastewater stabilization ponds, algal high-rate ponds, advanced integrated wastewater pond systems, turf

scrubbers, photo-bioreactors, and hybrid systems. In practice, the most used large-scale production technology is a high-rate algal pond or raceway pond. The critical benefit of raceway pond technology is its adaptability, high generation, simple design, and low operating and maintenance costs (Das et al., 2021). The brief description and significant pros and cons of various technologies have been discussed in **Table 2. 2** and a diagrammatic view of the cultivation technologies has been given in **Figure 2.2**. The waste stabilization pond is the cheapest and simplest technology for wastewater treatment. A series of stabilization ponds are a cost-effective alternative to nutrient removal and algae cultivation (Vagheei, 2021). In the facultative pond, approaching sunlight promotes the growth of algae. At this stage, the growth of algae is considered a problem as it increases the solids concentration and reduces the efficiency of biochemical oxygen demand removal. Photo-bioreactors are different from open raceway ponds as they hold the microalgae for growth in a clear container, and a more controlled environment is provided. The significant advantages of photo-bioreactors are that they provide ideal conditions for algae growth by controlling the required growth parameters (Zhu et al., 2016). However, one of the critical challenges of using photo-bioreactors is the high operational cost and, therefore, inappropriate for large-scale production (Mobin and Alam, 2014).

Table 2. 2 Current microalgae cultivation technologies: Summary of their advantages and disadvantages

Technology	Advantages	Disadvantages	References
Wastewater stabilization Pond/Open Pond	<ul style="list-style-type: none"> • Cost-effective • Treats domestic as well as industrial wastewater effectively 	<ul style="list-style-type: none"> • Requires sufficient land area • Requires regular maintenance to avoid odors 	(Vagheei, 2021)

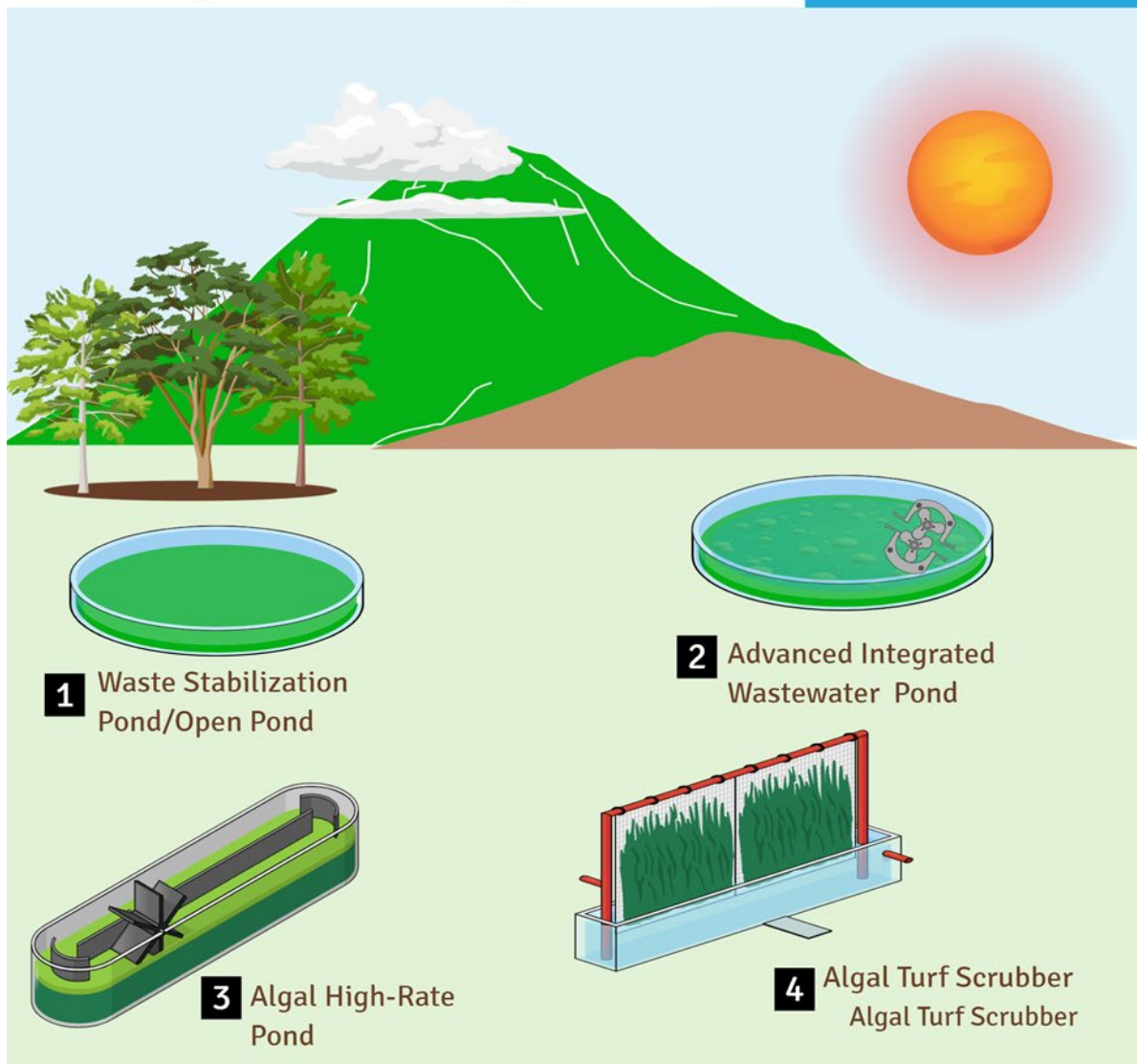
Advanced integrated wastewater pond system	<ul style="list-style-type: none"> • Significantly less accumulation of sludge and fewer odor problems • High algae growth • Cost-effective 	<ul style="list-style-type: none"> • Installation costs and energy requirements are higher in comparison with stabilization ponds. 	(Ponce, 2015)
Algal High-Rate Ponds (HRP)	<ul style="list-style-type: none"> • High algal productivity • Can receive a high loading rate • Easy operation and maintenance • Mixing exposes the algae to the sunlight High N & P removal 	<ul style="list-style-type: none"> • Incidence of light limited by increased cell density of algae • Evaporation losses affect cultural growth • CO₂ loss 	(Rawat et al., 2013)
Photo-bioreactor	<ul style="list-style-type: none"> • Artificial light stabilizes the intensity and improves total productivity. • Many parameters can be controlled to increase productivity • Allows growth of monoculture algae • No contamination risk 	<ul style="list-style-type: none"> • High cost • Extensive infrastructure • Large-scale production has not been developed yet • Need high maintenance and monitoring 	(Rawat et al., 2013)
Microalgal turf scrubber	<ul style="list-style-type: none"> • Meager operating and fuel production cost • Higher productivity over open ponds 	<ul style="list-style-type: none"> • Need sufficient waste space • Need considerable infrastructure 	(Lv et al., 2017)
Hybrid System	<ul style="list-style-type: none"> • High productivity • Fewer contamination risks 	<ul style="list-style-type: none"> • High cost and maintenance • Extensive infrastructure 	(Silva et al., 2009)

2.3.2 Downstream processes

2.3.2.1 Harvesting

The microalgae harvest is a two-stage process in which mass production and thickening are carried out. Harvesting the microalgae biomass is assessed to be around 30% of the total cost

Open Cultivation systems



Closed Cultivation systems

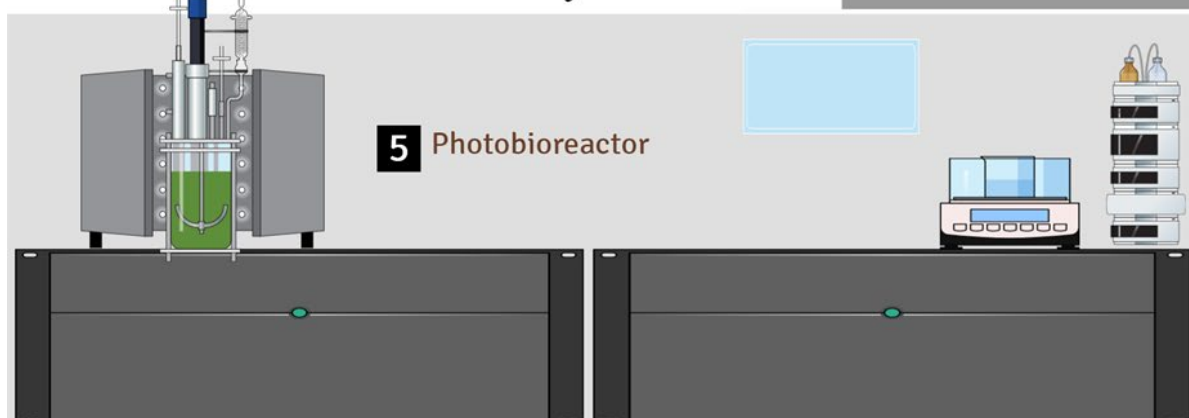


Figure 2. 2 Various micro-algae cultivation systems

for algal production in open ponds (Salim et al., 2011). However, microalgae harvesting poses a challenge due to the small size and low density of algae cells, increasing the cost of capital. Therefore, selecting methods for biomass production is demanding at a negligible cost (Jon K Pittman, Andrew P Dean, 2011). The description of various technologies and their advantages/disadvantages for both steps (algae-bulk and dewatering) have been discussed in **Table 2. 3.**

Table 2. 3 Methods for algae-bulk and dewatering harvesting methods

Method	Advantages	Disadvantages	References.
Algae-bulk Methods			
Coagulation/ Flocculation	<ul style="list-style-type: none"> • Simple and widely used method. • Can reach over 90% algae renewal. 	<ul style="list-style-type: none"> • Some coagulants emit GHG and have an impact on health. • Recycling of culture medium is limited. 	(Barros et al., 2015)
Auto and bio-flocculation	<ul style="list-style-type: none"> • Cheap method • Allows recycling of the cultural medium • Non-toxic to microalgae 	<ul style="list-style-type: none"> • Changes in cellular composition • Possibility of microbiological contamination 	(Salim et al., 2011)
Electro-coagulation	<ul style="list-style-type: none"> • 98% recovery of algae • Significantly less residual algae biomass contamination • Scaling up of the process is possible 	<ul style="list-style-type: none"> • Not helpful if the medium has less than 10% total solids. 	(Parmentier et al., 2020)
Flotation	<ul style="list-style-type: none"> • Preferred for large-scale applications. • Cost-effective method 	<ul style="list-style-type: none"> • Typically requires the use of chemical flocculants 	(Barros et al., 2015)
Sedimentation	<ul style="list-style-type: none"> • Commonly used harvesting method • Easy for large-scale biomass harvesting 	<ul style="list-style-type: none"> • Damage to biomass can occur at high temperatures. 	(Cheirsilp et al., 2019)

		<ul style="list-style-type: none"> • Low concentration of algal cake. 	
Dewatering Methods			
Filtration	<ul style="list-style-type: none"> • High Recovery efficiencies • Allows the separation of shear-sensitive species 	<ul style="list-style-type: none"> • The possibility of fouling/clogging increases operational costs. • Membranes should be regularly cleaned. • Highly costly 	(Barros et al., 2015; Cheirsilp et al., 2019)
Centrifugation	<ul style="list-style-type: none"> • Fast method • A wide range of algal species can be used. • High recovery efficiencies • Concentrates biomass into a solid cake 	<ul style="list-style-type: none"> • Expensive method. • High energy requirements. • Possibility of cell damage. 	(Makut, 2021)
Belt press	<ul style="list-style-type: none"> • Achieves 90% substantial recovery after dewatering. • Lower GHG emissions 	<ul style="list-style-type: none"> • High energy consumption. 	(Makut, 2021)

Chemical coagulation and flocculation are promising for economic microalgae biomass harvesting (Uduman et al., 2010). For efficient harvesting of biomass, coagulation/flocculation usually follows sedimentation. Various salts have been reported as coagulants for microalgae recovery, such as FeCl_3 , $\text{Al}_2(\text{SO}_4)_3$, and $\text{Fe}_2(\text{SO}_4)_3$. Auto flocculation is another method that appears to be an appealing alternative because of its various advantages. It is cheap, consumes less energy, is non-toxic to microalgae, and eliminates the utility of chemical flocculants (Irena Branyikova, Gita Prochazkova, Tomas Potocar, 2018). Regardless of numerous benefits, the method mentioned earlier is not favored at the industrial scale for harvesting microalgae biomass as flocculation cannot be controlled and can cause changes in the composition of cells (Salim et al., 2011). Flocculants produced by bacteria, viruses, or microalgae, known as bio-flocculants, can be essential for economic and cost-effective microalgae biofuel production as

they eliminate the need for chemical flocculants. However, the co-cultivation of microalgae with bio-flocculants bacteria results in microbial contamination that interferes with the use of microalgae biomass in food (Zhu et al., 2016). Bio-flocculation concerns the micro-algae flocculation caused by the secreted bio-polymers, particularly extracellular polymeric substances (EPS) (Christenson and Sims, 2011). Electro-flocculation is another versatile method appropriate to various algae (Parmentier et al., 2020). Despite several benefits of electro-flocculation, the energy and equipment costs for sustainable large-scale applications remain too high (Christenson and Sims, 2011).

Flotation imparts the necessary lifting power for particle transport and separation. It is often preceded by coagulation/flocculation. There are four main techniques under flotation: i) Dissolved air flotation ii) Dispersed air flotation (DIAF) iii) Electrolytic flotation iv) Ozone dispersed flotation (Chen et al., 2011). Recently, magnetic particles have been used as a means for harvesting biomass. However, a considerable amount of flocculant is required in this process, which limits its use on a large scale (Pandit and Fulekar, 2017). Sedimentation is an effective method for a wide variety of microalgae and is exceedingly energy efficient. However, the sedimentation process is a prolonged process that leads to biomass degradation and limits this technique to routine harvesting (Christenson and Sims, 2011). Applying the coagulation/ natural process before the sedimentation process is prevalent to fasten the biomass settling (Shi et al., 2019).

The dewatering of the harvested microalgae can be accomplished by several mechanical means, including different kinds of filtration processes, centrifugation, and belt press. Filtration is the primary dewatering method commonly used after coagulation/flocculation to improve harvest efficiency. However, over some time, algae deposits on the filtration membrane throughout the process may block the membrane pores (Christenson and Sims, 2011). The blockage represents the major drawback of filtration techniques that increase operating costs (Show et al., 2015).

Filtration is favorable for harvesting long-length and low-density colonies (Zhou et al., 2013). Mainly, very low-dense microalgae can be easily harvested by this method. However, this technique is usually not utilized for large-scale operations because of the costs of replacement and pumping (Uduman et al., 2010). Centrifugation is the fastest method to harvest microalgae. However, it is the most expensive as it consumes high energy (Show et al., 2015; Zhou et al., 2013).

2.3.2.2 Extraction and Transesterification Process

This process includes the production of biodiesel from the harvested algal biomass by performing a two-step protocol. Firstly, algae oil is extracted from the harvested biomass, and then extracted oil or lipids are converted to biodiesel using a catalyst in controlled parametric conditions (Ali et al., 2023). There are various chemical, physical, and biological techniques for extracting the oil from microalgae (Silva et al., 2014). The advantages and disadvantages of various techniques are described in **Table 2. 4**.

Table 2. 4 Lipid extraction techniques along with advantages and disadvantages of each technique

Method	Advantages	Disadvantages	References
Mechanical pressing	<ul style="list-style-type: none"> • Oil extracted from approx. 70-75% dried biomass. • Fast process 	<ul style="list-style-type: none"> • Expensive and technically challenged. 	(Cooney et al., 2011)
Chemical solvent extraction	<ul style="list-style-type: none"> • Low-cost and high efficiency • Efficient extraction of microalgal lipids 	<ul style="list-style-type: none"> • Toxicity among humans and the environment is high. • Must be coupled with other methods for efficiency. 	(Cheng et al., 2014; Lam and Lee, 2012)

Pyrolysis	<ul style="list-style-type: none"> • Uses whole algal biomass 	<ul style="list-style-type: none"> • Biomass must be dried before this process. • High energy intensive • Resulting biofuel has low energy content. 	(Kumar et al., 2017)
Supercritical fluid technology (SCF)	<ul style="list-style-type: none"> • Fast process. • High selectivity due to the flexibility of changing pressure and temperature 	<ul style="list-style-type: none"> • High maintenance and operational cost 	(Li et al., 2014; Yen et al., 2014)
Hydrothermal Liquefaction	<ul style="list-style-type: none"> • After harvesting, no drying is required. • Low ash content and sulphur emissions • The produced oil has a high calorific value 	<ul style="list-style-type: none"> • In addition, steps needed to transform oil products into biodiesel. • High NO_x emissions 	(Frank et al., 2013; Kumar et al., 2017)
Microwaving	<ul style="list-style-type: none"> • High efficiency in damaging algae cells • Simple method and efficient for scaling up 	<ul style="list-style-type: none"> • Energy-intensive 	(Lee et al., 2010; Silva et al., 2014)
Sonification	<ul style="list-style-type: none"> • When used with solvents, advances high absorption of the solvent and fast extraction of lipids 	<ul style="list-style-type: none"> • The sample has to be dried. • Energy intensive 	(Ghasemi Naghdi et al., 2016; Lee et al., 2010)

After the extraction process, lipids are converted to biodiesel in the next step i.e., the transesterification process. Transesterification is one of the most commonly used methods for biodiesel production; its purpose is to reduce the molecular weight of oils to alkyl esters of fatty acids when alcohol is present (usually ethanol or methanol) and a catalyst, the by-product is glycerol (Rawat et al., 2013).

2.3.2.3 In-situ or direct transesterification

Biodiesel conversion mainly includes two phases: extraction and transesterification. Conversely, direct or in-situ transesterification modifies the whole process of extraction and transesterification reaction into a single step in which the solvent used in oil extraction likewise acts as a reagent in the conversion to biodiesel fatty acids (Wahlen et al., 2011). The advantage is the reduction of biodiesel production steps, costs, and time, constraining the moisture content and production of the product (D'oca et al., 2011). In this process, several factors, like the type of solvents, catalysts, amount of reagents, temperature, and reaction time play an essential role in the quality of biodiesel production. Generally, high purification of solvents may result in better performance in the case of dry biomass, whereas in the case of wet biomass, solvent percolation can be impeded by water (Kim et al., 2013).

Notwithstanding, a study concluded that the FAME content of 90% (w / w) was obtained when wet microalgae biomass was used for direct transesterification (Im et al., 2014). Moreover, in-situ transesterification for *Synechococcus elongates* (cyanobacteria), *Tetraselmis suecica*, *Chaetoceros gracilis* (diatom), and municipal wastewater lagoons resulted in 78, 40, 82, and 74% FAME, respectively (D'oca et al., 2011). Biodiesel production from microalgae by the in-situ transesterification process will be more efficient by selecting high oil content and fast-growing algae strains (Skorupskaite et al., 2016). Simultaneous extraction and transesterification were studied for pond water algae using different solvents. Maximum biodiesel yield was obtained when hexane was used as a solvent (Nautiyal et al., 2014). Many researchers reported the synthesis of heterogeneous catalysts derived from waste material that produces a good yield of biodiesel from microalgae and can also be used in direct transesterification by some modifications (Laca et al., 2017; Marwaha et al., 2018; Pandit and Fulekar, 2017).

2.3.3 Catalysts utilized in the in-situ transesterification process.

2.3.3.1 Homogeneous base and acid catalysts

Homogeneous base catalysts like KOH and NaOH have been used to transesterify lipids (Cercado et al., 2018). However, the free fatty acids present in the lipids of microalgae have prevented the use of homogeneous catalysts because low fatty acids will form soap when react with the base catalyst, which will reduce the yield of biodiesel and make it challenging to separate biodiesel from glycerol (by-product) (Lam and Lee, 2012). On the other hand, acidic catalysts will do a better job because they are not sensitive to free fatty acid in the oil. Therefore, utilizing an acid-base catalyst will be an excellent alternative to enhance the conversion of microalgae lipids to biodiesel. In this method, the microalgae lipid is first subjected to acid pre-treatment to reduce the FFA level of the microalgae oil. Then, the base-catalyzed transesterification reaction takes place (Ganesan et al., 2021). Nevertheless, the major disadvantage of an acid-base catalyst is using an additional base catalyst to neutralize the acid catalyst during transesterification, which adds up to an additional cost for producing biodiesel.

2.3.3.2 Heterogeneous catalysts

Heterogeneous catalysts have been explored for the transesterification reaction to produce biodiesel. The heterogeneous catalyst can be recycled, regenerated, and reused for subsequent cycles of transesterification, unlike homogeneous catalyst that further improves the profitability of biodiesel production (Makareviciene et al., 2021). Besides, they are less sensitive to free fatty acids and moisture, do not require complicated separation and purification steps, and thus minimize product contamination and the number of water wash cycles (Kaur et al., 2018). The most used solid catalysts are alkaline earth metal oxides and, to a lesser extent, transition metal oxides and hydrotalcite. Furthermore, solid acid catalysts are preferred to base catalysts as they are less sensitive to free fatty acids and the water content of biomass (Xie and

Wan, 2019; Xie and Wang, 2021). Synthesizing solid magnetic catalysts using Fe_3O_4 as the core for biodiesel production proved very successful due to their easy recovery and oil conversion efficiency (Xie et al., 2018; Xie and Wang, 2020). CaO supported with Al_2O_3 attained good results as a heterogeneous catalyst for biodiesel production. Cobalt-doped CaO catalyst was approved for high catalytic activity in the transesterification of algal biomass to produce biodiesel (Das et al., 2020). Heterogeneous catalysts such as Mg-Zr, and hierarchical zeolites have been examined to transesterify microalgae oil; however, less than 30% biodiesel yield was achieved (Carrero et al., 2011; Talebian-Kiakalaieh et al., 2013). Red mud, waste from the aluminum industry, was used as a catalyst that improved the properties of the fuel (Senthil et al., 2016).

2.3.3.3 Bio-based catalysts

Biomass-derived catalysts are gaining much attention over conventionally available catalysts due to low stability and high-cost factors (Osman et al., 2023; Singh and Garg, 2021). These can be prepared from waste bioproducts that are less toxic, have high reactivity, and are more stable. Attempts have been made to synthesize bio-based catalysts by utilizing agricultural waste and animal bones (Chowdhury et al., 2021; Thangaraj et al., 2019). Natural dolomitic rock and waste seashells were investigated as renewable waste resources to prepare heterogeneous catalysts to produce biodiesel (Jaiyen et al., 2014). Kumar et al. attempted to utilize bio-based thermal plant's fly ash as a catalyst for the transesterification of Jatropha oil with optimized yield of 93.9% (Kumar et al., 2015). Ahmad et al. synthesized low-cost catalysts using waste eggshells, and a yield of 93.4% from algal oil was ascertained (Ahmad et al., 2020). Saleem et al. conducted a study in which waste egg shells were used as catalyst for biodiesel production from chicken fat (Saleem et al., 2022). Deeba et al. utilized yeast residue for biodiesel production and a 94.8% yield was obtained, they reported the synthesized catalyst was highly reactive and stable (Deeba et al., 2020). A study was conducted in which Branan

trunk ash was utilized as a catalyst for biodiesel production, and a yield of 98.39% was obtained under optimized conditions (Rajkumari and Rokhum, 2020).

2.3.3.4 Enzymatic catalysts

In-situ transesterification by the immobilized lipases has emerged as an economical technique to produce biodiesel in comparison with other catalysts (Ferreira Mota et al., 2022; Gufrana et al., 2022). An enzyme extracted from the fungal strain *Cladosporium tenuissimum* was used as a biological catalyst for enzymatic transesterification of diatom, and an efficiency of 87.2% was achieved (Saranya and Ramachandra, 2020). *Rhizomucor miehei* lipase expressed in *Pichia pastoris* was utilized to produce free enzyme lipase GH2. Produced lipase was then used to convert *Chlorella vulgaris* into biodiesel and a yield of 90% was observed. Furthermore, immobilized lipase (immobilization was done by magnetic particles coated over graphene oxide) was also utilized for biodiesel production, and an efficiency of 92.8% was achieved (Xie and Huang, 2018). Likewise, immobilized lipase impregnated on Fe₃O₄/Poly (styrene-methacrylic acid) by covalent coupling method to utilize it for biodiesel production with 86% oil conversion by covalent coupling method to utilize it for biodiesel production with 86% oil conversion (Xie and Wang, 2014)

2.4 Summary

Microalgal biodiesel has proved to be the most preferred renewable fuel among all the other renewable fuels. Yet its commercialization has not been recognized because of various factors like the selection of algae strains, energy-intensive cultivation, and conversion process. However, many researchers in the past have come up with advanced technology and affordable processing methods to make it more sustainable to proceed further in the microalgal biodiesel industry. Still, in the present scenario, the cost-effectiveness of algae biodiesel needs to be improved.

Sustainable algal biodiesel production is possible by improving all the high-cost bottleneck areas like algae strains, cultivation, and conversion processes. The utilization of indigenous algal species already grown in wastewater for the conversion process is a great alternative to pure cultures grown in a closed system photobioreactor. Rare studies addressed the utilization of indigenous algal species grown in ponds that are very common in Indian rural areas. In-situ transesterification has shown its higher credibility over the conventional two-step transesterification process of biodiesel production in terms of cutting down the extra step of oil extraction. Various catalysts have been studied to improve the in-situ transesterification process but very few studies are present on the bio-based catalyst synthesized from waste materials.

2.5 Research gaps

The extensive literature survey on the research studies of biodiesel production through algae reveals that some research has been conducted, but its production from indigenous algal culture by in-situ transesterification process utilizing the waste material-based catalyst has lagged. Hence, the following gaps have been identified where more research is needed:

- Most of the research is based on using specific single species or mixture of known freshwater algae species for cultivation in wastewater instead of mixed microalgae species isolated from wastewater. Focused studies are required to utilize the mixed algal species isolated from wastewater as they are already adapted and tolerant to wastewater.
- Recent studies on the exploration of heterogeneous catalysts derived from waste materials have attracted much attention. Therefore, the synthesis of catalysts from waste materials possesses enormous potential for the development of biodiesel generation.
- Direct or in-situ transesterification method is the efficient method used to produce biodiesel. Numerous studies have been carried out for the direct transesterification of

single-species algal biomass. However, the conversion of indigenous algal biomass to biodiesel by this method needs more exploration.

Chapter 3

Materials and Methods

The following chapter describes the materials and methods followed to produce microalgal biodiesel including wastewater and microalgae collection, cultivation, wastewater analysis, characterization of indigenous microalgal species, harvesting, synthesis, and characterization of catalyst utilized in in-situ transesterification. Additionally, the experimental setup for the cultivation of microalgae and the in-situ transesterification process are also discussed.

3.1 Collection of indigenous microalgae and wastewater

Wastewater containing indigenous microalgae cultures was collected by grab sampling from the rural oxidation pond (a unit of the wastewater treatment plant) located at Haripur Village, Jalandhar, India (31.0342° N, 75.3103° E). The location of the treatment plant is shown in **Figure 3. 1(a)** The existing oxidation pond was a part of three well-system wastewater treatment plants as shown in **Figure 3. 1(b)**. The samples were collected three times in May 2019 due to the abundance of microalgae in this season (Das et al., 2022). Subsurface (50 cm depth) water samples from 3 different locations were collected using a pre-cleaned plastic container (10L) and then mixed. Microalgae samples were procured by filtering collected water through a 0.45µm vacuum membrane filter (Subramanian et al., 2023).

3.2 Raw material for catalyst synthesis

Rice bran powder was utilized as a pioneer of activated carbon to develop a KOH/rice bran-derived activated carbon catalyst for the in-situ transesterification reaction. Rice bran was procured from rice sheller in the dry powder form from Durga Rice General Mills, Patiala, India.

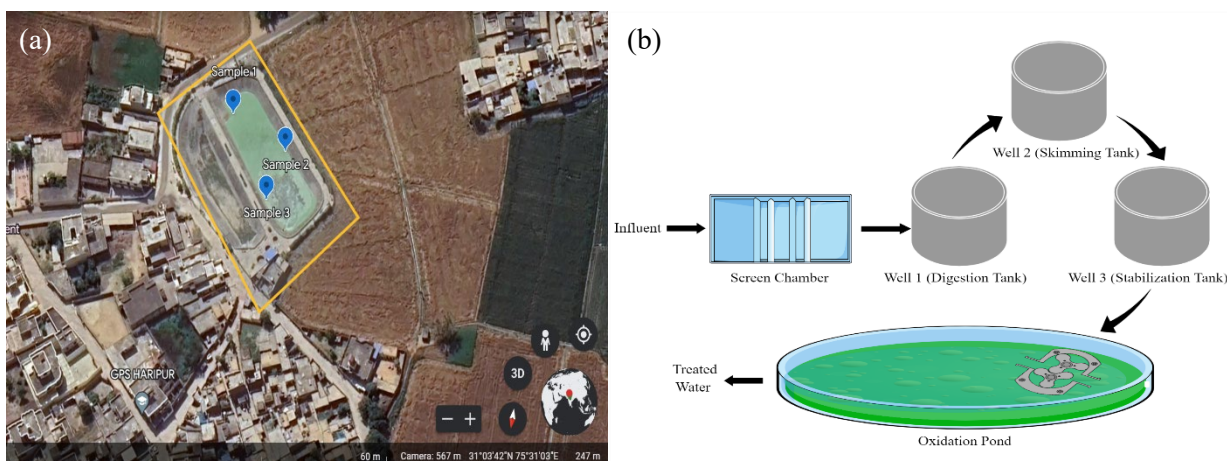


Figure 3. 1 (a) Location of the treatment plant; (b) three-well system wastewater treatment plant

3.3. Chemicals and reagents

Potassium dichromate ($K_2Cr_2O_7$) and concentrated sulphuric acid (H_2SO_4) from SD Fine Chemicals Ltd. (India) were used for chemical oxygen demand (COD) estimation. Calcium chloride ($CaCl_2$), phosphate buffer, ferric chloride ($FeCl_3$), sodium hydroxide ($NaOH$), and magnesium sulfate ($MgSO_4$) were used for the determination of dissolved oxygen and biochemical oxygen demand (BOD) and were also purchased from SD Fine Chemicals Ltd. (India). For nitrate determination, hydrochloric acid was used. Stannous chloride ($SnCl_2$) and ammonium molybdate ($(NH_4)_2MoO_4$) were used for the phosphate determination and were obtained from Loba Chemie Ltd (India).

Blue-green (BG-11) broth for the cultivation of indigenous microalgae (calcium chloride dihydrate, 36.7 mg/L, citric acid, 5.6 mg/L, dipotassium hydrogen phosphate, 31.4 mg/L, disodium magnesium EDTA, 1 mg/L, ferric ammonium citrate, 6 mg/L, magnesium sulfate, 36 mg/L, sodium carbonate, 20 mg/L, sodium nitrate, 1.5 g/L) was obtained from Millipore (Mumbai, India), and agar-agar from Hi-Media laboratories (Mumbai, India),

Solvents and co-solvents – methanol, ethanol, chloroform, and hexane for in-situ transesterification process were obtained from Fisher Scientific (Hyderabad, India), potassium

hydroxide (KOH) for catalyst synthesis was obtained from Fisher Scientific (Hyderabad, India). All these chemicals were of analytical grade.

3.4 Wastewater analysis

All the collected wastewater samples (three 5L samples) were characterized for their physio-chemical wastewater quality analysis as per American Public Health Association (APHA) standard methods (Rice et al., 2017). pH and conductivity were measured using a benchtop pH/conductivity meter, total dissolved solids were analyzed by the 2540-C Method (APHA, 2017), total chemical oxygen demand (COD) and soluble COD (CODs – filtered at 0.45 μm) were determined according to the 5220. C Method (APHA, 2017), biochemical oxygen demand (BOD) by the 5210-B Method (APHA, 2017), phosphates by stannous chloride method mentioned in section 4500 (P.D.) (APHA, 2017), nitrates by 4500-NO₃ Method (APHA, 2017) and dissolved oxygen (DO) by 4500-O Method (APHA, 2017). The wastewater analysis results were the average of all the samples collected from the oxidation pond.

3.5 Cultivation of microalgae

Filtered microalgae from the collected wastewater were brought to the laboratory. Microalgae were allowed to cultivate in triplicates in a 1000 ml Erlenmeyer flask containing 800 ml collected wastewater and 100 ml microalgae for 15 days. Microalgae cultivation setup, as shown in **Figure 3. 2**, was installed to replicate the atmospheric cultivation conditions present in the rural oxidation pond. Light emitting diode light (illumination: > 20,000 lx, light cycle: 12 h light: 12 h dark) was utilized to supply constant light intensity with a temperature of 28 ± 3 °C (De Blasio, 2019). Also, an air bubbler is provided to supply oxygen in the flasks with an attached tube and bubble diffuser at the end. The growth estimation of the microalgae was analysed every alternate day for two weeks by measuring the optical density of 10 ml samples

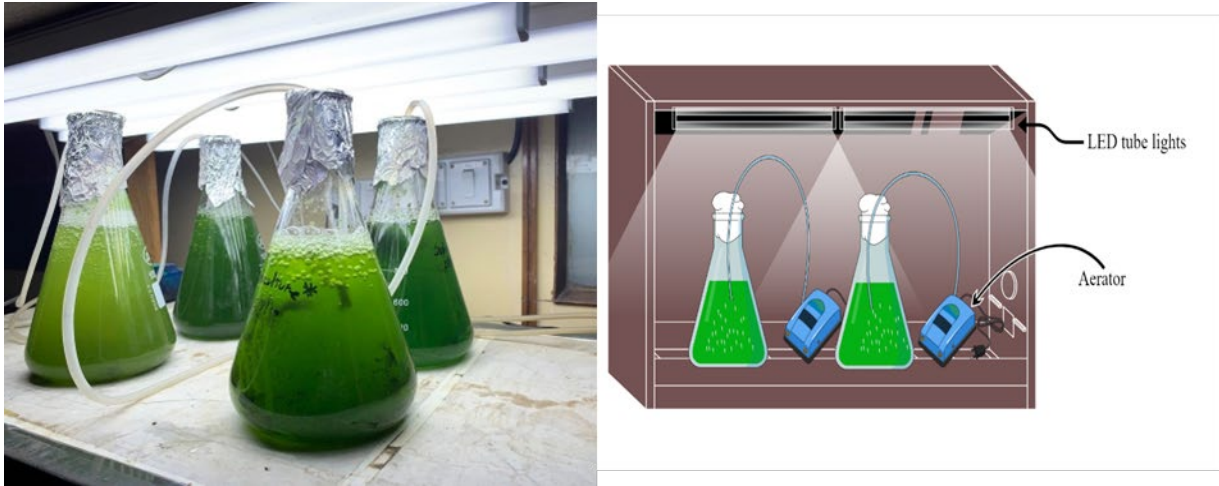


Figure 3. 2 Experimental setup for microalgae cultivation

using spectrophotometry at 680 nm (Ji et al., 2018; Mishra and Mohanty, 2019). The biomass concentration (g/l) was also calculated with absorbance at 680 nm using **Eq. 3. 1** (Rendón, 2013).

$$y = 0.7015x + 0.0362 \quad \text{Eq. 3. 1}$$

where y = biomass (g/l), x = OD at 680 nm, at $R^2 = 0.9987$.

In addition, chlorophyll-A content was also analysed in the grown cultures every alternate day for 2 weeks. Firstly, a sample of 10 ml was centrifuged at 4000 rpm for 10 min, and then the supernatant was discarded. Then, the acquired pellet was re-suspended in 10 ml of 90% acetone and then stored in the dark for 24 h at 4 °C. Finally, the suspension was centrifuged for 10 min at 4000 rpm. Furthermore, the supernatant was collected to determine chlorophyll-A concentration by taking OD (optical density) at different wavelengths of 630 nm, 645 nm, 663 nm, and 750 nm. The following equation calculate the chlorophyll-A concentration:

$$Chl - a = \frac{114 \times \{(OD_{663} - OD_{750}) - 2.16 \times (OD_{645} - OD_{750}) + 0.10 \times (OD_{630} - OD_{750})\}}{V} \quad \text{Eq. 3. 2}$$

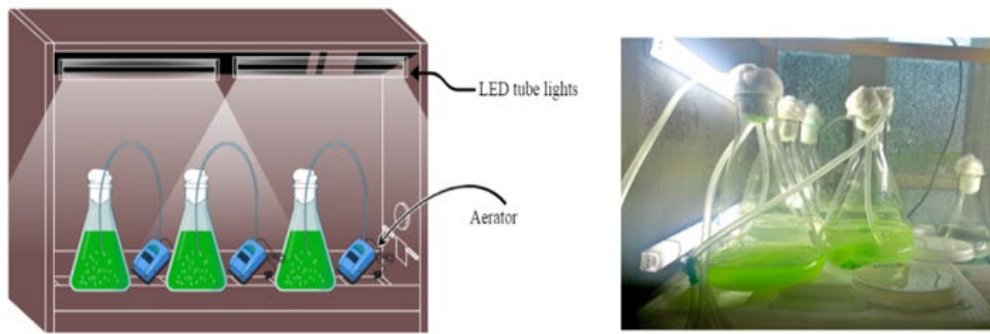
where V is the extracting volume in ml and OD is the optical density (wavelength in nm) (Johan et al., 2014). The presented data points for algal growth and chlorophyll -A in the results are the average of three replicates and the error bars represent the standard deviation from the mean.

3.5.1 Identification and characterization of microalgae

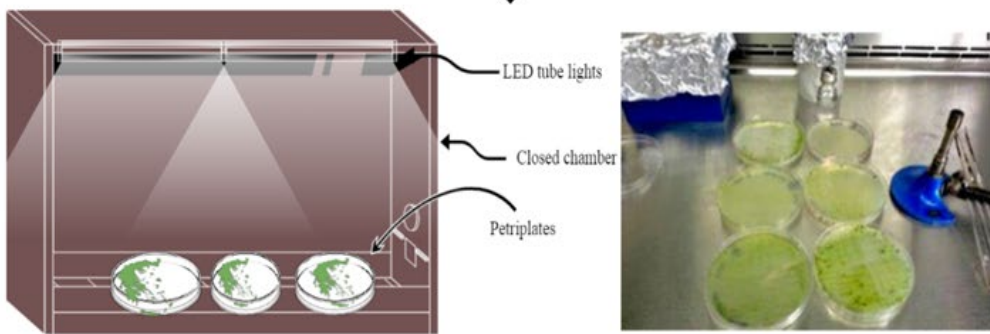
A standard plating method was employed to isolate and purify the collected algal samples for identification. The field samples were first cultivated in BG-11 media in triplicates for 15 days under the above-mentioned experimental conditions. After cultivation, the grown algae cultures were diluted (1ml in 10 ml ionized water) and then streaked in BG-11 media with 2% agar and finally allowed to grow in the same conditions but in a closed chamber to avoid contamination, see **Figure 3. 3**. The growth was allowed to take for 15 days, and then repeated subculturing was done by picking the individual grown colonies on solidified BG-11 media by pour plate technique. This method was repeated till all the present clones were isolated and purified culture plates were obtained. Finally, the purified cultures were examined for morphological and molecular identification. Morphological characterization was done with light microscopy and molecular identification was done by sequencing the isolated strains by 16SrRNA gene followed by BLASTN search of the GenBank database.

3.6 Harvesting and drying

The grown biomass was then separated from the media by filtering through a 0.45 μ m vacuum membrane filter. Afterward, centrifugation at 3000 rpm for 10 min was conducted to obtain

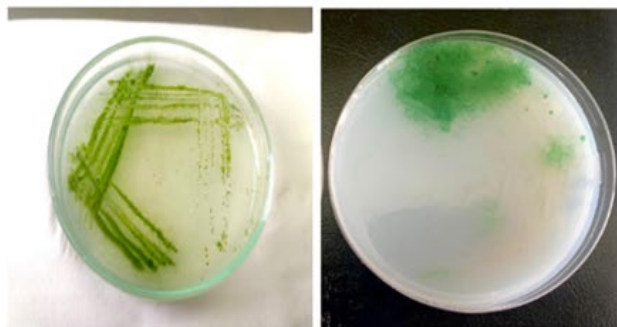


Cultivation in BG-11 media



Streaked petri plates cultures

After repeated
sub-culturing



Pure isolated cultures

Figure 3. 3 Experimental methodology for the isolation of microalgae species more concentrated biomass. Finally, the obtained pellets were washed 2–3 times to remove other present impurities. The harvested biomass was then allowed to dry in the sun for 24 hours.

Finally, the dried samples were used to produce biodiesel via the in-situ transesterification reaction.

3.7 Synthesis and characterization of KOH/rice bran activated carbon (AC heterogeneous catalyst catalyst used for in-situ transesterification process

Rice bran, a by-product of the rice milling process, was used in powder form to prepare activated carbon support. Then, KOH (potassium hydroxide) was impregnated over the prepared support by the wet impregnation method. The moisture content of the rice bran powder was 0.035 (wt%). The powder was further screened through a sieve size of 125 μ m to reject the larger particles. The filtered particles were treated with concentrated mineral acid (H₂SO₄) in the ratio of 5:1 (v/w) for the initial carbonization step under one hour of mixing (Wang et al., 2021). Furthermore, the same quantity of distilled water was further included in the blend to reduce viscosity, and then the obtained sludge composition was allowed to age for 24 hours. After aging, the mixture was further diluted by adding an identical volume of distilled water. A saturated KOH was supplemented to balance the acidity to make it neutral, the solid particles and the crystallized potassium salts were purified and dried at 110 °C for 2–3 hours. These solids were then shifted to the tube furnace for pyrolysis under nitrogen at 700 °C for 4 hours. Finally, the activated carbon was washed with hot distilled water 2–3 times and then further dried. Further, activation was executed by the wet impregnation method, in which activated carbon was allowed to plunge into saturated KOH solution and stirred for 4 h at 500 rpm and then kept under agitation for 24 h. After agitation, the wet-loaded carrier was separated from the solution by filtration and dried out in the oven at 110 °C for 2–4 h, further calcined under an inert atmosphere for 2 h at 450 °C. The whole methodology for the catalyst synthesis is shown in **Figure 3. 4**. Finally, the synthesized catalyst particles were characterized by a sieve analysis test, scanning electron microscopy (SEM (JEOL, Model: JSM-5800)), and X-ray diffraction (XRD) analysis, Bruker, D8 Advance Philips PW 1840 diffractometer, operated

with Cu-K α radiation having a wavelength of 1.542Å in the angular range of 20° - 120° with a step size of 0.019° for a period of 0.2 s per step.

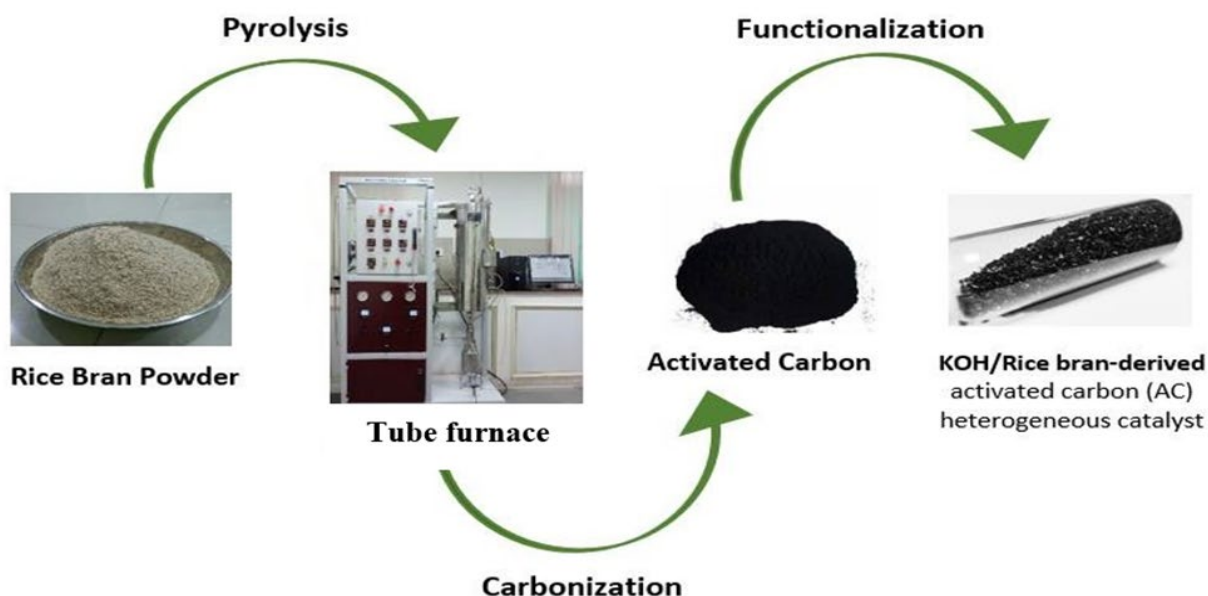


Figure 3. 4 Experimental methodology for the preparation of KOH/rice bran-derived activated carbon heterogeneous catalyst

3.8 In-situ transesterification

Experiments were conducted in a 250 ml round bottom flask placed in an oil bath for temperature maintenance. The setup had a stirrer for agitation and a thermometer dipped in the flask to observe process temperatures, see **Figure 3. 5**. In this experiment, a 1:10 w/v ratio of

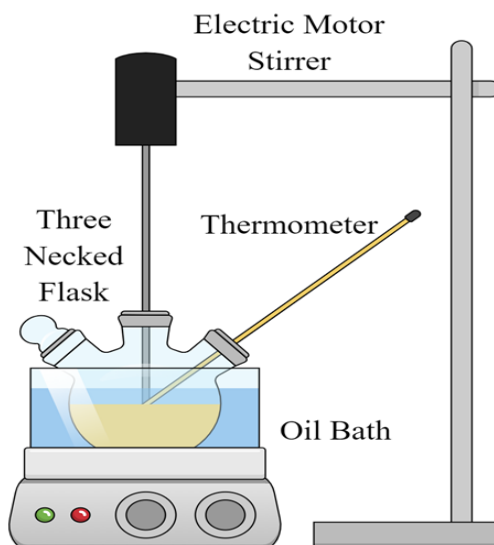


Figure 3. 5 Experimental set-up for in-situ transesterification

solvent and co-solvent was used according to the weight of dry biomass. After adding solvent, co-solvent, and biomass, the catalyst with varying concentrations was supplemented in various quantities for distinct reaction times, temperatures, and RPM (rotations per minute) as reported by the experimental design discussed in the below-mentioned section. In the last part of the process, the whole mixture was cooled down at room temperature and then transferred to the separating funnel. The mixture was then put to rest for some time until all the separate phases of esters, glycerol, residual biomass, and catalyst were visible. The ester layer was the topmost layer; after that, there was a layer of glycerol, and finally, the bottom layer was a mixture of waste biomass and the catalyst. The bottom two layers were removed from the flask. The uppermost ester layer was further filtered by drying it at 80 °C and washing it several times with distilled water to eliminate the remaining impurities. The biodiesel yield was calculated by the given equation (Ogunkunle et al., 2017).

$$\text{Yield \%} = \frac{M_O}{M_B} \times 100 \quad \text{Eq. 3.3}$$

where *Yield%* = biodiesel yield %; *MO* = mass of extracted oil (g); *MB* = mass of the dry algal biomass (g).

3.8.1 In-situ transesterification process utilizing commercially available catalyst (H₂SO₄)

This experiment was conducted to find the suitable solvent or solvent and co-solvent combinations by hit and trial method to be utilized in the in-situ transesterification by the synthesized catalyst in the succeeding section by utilizing a commercial catalyst (H₂SO₄). The in-situ transesterification process was conducted with the following solvents and co-solvents – methanol, ethanol, methanol + chloroform, methanol + hexane. These were added to the dry biomass in the ratio of 1:10 w/v along with 5% v/v H₂SO₄ for the reaction time of 120 min, 70 °C temperature at 400 RPM (Chamola, 2019). The in-situ transesterification process was

conducted under the aforementioned section conditions. The experiment was done in triplicates for each solvent and co-solvent combination. The biodiesel yield percentage for each case was obtained by equation. The combination of solvent and co-solvent with maximum biodiesel yield % was preferred for the in-situ transesterification by the synthesized catalyst.

3.8.2 In-situ transesterification process utilizing a synthesized catalyst

In this experiment, a suitable combination of solvent and co-solvent (found in the foregoing section), time, temperature, and synthesized catalyst were used for the in-situ transesterification process. The statistical regression analysis method was utilized to model and analyse all the parameters. All the experiments were performed by the aforementioned section conditions.

3.8.2.1 Experimental design for optimization

Regression analysis – a statistical technique was used to model and analyse dependent and independent variables (Singh and Verma, 2019a). The process parameters which control the quality of the biodiesel yield percentage (Y%) were time (T) (1–2 h), catalyst loading (CL) (3–5 wt%), (methanol + hexane) to dry algae biomass molar ratio (S + CS) (5:1–15:1), and temperature (Tp) (60–80 °C) (Chowdhury et al., 2021; Johan et al., 2014; Rendón, 2013). The ranges of these parameters were carefully chosen over a few trials, see **Table 3. 1** . The dependent variable was the biodiesel yield percentage estimated from Eq. 3. 3. In this study, the experiments were conducted using the central composite design (CCD) used in response surface methodology (RSM) (facilitates to detect the suitable approximation for the actual functional relationship between the response and the set of independent variables) for building the second-order polynomial regression model to a given response variable, instead of using complete three-level full factorial experiments. This methodology played a crucial role in studying the effect of input process parameters on the yield, especially in this biodiesel extraction process methodology. The variables or factors were set at the midpoint of their

respective ranges and trials at the extreme levels in response surface design. The number of centre points was taken as three, and three replicates were considered for each combination of process parameters. Therefore, a total of sequences of process parameters were taken for doing the experiments. The effectiveness of models was evaluated by determining R^2 (determination coefficient), and RSM attained ideal conditions of the reaction. After getting optimized values for every independent variable, the transesterification process was carried out using the optimized parameters.

Table 3. 1. Process parameters and their ranges used for quality of biodiesel yield percentage.

S. No	Inputs/unit	Coded symbol	Uncoded symbol	Minimum Value	Mid value	Maximum value
1.	Temperature (hrs.)	X_1	T_p	1	1.5	2
2.	Biomass molar Ratio	X_2	$S+CS$	0.06	0.13	0.2
3.	Catalyst Loading (wt.%)	X_3	CL	3	4	5
4.	Time (hrs.)	X_4	T	1	1.5	2

3.8.2.2 Experimental model development

Direct transesterification reaction was directed over conventional mechanical stirring utilizing the synthesized heterogeneous catalyst. Twenty-eight runs of experiments were carried out as per the suggested experimental design. All these runs of experimental design with the biodiesel yield are shown in the results and discussion chapter.

3.8.2.3 Method of analysis

Regression analysis: The implementation of non-linear statistical analysis using Minitab 18 software [44] to create input–output relationships of the process is shown below in Eq. 3. 4:

$$X = \alpha_0 + \sum_{i=1}^k \alpha_i x_i + \sum_{i=1}^k \alpha_{ii} x_i^2 + \sum_{i=1}^k \sum_{i < j}^k \alpha_{ij} x_i x_j + \varepsilon \quad \text{Eq. 3. 4}$$

where X is the response; $\alpha_0, \alpha_1, \alpha_2, \dots, \alpha_k$ represents coefficients of the variables to be computed using a least-squares method. Here, ε positions for the error in the fitting. During regression analysis, a significance test of each of the process parameters was conducted for each of the responses. The format of the significance test was obtained using MINITAB software. The adequacy of the developed model was tested through an analysis of variance (ANOVA). The effectiveness of the developed regression model was assessed by comparing the model's predicted values with the experimental ones. The time, catalyst loading, (methanol + hexane) to dry algae biomass molar ratio, and temperature were represented as the functions of input process parameters, respectively.

3.8.2.4 Input-output mapping using regression analysis

The mapping between inputs and the output was associated by using regression analysis. The significance test was executed to study the model's capability at a confidence level of 95%. Yield percentage (Y%) was characterized in the coded form primary as a non-linear function of input process parameters, namely, time (T) (1–2 h), CL (3–5 wt%), (methanol + hexane) to dry algae biomass molar ratio (S + CS) (5:1–15:1), and temperature (Tp) (60–80 °C), denoted by X_1, X_2, X_3 , and X_4 , respectively. The significance test results for Y% and ANOVA (Analysis of variance) are presented in the preceding chapter.

3.9 Characterization and analysis of biodiesel properties

The composition of the biodiesel sample attained at the most acceptable conditions for the instantaneous esterification-transesterification conditions was determined using Varian 450-GC coupled with a 240-MS mass spectrometer. Helium was used as carrier gas with a flow rate of 0.6 mL min⁻¹. The MS ion source temperature was kept at 180 °C. FAME components

present in the biodiesel were identified by corresponding spectra with similar spectra using the National Institute of Standards and Technology Research (NIST) library software.

Some of the important quality parameters of biodiesel were analysed to examine the performance of biodiesel (de Blasio, 2019b). The properties of algal biodiesel - kinematic viscosity (mm^2/s), relative density (kg/m^3), calorific value (MJ/kg), pour point ($^{\circ}\text{C}$), and flash point ($^{\circ}\text{C}$) were examined and followed as per ASTM standards (D6751) (de Jesus et al., 2020). Three trial tests for all properties were done and their means were calculated.

Chapter 4

Results and Discussion

This chapter documents all the results from the experimental research work carried out for the sustainable biodiesel production from indigenous microalgae procured from a wastewater oxidation pond. In the present study, attempts were made to improve the cost-effectiveness of microalgal biodiesel production. Utilization of the indigenous microalgae cultures collected from the rural oxidation ponds as an alternative to pure cultures was done for cultivation to save time, toil, and cost required to isolate the algae cultures. Furthermore, indigenous microalgae were cultivated in the wastewater to eliminate the need for freshwater, and nutrients. Additionally, a waste-based heterogeneous catalyst derived from rice bran powder was synthesized for the in-situ transesterification process to eliminate the need for expensive catalysts and reduce the environmental impact of the production process. Finally, optimization of process parameters was also done for the in-situ transesterification process to maximize the biodiesel yield.

At the onset, collected wastewater was characterized to verify the presence of essential nutrients for microalgae growth. Aftermath the cultivation of microalgae in wastewater was accomplished, the algae growth was analysed by determining the growth pattern, biomass growth, and chlorophyll-A. Simultaneously, isolation and identification of collected indigenous microalgae was carried out to compare the biodiesel yield by mixed indigenous cultures with the individual pure cultures.

In addition, the synthesis of a waste-based heterogeneous catalyst derived from rice bran powder was carried out, and the catalyst characterization was carried out with SEM and XRD. Furthermore, trial experiments were run for the in-situ transesterification with H_2SO_4 as a catalyst and various solvent and solvent-co-solvent combinations. A preferable solvent or solvent-co-solvent combination was selected and utilized further in the in-situ transesterification with a synthesized catalyst. Moreover, multiple experiments were carried out for the in-situ transesterification process with synthesized catalysts. The selected solvent-co-solvent combination and the optimization of the process parameters was accessed to

maximize biodiesel production yield. Also, the biodiesel yield with mixed indigenous culture was compared with the yield of pure isolated culture. Finally, the characterization of the produced biodiesel was performed by GC-MS, and some of the quality parameters of biodiesel were analysed to examine the performance of biodiesel. The experimental workflow is depicted in a flow chart **Figure 4. 1** to visually represent what and how the research has been described in subsequent sections.

4.1 Wastewater characterization

Numerous physical parameters like light intensity, temperature, oxygen, and nutrients affect microalgae growth. Parameters other than the micro-nutrients in wastewater were already controlled in the laboratory setup during cultivation. Although microalgae were already proliferating in the collected wastewater when present in the rural oxidation pond, it still necessitates analysis for the essential nutrients, nitrogen (N) and phosphorus (P) being the most important ones for algae cultivation. Characterization was conducted to make sure of any nutrient deprivation. Analysis of parameters like pH, conductivity, total dissolved solids, total chemical oxygen demand (COD) and soluble COD, biochemical oxygen demand (BOD), total phosphates, nitrates, and dissolved oxygen (DO) are analyzed as per APHA standard methods. The average values of all the parameters after the analysis of collected wastewater are depicted in **Table 4. 1**

As shown in **Table 4. 1**, the wastewater's mean pH value is 7.29. The pH range required for the growth of indigenous microalgae is between 7-9 (Mishra et al., 2015). In our study, the pH value was observed in the given range and was maintained throughout the growth period by providing continuous mixing. Failing to do so disrupts various cellular processes, leading to the inhibition of biomass growth. Dissolved oxygen is an additional important parameter for biomass productivity. Higher concentrations of dissolved oxygen can exhibit the growth of photosynthetic organisms. Kazbar et. al investigated the effect of higher concentrations of dissolved oxygen in the photobioreactor, and results showed a loss of biomass productivity at concentrations higher than 30 mg/l (Kazbar A., Cogne G., Urbain B, Marec H., Le-Gouic B, Tallec J.,Takache H., Ismail A., 2019). In the present study, dissolved oxygen

Research work flow

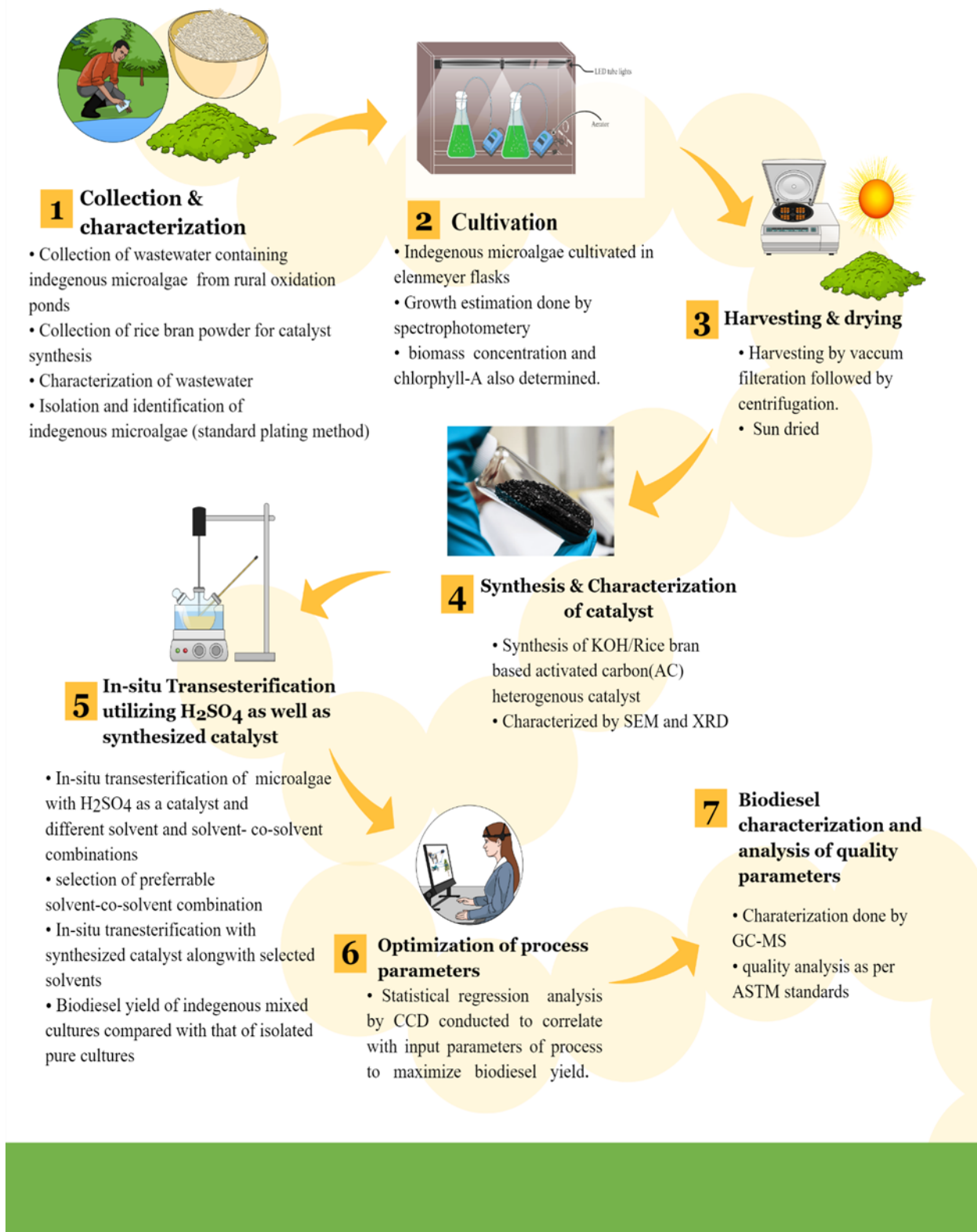


Figure 4. 1 Experimental Workflow

concentration was less than 30mg/l. Other crucial parameters for microalgal growth were nitrates (N-NO₃⁻) and phosphates (P-PO₄³⁻). Their ratio in the wastewater decides the optimum growth of microalgae. The N:P ratio for the growth of indigenous microalgae is given as 1:1.5 with no addition of extra carbon dioxide (Montoya-Vallejo et al., 2023). In the present study, the ratio was coming out to be approximately 1:15, which is considered acceptable for microalgae growth. Other parameters like conductivity and total dissolved solids are associated with the salinity of the wastewater, which is another crucial parameter for the algal growth (Xu et al., 2022). Bartley et. al researched to observe the effect of the salinity of the wastewater on the growth of microalgae. They found that the optimum growth of microalgae occurs between the salinity values of 22 and 34 PSU (Practical Salinity Unit) (Bartley et al., 2013). In this relation, conductivity and total dissolved solids were coming in range to neglect the effect on microalgae growth. Overall, all the characteristic parameters were coming within the ranges for optimum microalgae growth in the collected wastewater, and there is no need to provide additional nutrients.

Table 4. 1 Physio-chemical analysis of the collected wastewater

S. No.	Parameters	Measurement unit	Mean Values
1	pH	-	7.29
2	Conductivity	mS/cm	0.98
3	Total Dissolved Solids	mg/l	493.80
4	COD	mg/l	112
5	Soluble COD	mg/l	80
6	DO	mg/l	3.71
7	BOD	mg/l	17
9	Nitrates	mg/l	1.80
10	Phosphates	mg/l	2.71

4.2 Identification and characterization of indigenous microalgae collected from wastewater.

4.2.1 Cultivation of microalgae in BG-11 media

Microalgae was allowed to cultivate in triplicates in a 1000 ml Erlenmeyer flask containing 800 ml BG-11 media and 100 ml microalgae for 15 days. LED light (illumination: > 20,000 lx) was utilized to supply constant light intensity with a temperature of 28 ± 3 °C. Also, an air bubbler was provided for mixing to maintain pH in the flasks. Microalgae growth estimation and chlorophyll-A content in the cultivation media was observed and presented in the figures **Figure 4. 2** and **Figure 4. 3**.

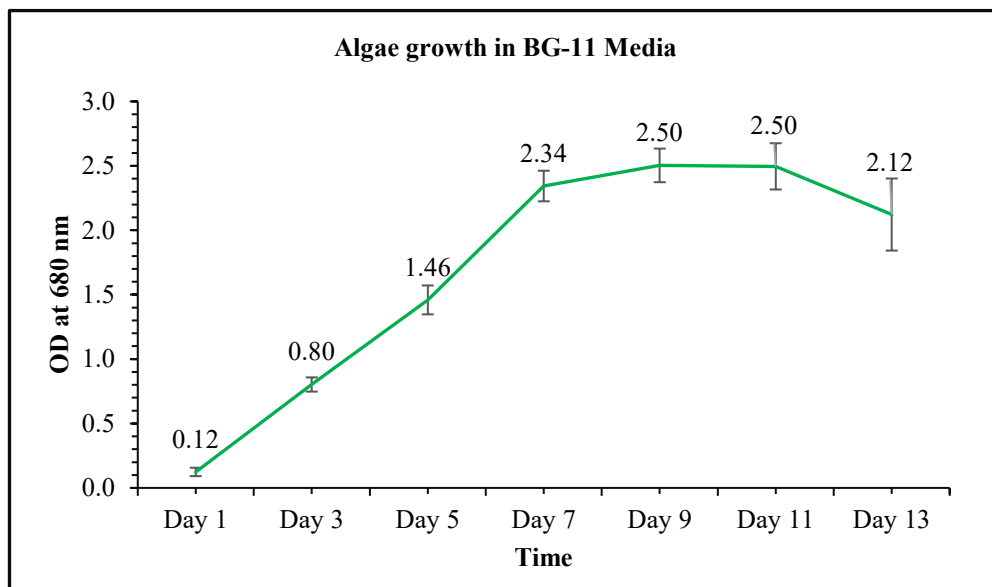


Figure 4. 2 Microalgae growth pattern in BG-11 media (optical density at 680nm vs. time)

It is clear from **Figure 4. 2** that the algal growth cultivated in BG-11 media was following the logarithmic growth pattern. It is ascertained that there was a significant growth of algae in the wastewater in which the lag phase lasted for 5 days. The exponential growth phase was sustained until the 9th day of the culture, and then the stationary phase was identified between the 9th and 11th day, and finally, after the 11th day, the death or decline phase was seen (Namitha et al., 2021; Putri et al., 2019). The presence of chlorophyll-

A was confirming the presence of algae and the increasing linear trend confirmed the increase in chlorophyll-A in the media over the observed time (Talapatra et al., 2023).

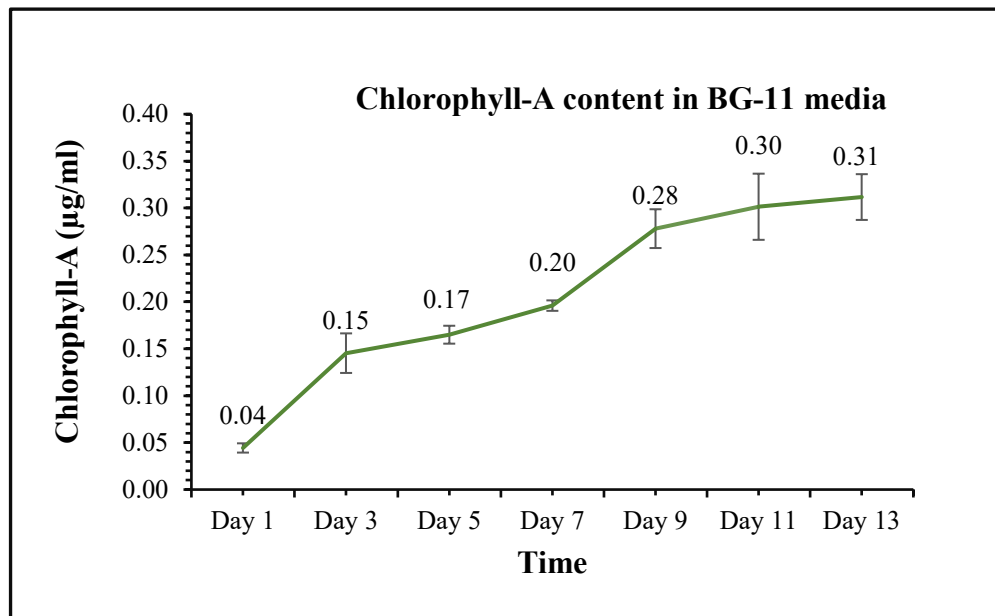


Figure 4. 3 Chlorophyll-A content for the grown culture in BG-11media (calculated from Equation 3.2)

4.2.2 Identification and characterization

After cultivation, the grown algae cultures were streaked on BG-11 media with 2% agar and finally allowed to grow for 15 days in the same conditions. Aftermath, repeated subculturing was done by picking the individual grown colonies by pour plate technique. This method was repeated till purified culture plates are obtained. Finally, purified cultures were examined for morphological and molecular identification. Morphological characterization was based on the size and shape under the microscope, as shown in **Figure 4. 4.** From the microscopic image in **Figure 4. 4 (a)**, it was observed that the culture has spherical single cells with visible cell walls. It was assumed to have a similarity with *Chlorella* sp. (Safi et al., 2014). In **Figure 4. 4(b)**, it was assumed that the culture has a pale to blue-green green color with densely thin entangled filaments that resemble *Desertiflum* sp. (Cai et al., 2017).

For the exact identification of the isolated cultures, molecular identification was done by sequencing the isolated strains by 16SrRNA gene followed by a basic BLASTN search of the GenBank databases. rDNA

sequences of the isolates showed query sequence NC140222 has 99.51% identity to *Desertiflum salkalinema*, and the query sequence NC21022 has 99.9% similarity to *Chlorella vulgaris*.

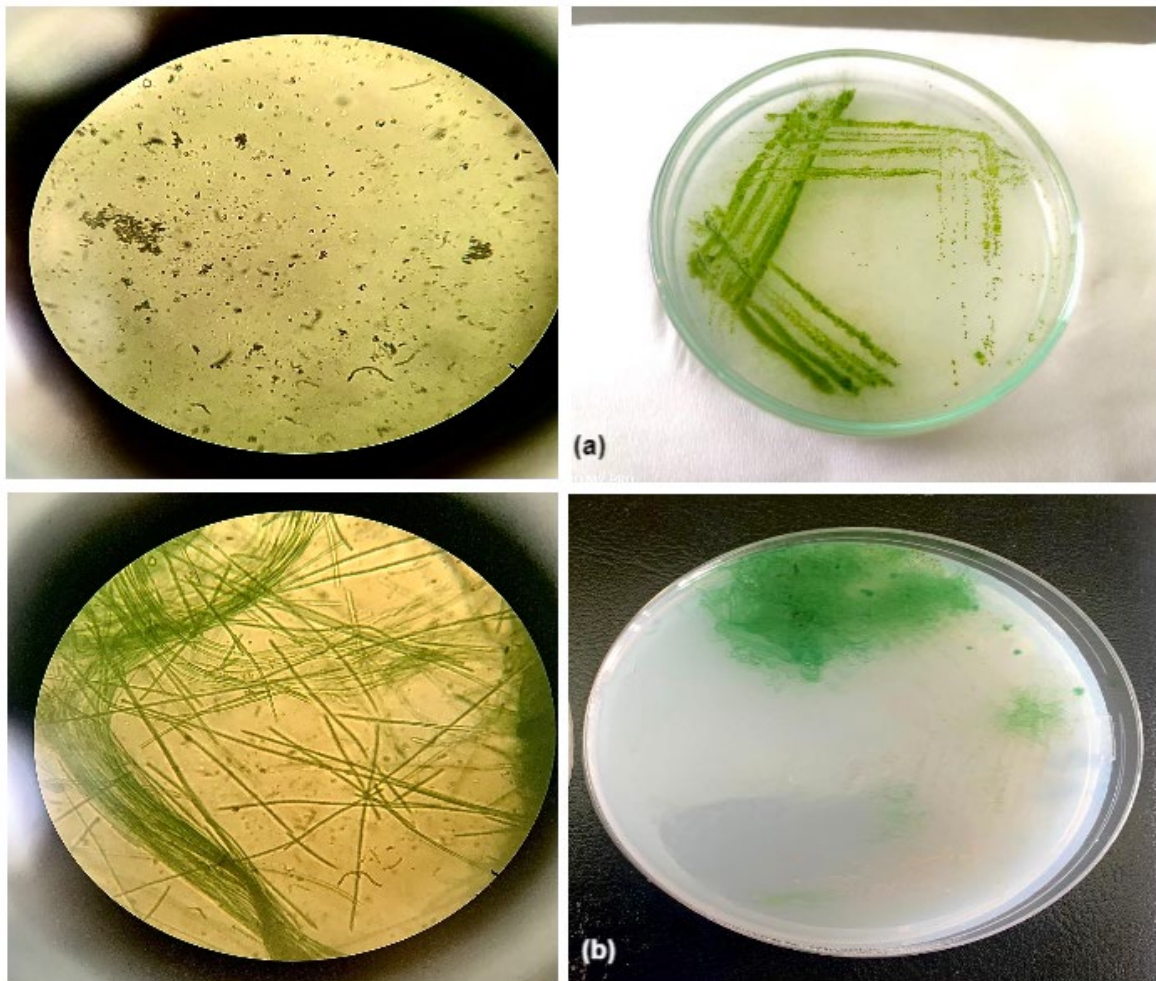


Figure 4. 4 Microscopic images of the isolated cultures at 100X

4.3 Microalgae cultivation in wastewater

Microalgae were allowed to cultivate in triplicates in a 1000 ml Erlenmeyer flask containing 800 ml collected wastewater and 100 ml microalgae for 15 days. A Microalgae cultivation setup was installed to fulfil all the essential factors like light intensity and temperature responsible for growth. It was set up to duplicate natural conditions present at the oxidation pond. LED light (illumination: > 20,000 lx) was utilized to supply constant light intensity with a temperature of 28 ± 3 °C. Also, an air bubbler was provided for mixing to maintain pH in the flasks. Microalgae growth estimation was evaluated every

alternate day for 2 weeks. The observed graph for the optical density using spectrophotometry at 680 nm with time is shown in **Figure 4. 5**.

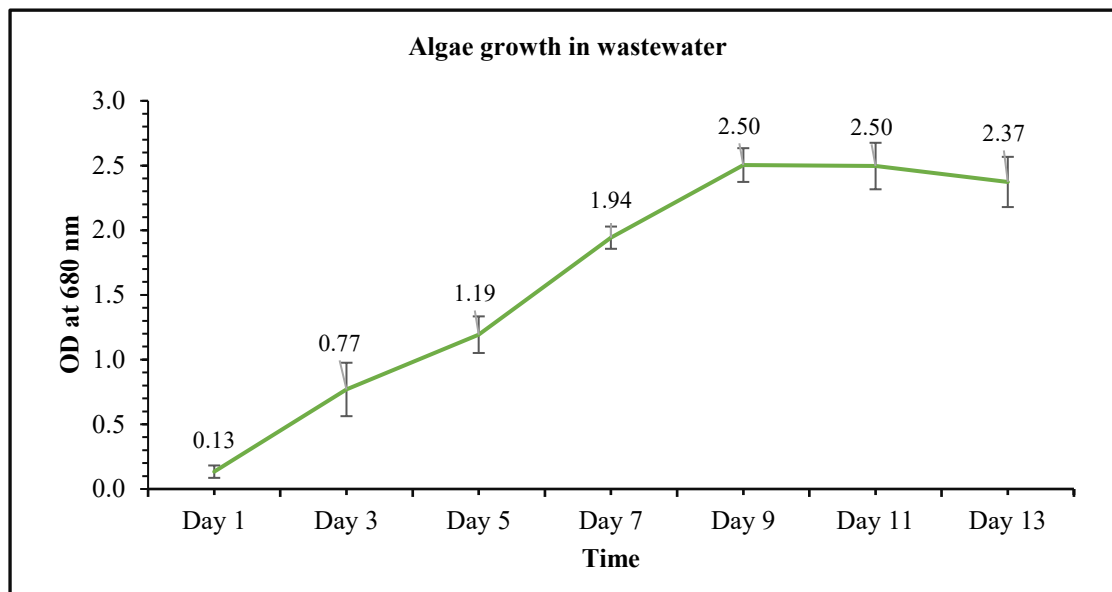


Figure 4. 5 Microalgae growth pattern in wastewater (optical density at 680nm vs. time)

From **Figure 4. 5**, it is ascertained that there is a significant growth of algae in the wastewater in which the lag phase has lasted for 5 days. The exponential growth phase was sustained until the 9th day of the culture, and then the stationary phase was identified between the 9th and 12th day, and finally, after the 12th day, the death or decline phase was seen (Namitha et al., 2021; Putri et al., 2019). The biomass concentration (g/l) is calculated using **Equation 3.1** for 15 days on every alternate day. The biomass concentration is shown in **Table 4. 2**. It was observed that the biomass content was increasing with the growth of microalgae in the wastewater. However, at the end of the observation time, when growth started to decline, total biomass increased due to the accumulation of the dead biomass in the media. Moreover, the chlorophyll-A content of the grown culture was determined by **Equation 3. 2**. The corresponding graph for chlorophyll-A content against the number of days is represented in **Figure 4. 6**., It represented the same trend mentioned in the forgoing section regarding chlorophyll-A content in BG-11 media.

Table 4. 2 Biomass production (g/l) in relation to absorbance at 680nm

No. of Days	Average OD at 680 nm	Biomass Production (g/l)
Day 1	0.13	0.13
Day 3	0.77	0.58
Day 5	1.19	0.87
Day 7	1.94	1.40
Day 9	2.50	1.79
Day 11	2.50	1.79
Day 13	2.37	1.89

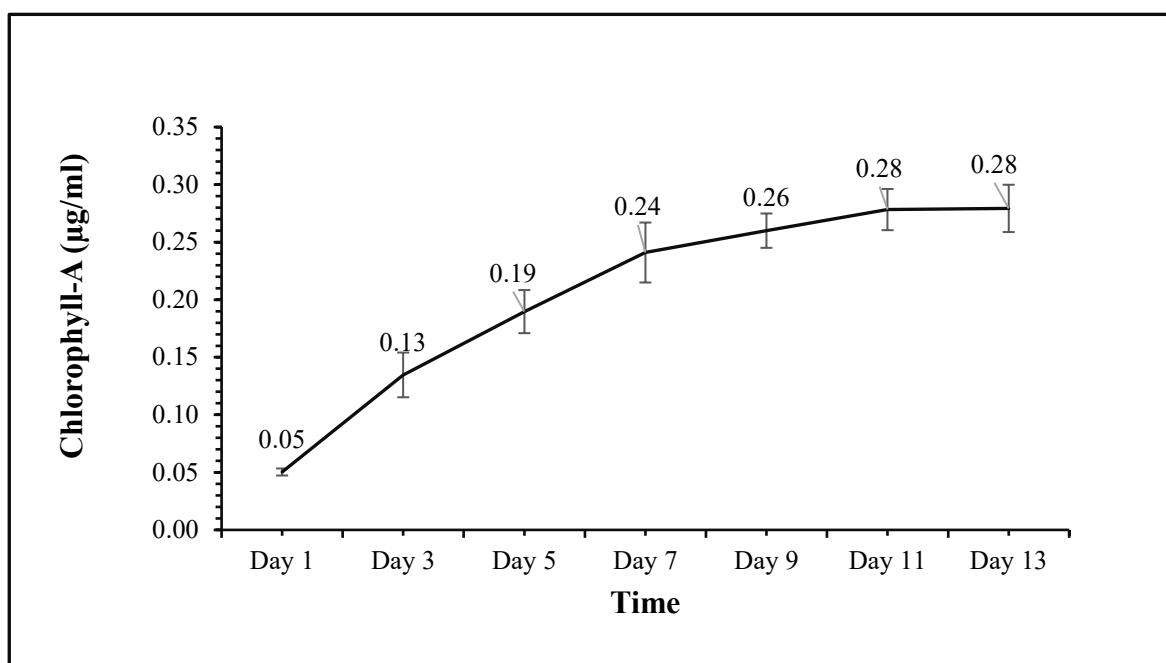


Figure 4. 6 Chlorophyll-A content for the grown culture in wastewater (calculated from Equation 3.2)

4.4 Characterization of KOH/rice bran AC heterogeneous catalyst

The surface morphology of the catalyst was analyzed using SEM at 2 µm (**Figure 4. 7(a)** and **Figure 4. 7(b)**). It was observed that the activated carbon carrier, which was highly porous before, is now finally packed and enclosed with KOH (potassium hydroxide). As per the following images, it was observed that the synthesized catalyst is comparatively non-porous, and the reactions will now typically occur on its surface. Furthermore, **Figure 4. 8** displays the XRD analysis of the KOH/rice bran AC catalyst. It shows

a clear, solid KOH peak at about 32.9° , besides other weak peaks at 29.8° and 13.1° because of traces of potassium lead oxide and potassium uranyl fluoride sulphate hydrate. The other weak peaks may be due to the traces of pesticides or insecticides in the rice bran powder. The results reported sharp XRD reflections with (0 1 1), (0 2 1), (2 2 0), (1 1 0), and (2 2 2) orientations, suggesting that the calcined material is well crystallized throughout the process.

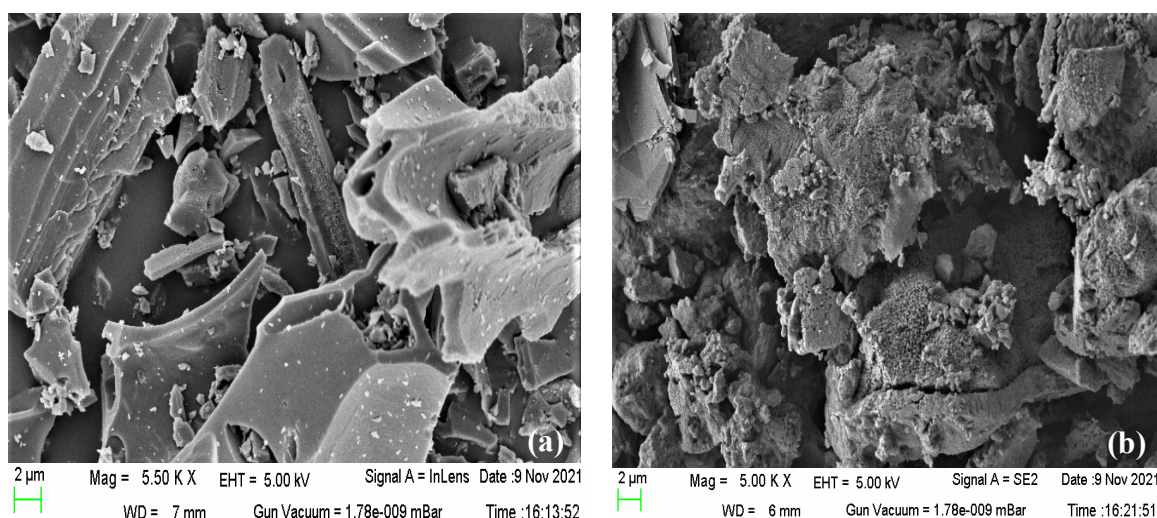


Figure 4. 7(a) SEM image of activated carbon (carrier) at 2 μm, (b) SEM image of the synthesized catalyst at 2 μm.

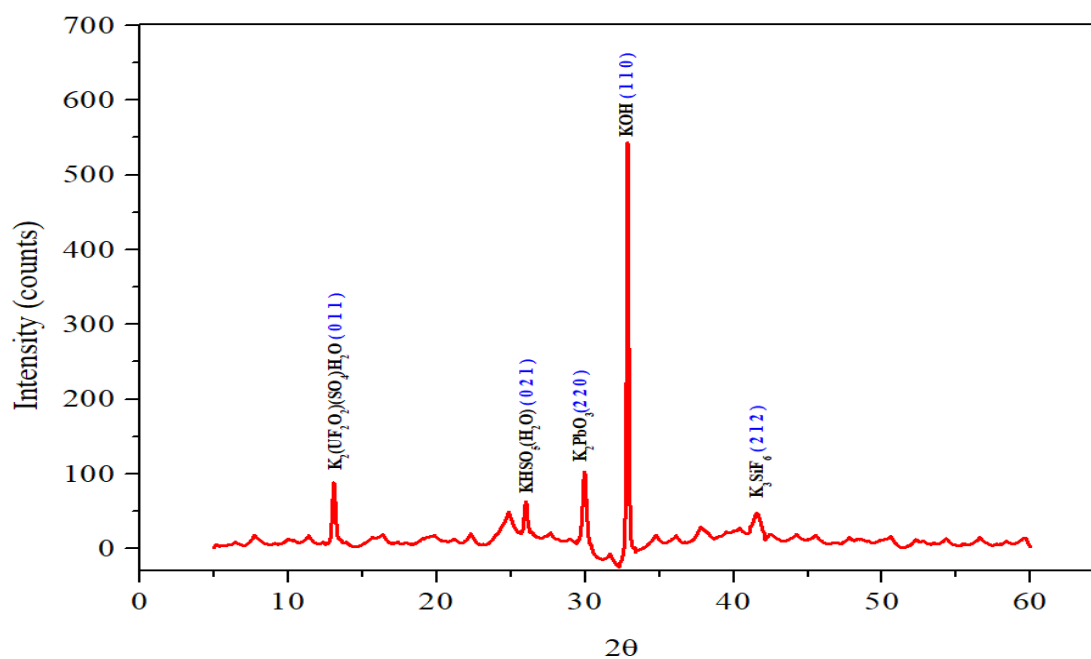


Figure 4. 8 XRD analysis of the “KOH/Rice-bran activated carbon” catalyst.

4.5 In-situ transesterification by (H₂SO₄)

This experiment was conducted to find the suitable solvent or solvent and co-solvent combinations to be utilized in the in-situ transesterification by the synthesized catalyst. The experiments were conducted by selecting the 10: 1 of solvents to dry biomass ratio, with 5% H₂SO₄, for 120 minutes at 70°C temperature with 500 rpm. The experiment was done in triplicates for each solvent and co-solvent combination. The biodiesel yield percentage for each case was obtained and the maximum yield was observed for the combination of methane and hexane **Figure 4. 9** (Ummu Kalsum et al., 2022).

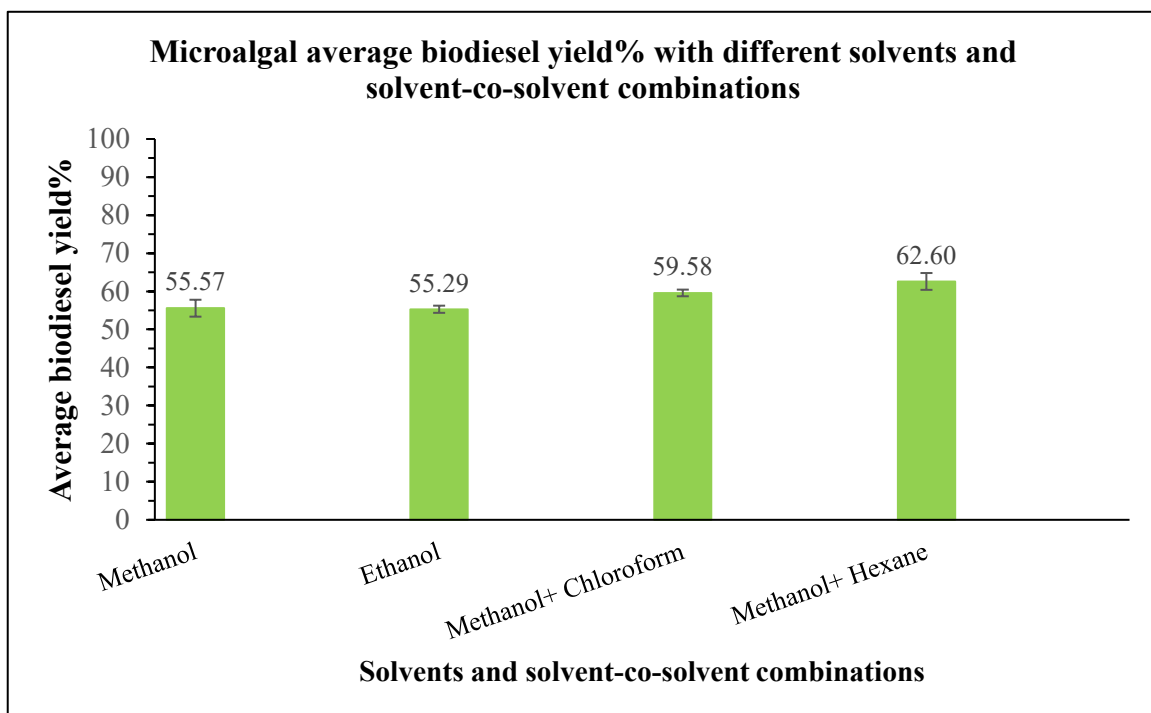


Figure 4. 9 Biodiesel yield with different solvent and solvent-co-solvent combinations

4.4. In-situ transesterification by KOH/rice bran AC heterogeneous catalyst

This experiment used a suitable combination of solvent and co-solvent (methanol + hexane in the preceding section), time, temperature, and synthesized catalyst for the in-situ transesterification process. The statistical regression analysis with CCD was utilized to model and analyse all the parameters.

4.5.1 Statistical Regression Analysis

Direct transesterification reaction was directed over conventional mechanical stirring utilizing the synthesized heterogeneous catalyst.

Table 4. 3 has been obtained by conducting various runs per the suggested experimental design.

Table 4. 3 Results of experimental design.

Run Order	Temp	S+CS	Caty	Time	Y%
1	70	0.13	3	1.5	74.39
2	60	0.2	3	2	66.05
3	80	0.2	3	2	69
4	80	0.06	3	1	52.92
5	70	0.13	4	2	71
6	80	0.06	5	2	71.96
7	60	0.2	3	1	58.4
8	60	0.13	4	1.5	66.7
9	80	0.06	3	2	57.39
10	70	0.13	4	1	70.5
11	80	0.2	5	2	75.05
12	70	0.13	4	1.5	70.9
13	70	0.13	4	1.5	70.9
14	70	0.13	4	1.5	70.9
15	70	0.2	4	1.5	70.9
16	60	0.06	3	2	61.32
17	80	0.2	3	1	59.58
18	60	0.2	5	1	69.5
19	70	0.13	4	1.5	70.9
20	70	0.06	4	1.5	67.89
21	80	0.2	5	1	74
22	80	0.06	5	1	70.01
23	60	0.06	5	2	72.26
24	60	0.06	3	1	49.63
25	80	0.13	4	1.5	72.2
26	60	0.06	5	1	65.37
27	70	0.13	5	1.5	81.8
28	60	0.2	5	2	68.4

4.5.2 Input-output mapping using regression analysis

The mapping between inputs and the output were associated by using regression analysis. The significance test was executed to study the model's capability at a confidence level of 95 %. Yield percentage ($Y\%$) was characterized in the coded form primary as a non-linear function of input process parameters, namely, time (T) (1–2 h), catalyst loading (CL) (3–5 wt.%), (methanol + hexane) to dry algae biomass molar ratio (S+CS) (5:1–15:1), and temperature (T_p) (60–80 °C) denoted by X_1 , X_2 , X_3 , X_4 , respectively. The significance test results for $Y\%$ and analysis of variance by ANOVA are presented in **Table 4. 4** and **Table 4. 5**, respectively.

Table 4. 4 Results of significance test for biodiesel yield %

S.no	Term	Coeff.	SE Coeff.	T	p
1	Constant	72.6283	0.7995	90.841	0.000
2	X_1	1.3600	0.5450	2.495	0.027
3	X_2	2.3406	0.5450	4.295	0.001
4	X_3	5.5372	0.5450	10.160	0.000
5	X_4	2.3622	0.5450	4.334	0.001
6	X_1^2	-4.3304	1.4397	-3.008	0.010
7	X_2^2	-4.3854	1.4397	-3.046	0.009
8	X_3^2	4.3146	1.4397	2.997	0.010
9	X_4^2	-3.0304	1.4397	-2.105	0.055
10	$X_1 X_2$	0.7238	0.5780	1.252	0.233
11	$X_1 X_3$	0.7500	0.5780	1.297	0.217
12	$X_1 X_4$	-0.5150	0.5780	-0.891	0.389
13	$X_2 X_3$	-1.5263	0.5780	-2.640	0.020
14	$X_2 X_4$	-0.4987	0.5780	-0.863	0.404
15	$X_3 X_4$	-1.5275	0.5780	-2.643	0.020
S = 2.31218;		R-Sq = 94.77%;		R-Sq (adj.) = 89.15%	

Table 4. 5 Analysis of variance (ANOVA) test results for biodiesel yield %

Source	DoF	Seq. SS	Adj. SS	Adj. MS	F-value	p
Regression	14	1260.61	1260.606	90.043	16.84	0.000
Linear	4	784.24	784.237	196.059	36.67	0.000
Square	4	376.16	376.161	94.040	17.59	0.000

Interaction	6	100.21	100.208	16.701	3.12	0.040
Residual Error	13	69.50	69.500	5.346		
Lack-of-fit	10	69.50	69.500	6.950		
Pure Error	3	0.000	0.0000	0.0000		
Total	27	1330.11				

$X_1, X_2, X_3, X_4, X_1^2, X_2^2, X_3^2, X_4^2, X_2 X_3, X_3 X_4$ is significant. It suggests that the relationship between yield percentage ($Y\%$) found to be non-linear with temperature (X_1), biomass molar ratio (X_2), (methanol + Hexane) to dry algae biomass molar ratio (X_3), and time (X_4). The model was statistically acceptable for making predictions, as the coefficient of correlation was attained as 0.9477. The F values seemed greater than those in the standard statistical table. Therefore, the model was statistically acceptable (Singh and Verma, 2019b). The un-coded form of yield percentage ($Y\%$) was found to be in the *Eq. 4. 1*.

$$\begin{aligned}
Y\%_{Un-coded} = & -168.884 + 5.91871(T_p) + 302.347(S + CS) - 26.8123(CL) + 62.3722(T) - \\
& 0.0433043(T_p)^2 - 894.987(S + CS)^2 + 4.31457(CL)^2 - 12.1217(T)^2 + 1.03393(T_p)(S + CS) + \\
& 0.075(T_p)(CL) - 0.1030(T_p)(T) - 21.8036(S + CS)(CL) - 14.2500(S + CS)(T) - \\
& 3.05500(CL)(T)
\end{aligned}
\tag{Eq. 4. 1}$$

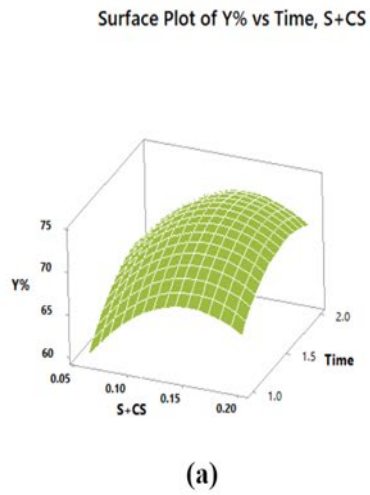
4.5.3 Effect of reaction parameters on the process yield

The interactions of the four reaction parameters, time, solvent + co-solvent to biomass molar ratio, catalyst ratio, and temperature, were examined through response surface methodology. Response surfaces and contour lines that associate biodiesel yield to the considered parameters is given in **Figure 4. 10**. In **Figure 4. 10 (a)**, during the *in-situ* transesterification process, the biodiesel yield is directly proportional to the increasing reaction time until the point when yield gets inversely proportional. After that, a higher reaction time might not accurately generate more biodiesel. This trend was due to excessive solvent + co-solvent losses, overheating reaction mixture, by-product formation, and other energy losses (Cao et al., 2013). Therefore, biodiesel yield was marginally decreased with the increase in the reaction time in the experiment. The results agreed with the preceding research, suggesting that the time reaching the highest

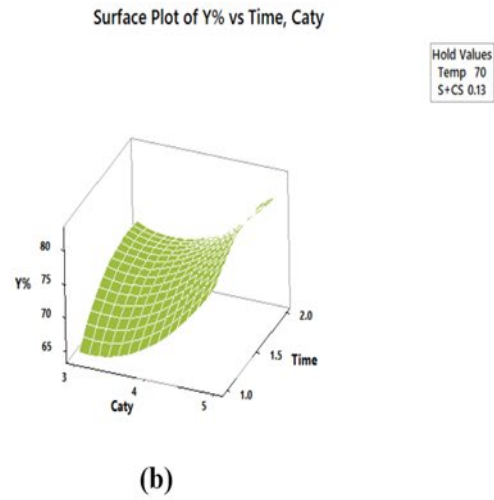
biodiesel yield has been reduced (Wahlen et al., 2011). In **Figure 4. 10 (b)**, the biodiesel yield increased to a molar ratio 0.13. In contrast, an additional increase in the molar ratio decreased the output due to surplus accessibility of alcohol emerging in ester-glycerol recombination. Similarly, an increase in time allowed the yield to increase with an increase in molar ratio, but a further rise in time exposure causes solvent loss that affects the yield (Jambulingam et al., 2020). **Figure 4. 10 (c)**, a gradual increase in biodiesel yield was observed up to a catalyst concentration of 4% due to increased catalytic activity. Still, the yield of biodiesel soon decreased with the additional increase in catalyst concentration due to soap formation (Jambulingam et al., 2020; Naeem et al., 2021). **Figure 4. 10 (d)**, the increase in the yield was noticed with the rise in the amount of catalyst irrespective of time. However, the overall yield gets affected when excess solvents were present along with catalysts. Nonetheless, in this case, the solvent ratio was not in excess; yield increases can be seen (Degfie et al., 2019). In **Figure 4. 10 (e)**, the catalytic activity of the synthesized catalyst was not affected by the temperature as it is prepared to bear extremely high range temperatures. However, the overall yield is affected by the increase in temperature as it causes the solvent and co-solvent to evaporate from the reaction process (Mohamed et al., 2020). In **Figure 4. 10 (f)**, it was observed that the yield first increases with an increase in temperature and the S+CS ratio. Still, it decreased with a further rise in temperature because of various energy losses and S+CS evaporation. Furthermore, with a further increase in molar ratio concerning temperature, the yield gets affected due to the formation of ester-glycerol by surplus alcohol (Anwar et al., 2018).

4.5.4 Optimization of biodiesel yield%

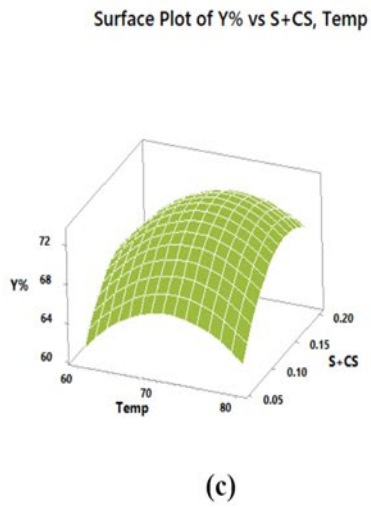
It was observed that the best finest conditions are the time of 1.5 h, S + CS to biomass molar ratio of 10:1, 5 wt% of catalyst loading, and a temperature of 70 °C, upon which the highest predicted yield was 81.8%.



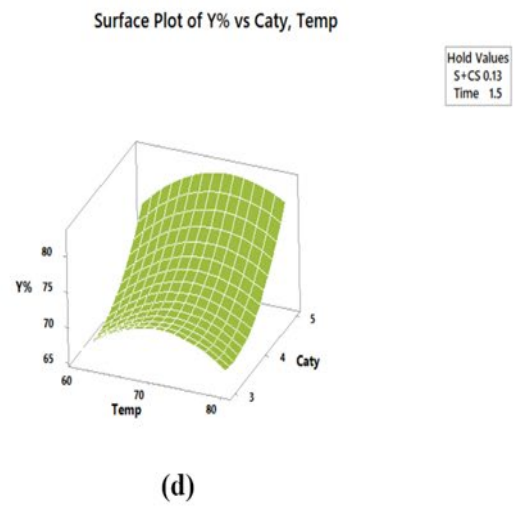
Hold Values
Temp 70
Caty 4



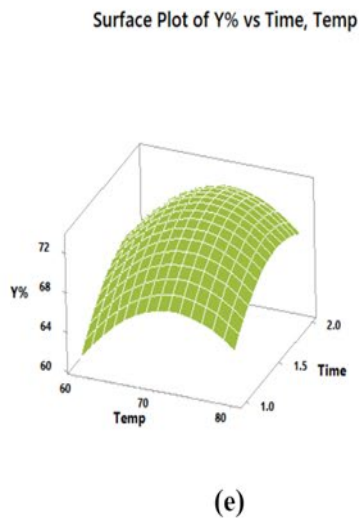
Hold Values
Temp 70
S+CS 0.13



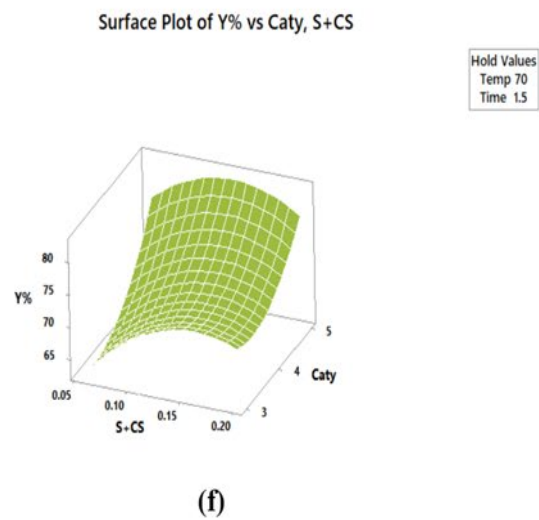
Hold Values
Caty 4
Time 1.5



Hold Values
S+CS 0.13
Time 1.5



Hold Values
S+CS 0.13
Caty 4



Hold Values
Temp 70
Time 1.5

Figure 4. 10 Surface plots of Y% with (a) Tp and T, (b) T and S+CS, (c) S+CS and CL (d) CL

4.6 Comparison study of biodiesel yield%

Finally, an experiment was also performed based on these optimized conditions, and the observed yield was 80.9% with a prediction error lesser than 0.9%. Hence, it can be ascertained that the proposed RSM model was satisfactory to both forecasts of the system behaviour and the optimal conditions. Furthermore, a comparison between the biodiesel yield of mixed algal culture and isolated pure cultures was done, and it was found that yield was maximum in the case of mixed algae culture **Figure 4. 11**.

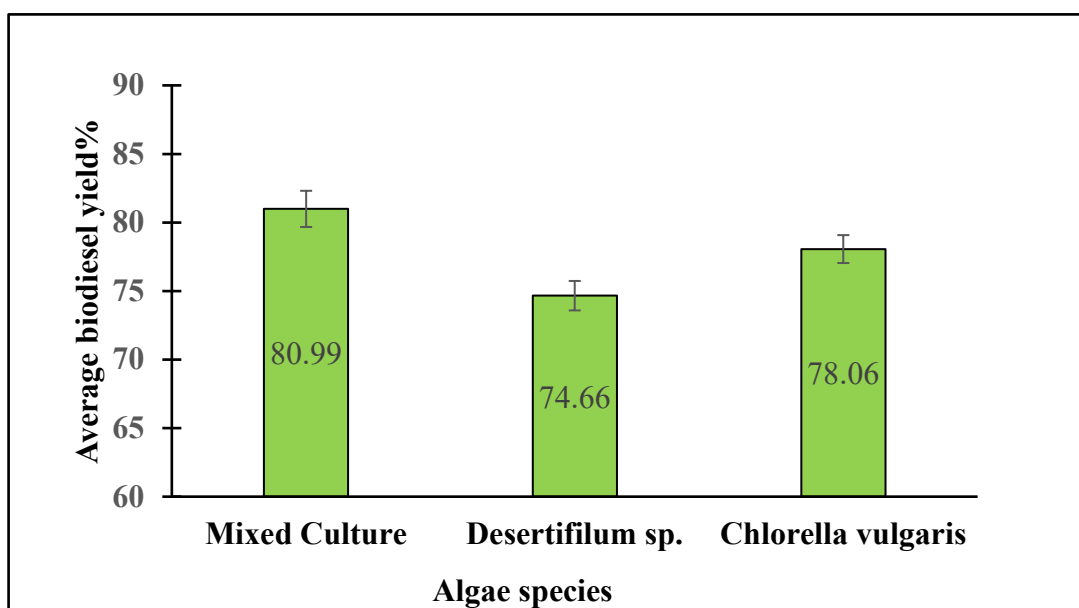


Figure 4. 11 Comparison of biodiesel yield of different algal species

4.7 Biodiesel characterization

The composition of the biodiesel sample attained at the most acceptable conditions for the instantaneous esterification-transesterification conditions was determined using Varian 450-GC coupled with a 240-MS mass spectrometer. Helium was used as a carrier gas with a flow rate of 0.6 mL min⁻¹. The MS ion source temperature was kept at 180 °C. FAME components present in the biodiesel were identified by the corresponding spectra with similar spectra using the National Institute of Standards and Technology Research (NIST) library software. **Table 4. 6** demonstrates the fatty acid compositions acquired by the gas chromatogram of the developed biodiesel. The obtained biodiesel mainly consisted of FAMEs (fatty acid methyl esters) from the C16 and C18 groups. 28.53% of linoleic acid (C18:2) followed by 13.29%

of palmitic acid (C16:0) and 12.17% of palmitoleic acid (C16:1) is present in algal biodiesel. Obtained biodiesel contains 26.79% saturated fatty acids and 53.82% unsaturated fatty acids. It has been reported that oil containing more significant levels of saturated fatty acids in contrast with unsaturated fatty acids may get solidified, and fuel lines can get clogged during low-temperature conditions. In comparison, oils with high unsaturated fatty acid composition are less viscous, making the biodiesel work in warm and cold weather (Nautiyal et al., 2014). Therefore, the produced biodiesel has been considered a high-quality fuel.

Table 4. 6 FAME composition of obtained biodiesel

S. No	FAME	Structure	Wt. % of FAME in biodiesel
1.	Caprylic	8:0	3.54
2.	Lauric	12:0	1.23
3.	Myristic	14:0	3.35
4.	Cis-10 Pentadecanoic	15:1	2.26
5.	Palmitic	16:0	13.29
6.	Palmitole	16:1	11.17
7.	Stearic	18:0	3.12
8.	Oleic	18:1	9.71
9.	Linoleic	18:2	28.53
10.	Linolenic	18:3	3.43
11.	Eicosapentaenoic	20:5	1.27

Some of the critical quality parameters of biodiesel were analyzed to examine the performance of biodiesel. **Table 4. 7** represents the physical and combustion characteristics of the specimen. The entire properties of algal biodiesel were followed according to the ASTM standards (D6751) (de Jesus et al., 2020). The density of the fuel affects the fuel injection system. A change in the mass of injected fuel will change the density, thus affecting engine performance (Pandit and Fulekar, 2019). In the current study, density is coming under the ASTM standard values. Pour point is defined as the point where the fuel loses its flowing properties. Kinematic viscosity was determined as 5.7 mm²/s, within the ASTM standard value range. Furthermore, the calorific value of fuel is an essential parameter that decides the energy content of the fuel. Presently, the calorific value came out to be satisfactory in comparison with the standard values.

In the current scenario, pour points for microalgae-based fuels have been observed to be less than conventional diesel. The lower pour point indicates the suitability of the biodiesel in cold conditions (Namitha et al., 2021). Equally important is the flash point property, which represented how easily a fuel can burn. In the present study, a higher flash point indicates less flammability and hazards that ensure safe handling and storage (Hassan et al., 2018; Saikia et al., 2019).

Table 4. 7 Physio-chemical properties of obtained biodiesel.

S. No	Parameter	Algae Biodiesel	ASTM 6751 Method & Standard Value
1.	Density (kg/m ³)	870	860-900 (D1298)
2.	Viscosity (mm ² /s) at 40°C	5.7	4.0-6.0 (D445)
3.	Calorific Value (MJ/kg)	36.9	At least 35 (D240)
4.	Pour Point(°C)	-12	-15 to 10 (D5773)
5.	Flash Point(°C)	130	100-170 (D93)

Chapter 5

Conclusions and Future Recommendations

5.1 Conclusions

The study was conducted for the sustainable production of biodiesel from indigenous microalgae procured from a wastewater oxidation pond of a village in Punjab Province of India. An attempt was made to improve the cost-effectiveness of microalgal biodiesel production by utilizing indigenous microalgae cultures collected from the rural oxidation ponds to save time, toil, and cost required to isolate the pure algae cultures. Furthermore, indigenous microalgae were cultivated in the wastewater to eliminate the need for freshwater and nutrients. Additionally, a waste-based heterogeneous catalyst derived from rice bran powder was synthesized for the in-situ transesterification process to eliminate the need for expensive catalysts and reduce the environmental impact of the production process. Finally, optimization of process parameters was also done for the in-situ transesterification process to maximize the biodiesel yield. After observing the optimized biodiesel yield from mixed indigenous microalgae by performing an in-situ transesterification process in the presence of a KOH/rice-bran-based AC heterogeneous catalyst, the following conclusions based on the results have been listed below:

Successful microalgae cultivation: The study demonstrated the successful cultivation of microalgae in wastewater (collected from a rural oxidation pond) over two weeks, with a logistic growth pattern. Cultivation in wastewater curtailed the need for supplementing additional nutrients and eliminated the utilization of fresh water. The presence of chlorophyll-A in the wastewater confirmed the presence of microalgae. This was a crucial step in the biodiesel production process, as it provides a sustainable source of biomass for the in-situ transesterification process.

Identification and characterization of indigenous species: The morphological characterization of the isolated cultures under the microscope provided valuable visual clues. Notably, spherical single cells with visible cell walls observed a resemblance to *Chlorella* sp. Furthermore, other pure cultures displayed a distinctive pale to blue-green color and densely thin entangled filaments, closely resembling *Desertifilum* sp.

For precise identification, molecular techniques were employed, explicitly sequencing the 16S rRNA genes of the isolated strains. The subsequent BLASTN search against GenBank databases confirmed the genetic identity of the microalgae strains beyond any doubt. Query sequences exhibited an identity to *Desertifilum salkalinema* and *Chlorella vulgaris*, unequivocally establishing its molecular identity.

Utilization of indigenous mixed species: The use of indigenous mixed microalgae species procured from the rural oxidation pond represents a significant advancement in biodiesel production. This approach reduces the complexity and cost of isolating pure species from mixed consortia. The study also showed that mixed indigenous cultures yielded the higher biodiesel production, supporting the choice of mixed cultures for sustainable biodiesel production.

Innovative catalyst development: Developing a bio-based waste material activated carbon support material impregnated with KOH as a catalyst is noteworthy. SEM images confirmed the effective filling of the porous structure with KOH, allowing surface reactions and improving the efficiency of the in-situ transesterification process. XRD analysis confirmed the presence of KOH in the synthesized catalyst and confirmed that the calcined material has a crystalline structure.

Suitable solvent or solvent-co-solvent combination: Exploration of solvent mixtures for direct transesterification with synthesized catalyst was carried out. In-situ transesterification

with H₂SO₄ was carried out to find the most suitable combination. Successful identification of methanol and hexane as the optimal solvent duo has not only maximized the efficiency of the direct transesterification process but also paved the way for its integration with our innovative synthesized catalyst.

Optimized Process Parameters: By applying response surface methodology based on the central composite design (CCD-RSM), the study identified optimal process conditions for direct transesterification. These conditions included a 1.5-hour reaction time, a 10:1 S + CS to biomass molar ratio, 5 wt% catalyst loading, and a temperature of 70°C. Under these conditions, the predicted biodiesel yield was 81.8%, closely matching the observed yield of 80.9% with a prediction error of only 0.9%. Establishing a statistical model for predicting biodiesel yield in the direct transesterification process is valuable for process optimization and control. The high R² value of 0.94 indicates the model's accuracy in making predictions, ensuring consistent and reliable biodiesel production.

Biodiesel quality and composition: Characterization of the obtained biodiesel through GC–MS analysis revealed a composition containing 53.82% unsaturated fatty acids, making it suitable for extreme weather conditions. Additionally, all biodiesel properties met ASTM standards, ensuring compliance with industry quality requirements.

In conclusion, this study succeeded in optimizing algal biodiesel production and offered sustainable and environmentally friendly solutions. The use of indigenous mixed microalgae species, the innovative catalyst development, and the establishment of a robust statistical model for process optimization collectively contribute to the advancement of biodiesel production. The findings of this research have significant implications for the renewable energy sector and further underscore the feasibility of microalgae-based biodiesel as a green and sustainable energy source.

5.2 Future recommendations

Indeed, based on the findings and achievements of your study, here are some future research directions and recommendations:

Exploration of other indigenous microalgae species: While this study successfully utilized indigenous mixed microalgae species like *Chlorella sorokiniana*, green algae (Chlorophyceae) and *Cylindrocapsa geminella*. Further research could explore additional native microalgae strains. Investigating a wider variety of species could lead to the discovery of strains with even higher lipid content and better suitability for biodiesel production.

Catalyst development and enhancement: The innovative use of bio-based waste material as a catalyst support material is promising. Future research can focus on optimizing the catalyst synthesis process further or exploring alternative eco-friendly catalysts to improve the in-situ transesterification process efficiency.

Process scaling and techno-economic analysis: Scaling up microalgae-based biodiesel production from laboratory to industrial levels is critical. Conducting comprehensive techno-economic research to evaluate large-scale production's feasibility, cost-effectiveness, and environmental impact is essential.

Sustainability Assessment: Investigate the overall sustainability of microalgae-based biodiesel production, considering factors such as land and water use, carbon footprint, and social impacts. This holistic assessment can help ensure that biodiesel production remains environmentally and socially responsible.

In conclusion, the future of microalgae-based biodiesel research holds great promise for addressing sustainable energy needs. Continued innovation, collaboration, and a focus on sustainability will be vital in advancing this field and realizing the full potential of microalgae as a renewable energy source.

References:

1. Adeniyi, O.M., Azimov, U., Burluka, A., 2018. Algae biofuel: Current status and future applications. *Renew. Sustain. Energy Rev.* 90, 316–335. <https://doi.org/10.1016/j.rser.2018.03.067>
2. Adetunji, C.O., Adenigba, V.O., Thangadura, D., Ahamed, M.I., 2021. Microalgae for Biodiesel Production. *Biodiesel Technol. Appl.* 429–445. <https://doi.org/10.1002/9781119724957.ch16>
3. Ahmad, S., Chaudhary, S., Pathak, V. V., Kothari, R., Tyagi, V. V., 2020. Optimization of direct transesterification of *Chlorella pyrenoidosa* catalyzed by waste egg shell based heterogenous nano – CaO catalyst. *Renew. Energy* 160, 86–97. <https://doi.org/10.1016/j.renene.2020.06.010>
4. Al-Samaraae, R.R., Atabani, A.E., Uguz, G., Kumar, G., Arpa, O., Ayanoglu, A., Mohammed, M.N., Farouk, H., 2017. Perspective of safflower (*Carthamus tinctorius*) as a potential biodiesel feedstock in Turkey: characterization, engine performance and emissions analyses of butanol–biodiesel–diesel blends. *Biofuels* 0, 1–17. <https://doi.org/10.1080/17597269.2017.1398956>
5. Ali, Z., Subeshan, B., Alam, M.A., Asmatulu, E., Xu, J., 2023. Recent progress in extraction/transesterification techniques for the recovery of oil from algae biomass. *Biomass Convers. Biorefinery* 13, 2553–2569. <https://doi.org/10.1007/s13399-021-01326-y>
6. Amani, A., Rahmati, S., Fakhlaei, R., Barati, B., Wang, S., Doherty, W., Ken, K., 2022. Biomass and Bioenergy Emerging technologies for biodiesel production: Processes , challenges , and opportunities. *Biomass and Bioenergy* 163, 106521. <https://doi.org/10.1016/j.biombioe.2022.106521>
7. Amin, A., Gadallah, A., El Morsi, A.K., El-Ibiari, N.N., El-Diwani, G.I., 2016. Experimental and empirical study of diesel and castor biodiesel blending effect, on kinematic

- viscosity, density and calorific value. *Egypt. J. Pet.* 25, 509–514.
<https://doi.org/10.1016/j.ejpe.2015.11.002>
8. Anwar, M., Rasul, M.G., Ashwath, N., Rahman, M.M., 2018. Optimisation of Second-Generation Biodiesel Production from Australian Native Stone Fruit Oil Using Response Surface Method. *Energies* 11. <https://doi.org/10.3390/en11102566>
 9. Athayde Neto, L.S., Souza, L.R. de, Muniz, P.B.V., Câmara, J.C.C., 2022. Use of Hydrogen As Energy Source: a Literature Review. *J Bioeng. Tech. Heal.* 121–126. <https://doi.org/10.5151/siintec2021-208273>
 10. Barros, A.I., Gonçalves, A.L., Simões, M., Pires, J.C.M., 2015. Harvesting techniques applied to microalgae: A review sedimentation shou. *Renew. Sustain. Energy Rev.* 41, 1489–1500. <https://doi.org/10.1016/j.rser.2014.09.037>
 11. Bartley, M.L., Boeing, W.J., Corcoran, A.A., Holguin, F.O., Schaub, T., 2013. Effects of salinity on growth and lipid accumulation of biofuel microalga *Nannochloropsis salina* and invading organisms. *Biomass and Bioenergy* 54, 83–88. <https://doi.org/10.1016/j.biombioe.2013.03.026>
 12. Cai, F.F., Chen, Y.X., Zhu, M.L., Li, X.C., Li, R.H., 2017. *Desertifilum salkalinema* sp. nov. (Oscillatoriales, cyanobacteria) from an alkaline pool in China. *Phytotaxa* 292, 262–270. <https://doi.org/10.11646/phytotaxa.292.3.6>
 13. Canabarro, N.I., Silva-Ortiz, P., Nogueira, L.A.H., Cantarella, H., Maciel-Filho, R., Souza, G.M., 2023. Sustainability assessment of ethanol and biodiesel production in Argentina, Brazil, Colombia, and Guatemala. *Renew. Sustain. Energy Rev.* 171, 113019. <https://doi.org/10.1016/j.rser.2022.113019>
 14. Cao, H., Zhang, Z., Wu, X., Miao, X., 2013. Direct biodiesel production from wet microalgae biomass of *Chlorella pyrenoidosa* through in situ transesterification. *Biomed Res. Int.* 2013. <https://doi.org/10.1155/2013/930686>
 15. Carrero, A., Vicente, G., Rodríguez, R., Linares, M., Del Peso, G.L., 2011. Hierarchical

- zeolites as catalysts for biodiesel production from *Nannochloropsis* microalga oil. *Catal. Today* 167, 148–153. <https://doi.org/10.1016/j.cattod.2010.11.058>
16. Cercado, A.P.I., Ballesteros, F.C., Capareda, S.C., 2018. Biodiesel from three microalgae transesterification processes using different homogenous catalysts. *Int. J. Technol.* 9, 645–651. <https://doi.org/10.14716/ijtech.v9i4.1145>
17. Chamola, R., 2019. DIRECT TRANSESTERIFICATION FOR BIODIESEL EXTRACTION FROM MICRO-ALGAL BIOMASS : A REVIEW. *Int. J. Appl. Eng. Res.* 14, 180–184.
18. Chamola, R., Khan, M.F., Raj, A., Verma, M., Jain, S., 2019. Response surface methodology based optimization of in situ transesterification of dry algae with methanol, H₂SO₄ and NaOH. *Fuel* 239, 511–520. <https://doi.org/10.1016/j.fuel.2018.11.038>
19. Chandra, Y., Singh, V., 2017. Microalgal biodiesel : A possible solution for India ' s energy security. *Renew. Sustain. Energy Rev.* 67, 72–88. <https://doi.org/10.1016/j.rser.2016.08.031>
20. Cheirsilp, B., Srinuanpan, S., Mandik, Y.I., 2019. Efficient harvesting of microalgal biomass and direct conversion of microalgal lipids into biodiesel, *Microalgae Cultivation for Biofuels Production*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-817536-1.00006-0>
21. Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J., Chang, J.S., 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresour. Technol.* 102, 71–81. <https://doi.org/10.1016/j.biortech.2010.06.159>
22. Chen, H., Wang, J., Zheng, Y., Zhan, J., He, C., Wang, Q., 2018. Algal biofuel production coupled bioremediation of biomass power plant wastes based on *Chlorella* sp. C2 cultivation. *Appl. Energy* 211, 296–305. <https://doi.org/10.1016/j.apenergy.2017.11.058>
23. Cheng, J., Huang, R., Li, T., Zhou, J., Cen, K., 2014. Biodiesel from wet microalgae: Extraction with hexane after the microwave-assisted transesterification of lipids. *Bioresour. Technol.* 170, 69–75. <https://doi.org/10.1016/j.biortech.2014.07.089>

24. Chhandama, M.V.L., Satyan, K.B., Changmai, B., Vanlalveni, C., Rokhum, S.L., 2021. Microalgae as a feedstock for the production of biodiesel: A review. *Bioresour. Technol. Reports* 15, 100771. <https://doi.org/10.1016/j.biteb.2021.100771>
25. Choo, M.Y., Oi, L.E., Ling, T.C., Ng, E.P., Lee, H.V., Juan, J.C., 2019. Conversion of microalgae biomass to biofuels, *Microalgae Cultivation for Biofuels Production*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-817536-1.00010-2>
26. Chowdhury, H., Loganathan, B., 2019. Third-generation biofuels from microalgae: a review. *Curr. Opin. Green Sustain. Chem.* 20, 39–44. <https://doi.org/10.1016/j.cogsc.2019.09.003>
27. Chowdhury, S., Dhawane, S.H., Jha, B., Pal, S., Sagar, R., Hossain, A., Halder, G., 2021. Biodiesel synthesis from transesterified *Madhuca indica* oil by waste egg shell–derived heterogeneous catalyst: parametric optimization by Taguchi approach. *Biomass Convers. Biorefinery* 11, 1171–1181. <https://doi.org/10.1007/s13399-019-00512-3>
28. Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnol. Adv.* 29, 686–702. <https://doi.org/10.1016/j.biotechadv.2011.05.015>
29. Cooney, M.J., Young, G., Pate, R., 2011. Bio-oil from photosynthetic microalgae: Case study. *Bioresour. Technol.* 102, 166–177. <https://doi.org/10.1016/j.biortech.2010.06.134>
30. D’oca, M.G.M., Viêgas, C. V., Lemões, J.S., Miyasaki, E.K., Morón-Villarreyes, J.A., Primel, E.G., Abreu, P.C., 2011. Production of FAMES from several microalgal lipidic extracts and direct transesterification of the *Chlorella pyrenoidosa*. *Biomass and Bioenergy* 35, 1533–1538. <https://doi.org/10.1016/j.biombioe.2010.12.047>
31. Das, M., Semy, K., Kuotsu, K., 2022. Seasonal monitoring of algal diversity and spatiotemporal variation in water properties of Simsang river at South Garo Hills, Meghalaya, India. *Sustain. Water Resour. Manag.* 8, 1–9. <https://doi.org/10.1007/s40899-022-00611-6>
32. Das, P.K., Rani, J., Rawat, S., Kumar, S., 2021. Microalgal Co-cultivation for Biofuel

Production and Bioremediation: Current Status and Benefits. *Bioenergy Res.*
<https://doi.org/10.1007/s12155-021-10254-8>

33. Das, V., Tripathi, A.M., Borah, M.J., Dunford, N.T., Deka, D., 2020. Cobalt-doped CaO catalyst synthesized and applied for algal biodiesel production. *Renew. Energy* 161, 1110–1119. <https://doi.org/10.1016/j.renene.2020.07.040>

34. de Blasio, C., 2019a. Fischer–Tropsch (FT) synthesis to biofuels (BtL Process). *Green Energy Technol.* 287–306. https://doi.org/10.1007/978-3-030-11599-9_20

35. de Blasio, C., 2019b. Some chemical analyses in biodiesel production and biofuel characteristics. *Green Energy Technol.* 267–285. https://doi.org/10.1007/978-3-030-11599-9_19

36. De Blasio, C., 2019. *Fundamentals of Biofuels Engineering and Technology*, Springer.

37. de Jesus, S.S., Ferreira, G.F., Moreira, L.S., Filho, R.M., 2020. Biodiesel production from microalgae by direct transesterification using green solvents. *Renew. Energy* 160, 1283–1294. <https://doi.org/10.1016/j.renene.2020.07.056>

38. Deeba, F., Kumar, B., Arora, N., Singh, S., Kumar, A., Han, S.S., Negi, Y.S., 2020. Novel bio-based solid acid catalyst derived from waste yeast residue for biodiesel production. *Renew. Energy* 159, 127–139. <https://doi.org/10.1016/j.renene.2020.05.029>

39. Degfie, T.A., Mamo, T.T., Mekonnen, Y.S., 2019. Optimized Biodiesel Production from Waste Cooking Oil (WCO) using Calcium Oxide (CaO) Nano-catalyst. *Sci. Rep.* 9, 1–8. <https://doi.org/10.1038/s41598-019-55403-4>

40. Dewangan, A., Yadav, A.K., Mallick, A., 2018. Current scenario of biodiesel development in India: prospects and challenges. *Energy Sources, Part A Recover. Util. Environ. Eff.* 40, 2494–2501. <https://doi.org/10.1080/15567036.2018.1502849>

41. Dieterich, V., Buttler, A., Hanel, A., Spliethoff, H., Fendt, S., 2020. Power-to-liquid via synthesis of methanol, DME or Fischer–Tropsch-fuels: a review. *Energy Environ. Sci.* 13, 3207–3252. <https://doi.org/10.1039/d0ee01187h>

42. Faried, M., Samer, M., Abdelsalam, E., Yousef, R.S., Attia, Y.A., Ali, A.S., 2017. Biodiesel production from microalgae: Processes, technologies and recent advancements. *Renew. Sustain. Energy Rev.* 79, 893–913. <https://doi.org/10.1016/j.rser.2017.05.199>
43. Ferreira Mota, G., Germano de Sousa, I., Luiz Barros de Oliveira, A., Luthierre Gama Cavalcante, A., da Silva Moreira, K., Thálysson Tavares Cavalcante, F., Erick da Silva Souza, J., Rafael de Aguiar Falcão, Í., Guimarães Rocha, T., Bussons Rodrigues Valério, R., Cristina Freitas de Carvalho, S., Simão Neto, F., de França Serpa, J., Karolinny Chaves de Lima, R., Cristiane Martins de Souza, M., dos Santos, J.C.S., 2022. Biodiesel production from microalgae using lipase-based catalysts: Current challenges and prospects. *Algal Res.* 62. <https://doi.org/10.1016/j.algal.2021.102616>
44. Frank, E.D., Elgowainy, A., Han, J., Wang, Z., 2013. Life cycle comparison of hydrothermal liquefaction and lipid extraction pathways to renewable diesel from algae. *Mitig. Adapt. Strateg. Glob. Chang.* 18, 137–158. <https://doi.org/10.1007/s11027-012-9395-1>
45. Ganesan, R., Manigandan, S., Shanmugam, S., Chandramohan, V.P., Sindhu, R., Kim, S.H., Brindhadevi, K., Pugazhendhi, A., 2021. A detailed scrutinize on panorama of catalysts in biodiesel synthesis. *Sci. Total Environ.* 777, 145683. <https://doi.org/10.1016/j.scitotenv.2021.145683>
46. Gao, C., Xin, H., Yang, S., Li, Z., Liu, S., Xu, B., Zhang, T., Dutta, S., Tang, Y., 2022. Trends and Performances of the Algal Biofuel: a Bibliometric Approach. *J. Environ. Eng. Landsc. Manag.* 30, 284–300. <https://doi.org/10.3846/jeelm.2022.16746>
47. Garcia, N.H., Mattioli, A., Gil, A., Frison, N., Battista, F., Bolzonella, D., 2019. Evaluation of the methane potential of different agricultural and food processing substrates for improved biogas production in rural areas. *Renew. Sustain. Energy Rev.* 112, 1–10. <https://doi.org/10.1016/j.rser.2019.05.040>
48. Ghaffar, I., Deepanraj, B., Sundar, L.S., Vo, D.-V.N., Saikumar, A., Hussain, A., 2023.

A review on the sustainable procurement of microalgal biomass from wastewaters for the production of biofuels. *Chemosphere* 311, 137094.

<https://doi.org/10.1016/j.chemosphere.2022.137094>

49. Ghasemi Naghdi, F., González González, L.M., Chan, W., Schenk, P.M., 2016. Progress on lipid extraction from wet algal biomass for biodiesel production. *Microb. Biotechnol.* 9, 718–726. <https://doi.org/10.1111/1751-7915.12360>

50. Goh, B.H.H., Ong, H.C., Cheah, M.Y., Chen, W.H., Yu, K.L., Mahlia, T.M.I., 2019. Sustainability of direct biodiesel synthesis from microalgae biomass: A critical review. *Renew. Sustain. Energy Rev.* 107, 59–74. <https://doi.org/10.1016/j.rser.2019.02.012>

51. Gufrana, T., Islam, H., Khare, S., Pandey, A., 2022. In-situ transesterification of single-cell oil for biodiesel production: a review. *Prep. Biochem. Biotechnol.* <https://doi.org/10.1080/10826068.2022.2065684>

52. Halder, P., Azad, A.K., 2019. Recent trends and challenges of algal biofuel conversion technologies, *Advanced Biofuels: Applications, Technologies and Environmental Sustainability*. Elsevier Ltd. <https://doi.org/10.1016/B978-0-08-102791-2.00007-6>

53. Hassan, U., Al-Zubaidi, I., Ibrahim, H., 2018. The effect of off-spec canola biodiesel blending on fuel properties for cold weather applications. *ChemEngineering* 2, 1–11. <https://doi.org/10.3390/chemengineering2030030>

54. Hirani, A.H., Javed, N., Asif, M., Basu, S.K., Kumar, A., 2018. A review on first- and second- generation biofuel productions. *Biofuels Greenh. Gas Mitig. Glob. Warm. Next Gener. Biofuels Role Biotechnol.* 141–154. https://doi.org/10.1007/978-81-322-3763-1_8

55. Hossain, N., Hasan, M.H., Mahlia, T.M.I., Shamsuddin, A.H., Silitonga, A.S., 2020. Feasibility of microalgae as feedstock for alternative fuel in Malaysia : A review. *Energy Strateg. Rev.* 32, 100536. <https://doi.org/10.1016/j.esr.2020.100536>

56. Im, H.J., Lee, H.S., Park, M.S., Yang, J.W., Lee, J.W., 2014. Concurrent extraction and reaction for the production of biodiesel from wet microalgae. *Bioresour. Technol.* 152, 534–

537. <https://doi.org/10.1016/j.biortech.2013.11.023>

57. Inamuddin Abdullahm, 2020. Sustainable Green Chemical Processes and their Allied Applications, Nanotechnology in the Life Sciences.

58. Irena Branyikova, Gita Prochazkova, Tomas Potocar, Z.J. and T.B., 2018. Harvesting of Microalgae by Means of Flocculation 4. https://doi.org/10.1007/978-3-319-16640-7_12

59. Jaiyen, S., Naree, T., Ngamcharussrivichai, C., 2014. Comparative study of natural dolomitic rock and waste mixed seashells as heterogeneous catalysts for the methanolysis of palm oil to biodiesel. *Renew. Energy* 74, 433–440. <https://doi.org/10.1016/j.renene.2014.08.050>

60. Jambulingam, R., Srinivasan, G.R., Palani, S., Munir, M., Saeed, M., Mohanam, A., 2020. Process optimization of biodiesel production from waste beef tallow using ethanol as co-solvent. *SN Appl. Sci.* 2, 1–18. <https://doi.org/10.1007/s42452-020-03243-7>

61. Jamil, F., Saleem, M., Ali Qamar, O., Khurram, M.S., Al-Muhtaseb, A.H., Inayat, A., Akhter, P., Hussain, M., Rafiq, S., Yim, H., Park, Y.K., 2023. State-of-the-art catalysts for clean fuel (methyl esters) production—a comprehensive review. *JPhys Energy* 5. <https://doi.org/10.1088/2515-7655/aca5b3>

62. Ji, X., Jiang, M., Zhang, J., Jiang, X., Zheng, Z., 2018. The interactions of algae-bacteria symbiotic system and its effects on nutrients removal from synthetic wastewater. *Bioresour. Technol.* 247, 44–50. <https://doi.org/10.1016/j.biortech.2017.09.074>

63. Johan, F., Jafri, M.Z., Lim, H.S., Wan Maznah, W.O., 2014. Laboratory measurement: Chlorophyll-a concentration measurement with acetone method using spectrophotometer. *IEEE Int. Conf. Ind. Eng. Eng. Manag.* 2015-Janua, 744–748. <https://doi.org/10.1109/IEEM.2014.7058737>

64. Jon K Pittman, Andrew P Dean, O.O., 2011. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour. Technol.* 102, 17–25. <https://doi.org/10.1016/j.biortech.2010.06.035>

65. Kaur, M., Malhotra, R., Ali, A., 2018. Tungsten supported Ti/SiO₂ nanoflowers as reusable heterogeneous catalyst for biodiesel production. *Renew. Energy* 116, 109–119. <https://doi.org/10.1016/j.renene.2017.09.065>
66. Kazbar A., Cogne G., Urbain B, Marec H., Le-Gouic B, Tallec J., Takache H., Ismail A., P.J., 2019. Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. *Algal Res.* 39.
67. Kim, J., Yoo, G., Lee, H., Lim, J., Kim, K., Kim, C.W., Park, M.S., Yang, J.W., 2013. Methods of downstream processing for the production of biodiesel from microalgae. *Biotechnol. Adv.* 31, 862–876. <https://doi.org/10.1016/j.biotechadv.2013.04.006>
68. Konarova, M., Aslam, W., Perkins, G., 2022. Fischer-Tropsch synthesis to hydrocarbon biofuels: Present status and challenges involved. *Hydrocarb. Biorefinery Sustain. Process. Biomass Hydrocarb. Biofuels* 77–96. <https://doi.org/10.1016/B978-0-12-823306-1.00006-6>
69. Kumar, B.R., Mathimani, T., Sudhakar, M.P., Rajendran, K., Nizami, A.S., Brindhadevi, K., Pugazhendhi, A., 2021. A state of the art review on the cultivation of algae for energy and other valuable products: Application, challenges, and opportunities. *Renew. Sustain. Energy Rev.* 138, 110649. <https://doi.org/10.1016/j.rser.2020.110649>
70. Kumar, G., Shobana, S., Chen, W.H., Bach, Q.V., Kim, S.H., Atabani, A.E., Chang, J.S., 2017. A review of thermochemical conversion of microalgal biomass for biofuels: Chemistry and processes. *Green Chem.* 19, 44–67. <https://doi.org/10.1039/c6gc01937d>
71. Kumar, M., Sun, Y., Rathour, R., Pandey, A., Thakur, I.S., Tsang, D.C.W., 2020. Algae as potential feedstock for the production of biofuels and value-added products: Opportunities and challenges. *Sci. Total Environ.* 716, 137116. <https://doi.org/10.1016/j.scitotenv.2020.137116>
72. Kumar, N., Sidharth, 2018. Some Studies on use of ternary blends of diesel, biodiesel and n-octanol. *Energy Sources, Part A Recover. Util. Environ. Eff.* 40, 1721–1728. <https://doi.org/10.1080/15567036.2018.1486902>

73. Kumar, P., Aslam, M., Singh, N., Mittal, S., Bansal, A., Jha, M.K., Sarma, A.K., 2015. Characterization, activity and process optimization with a biomass-based thermal power plant's fly ash as a potential catalyst for biodiesel production. *RSC Adv.* 5, 9946–9954. <https://doi.org/10.1039/c4ra13475c>
74. Laca, Amanda, Laca, Adriana, Díaz, M., 2017. Eggshell waste as catalyst: A review. *J. Environ. Manage.* 197, 351–359. <https://doi.org/10.1016/j.jenvman.2017.03.088>
75. Lam, M.K., Lee, K.T., 2012. Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnol. Adv.* 30, 673–690. <https://doi.org/10.1016/j.biotechadv.2011.11.008>
76. Lee, J.Y., Yoo, C., Jun, S.Y., Ahn, C.Y., Oh, H.M., 2010. Comparison of several methods for effective lipid extraction from microalgae. *Bioresour. Technol.* 101, 2009–2011. <https://doi.org/10.1016/j.biortech.2009.03.058>
77. Li, Y., Ghasemi Naghdi, F., Garg, S., Adarme-Vega, T.C., Thurecht, K.J., Ghafor, W.A., Tannock, S., Schenk, P.M., 2014. A comparative study: The impact of different lipid extraction methods on current microalgal lipid research. *Microb. Cell Fact.* 13, 1–9. <https://doi.org/10.1186/1475-2859-13-14>
78. Liu, J., Zhu, Y., Tao, Y., Zhang, Y., Li, A., Li, T., Sang, M., Zhang, C., 2013. Freshwater microalgae harvested via flocculation induced by pH decrease. *Biotechnol. Biofuels* 6, 1–11. <https://doi.org/10.1186/1754-6834-6-98>
79. Lv, J., Feng, J., Liu, Q., Xie, S., 2017. Microalgal cultivation in secondary effluent: Recent developments and future work. *Int. J. Mol. Sci.* 18, 1–17. <https://doi.org/10.3390/ijms18010079>
80. Mahmoudi, H., Mahmoudi, M., Doustdar, O., Jahangiri, H., Tsolakis, A., Gu, S., LechWyszynski, M., 2017. A review of Fischer Tropsch synthesis process, mechanism, surface chemistry and catalyst formulation. *Biofuels Eng.* 2, 11–31. <https://doi.org/10.1515/bfuel-2017-0002>

81. Makareviciene, V., Sendzikiene, E., Gaide, I., 2021. Application of heterogeneous catalysis to biodiesel synthesis using microalgae oil. *Front. Environ. Sci. Eng.* 15. <https://doi.org/10.1007/s11783-020-1343-9>
82. Makut, B.B., 2021. Algal Biofuel: Emergent Applications in Next-Generation Biofuel Technology. *Liq. Biofuels* 119–144. <https://doi.org/10.1002/9781119793038.ch4>
83. Malode, S.J., Gaddi, S.A.M., Kamble, P.J., Nalwad, A.A., Muddapur, U.M., Shetti, N.P., 2022. Recent evolutionary trends in the production of biofuels. *Mater. Sci. Energy Technol.* 5, 262–277. <https://doi.org/10.1016/j.mset.2022.04.001>
84. Marwaha, A., Dhir, A., Mahla, S.K., Mohapatra, S.K., 2018. An overview of solid base heterogeneous catalysts for biodiesel production. *Catal. Rev. - Sci. Eng.* 60, 594–628. <https://doi.org/10.1080/01614940.2018.1494782>
85. Mendiburu, A.Z., Lauermann, C.H., Hayashi, T.C., Mariños, D.J., Rodrigues da Costa, R.B., Coronado, C.J.R., Roberts, J.J., de Carvalho, J.A., 2022. Ethanol as a renewable biofuel: Combustion characteristics and application in engines. *Energy* 257. <https://doi.org/10.1016/j.energy.2022.124688>
86. Ministry of Petroleum & Natural Gas, G. of I., 2020. *Energizing India's Progress*. Ministry of Petroleum and Natural Gas, Government of India.
87. Mishra, S., Mohanty, K., 2019. Comprehensive characterization of microalgal isolates and lipid-extracted biomass as zero-waste bioenergy feedstock : An integrated bioremediation and biorefinery approach. *Bioresour. Technol.* 273, 177–184. <https://doi.org/10.1016/j.biortech.2018.11.012>
88. Mishra, S., Panda, S., Pradhan, N., Biswal, S.K., Sukla, L.B., Mishra, B.K., 2015. *Microbe–Mineral Interactions: Exploring Avenues Towards Development of a Sustainable Microbial Technology for Coal Beneficiation*. https://doi.org/10.1007/978-3-319-19018-1_2
89. Misra, R.D., Murthy, M.S., 2010. Straight vegetable oils usage in a compression ignition engine - A review. *Renew. Sustain. Energy Rev.* 14, 3005–3013.

<https://doi.org/10.1016/j.rser.2010.06.010>

90. Mobin, S., Alam, F., 2014. Biofuel Production from Algae Utilizing Wastewater. Australas. Fluid Mech. Conf. 1–7. <https://doi.org/10.1287/isre.1080.0219>

91. Mofijur, M., Rasul, M.G., Pina, A., Ferrão, P., Fournier, J., Lacarrière, B., Corre, O. Le, Andri, A.I., 2019. Recent Development in the Production of Third Generation Recent The Development in Symposium the Production Third Heating Cooling Biodiesel from Microalgae Biodiesel from Microalgae Assessing the feasibility of using the district heat demand forecast. Energy Procedia 156, 53–58. <https://doi.org/10.1016/j.egypro.2018.11.088>

92. Mohamed, R.M., Kadry, G.A., Abdel-Samad, H.A., Awad, M.E., 2020. High operative heterogeneous catalyst in biodiesel production from waste cooking oil. Egypt. J. Pet. 29, 59–65. <https://doi.org/10.1016/j.ejpe.2019.11.002>

93. Mondal, P., Basu, M., Balasubramanian, N., 2008. Direct use of vegetable oil and animal fat as alternative fuel in internal combustion engine. Biofuels, Bioprod. Biorefining 2, 155–174. <https://doi.org/10.1002/bbb>

94. Montoya-Vallejo, C., Guzmán Duque, F.L., Quintero Díaz, J.C., 2023. Biomass and lipid production by the native green microalgae *Chlorella sorokiniana* in response to nutrients, light intensity, and carbon dioxide: experimental and modeling approach. Front. Bioeng. Biotechnol. 11, 1–16. <https://doi.org/10.3389/fbioe.2023.1149762>

95. Naeem, M.M., Al-Sakkari, E.G., Boffito, D.C., Gadalla, M.A., Ashour, F.H., 2021. One-pot conversion of highly acidic waste cooking oil into biodiesel over a novel bio-based bi-functional catalyst. Fuel 283. <https://doi.org/10.1016/j.fuel.2020.118914>

96. Naik, D. V., Singh, K.K., Kumar, V., Prasad, B., Behera, B., Bangwal, D.P., Atheya, N., Garg, M.O., 2014. Catalytic cracking of glycerol to fine chemicals over equilibrium fluid catalytic cracking catalyst. Energy Procedia 54, 593–598. <https://doi.org/10.1016/j.egypro.2014.07.300>

97. Namitha, B., Sathish, A., Senthil Kumar, P., Nithya, K., Sundar, S., 2021. sMicro algal

biodiesel synthesized from *Monoraphidium* sp., and *Chlorella sorokiniana*: Feasibility and emission parameter studies. *Fuel* 301, 121063. <https://doi.org/10.1016/j.fuel.2021.121063>

98. Nautiyal, P., Subramanian, K.A., Dastidar, M.G., 2014. Production and characterization of biodiesel from algae. *Fuel Process. Technol.* 120, 79–88. <https://doi.org/10.1016/j.fuproc.2013.12.003>

99. Nautiyal, P., Subramanian, K.A., Dastidar, M.G., Kumar, A., 2019. Effective utilization of algae biomass from wastewater for biodiesel production by direct transesterification: A promising approach for sustainable bioenergy production. *Asian J. Chem.* 31, 2065–2068. <https://doi.org/10.14233/ajchem.2019.22101>

100. Ogbeide Ebhodaghe, S., Evans Imanah, O., Ndibe, H., 2021. Biofuels from Microalgae Biomass: A Review of Conversion Processes and Procedures. *Arab. J. Chem.* 15, 103591. <https://doi.org/10.1016/j.arabjc.2021.103591>

101. Ogunkunle, O., Oniya, O.O., Adebayo, A.O., 2017. Yield Response of Biodiesel Production from Heterogeneous and Homogeneous Catalysis of Milk Bush Seed (*Thevetia peruviana*) Oil. *Energy Policy Res.* 4, 21–28. <https://doi.org/10.1080/23815639.2017.1319772>

102. Osman, A.I., Elgarahy, A.M., Eltaweil, A.S., Abd El-Monaem, E.M., El-Aqapa, H.G., Park, Y., Hwang, Y., Ayati, A., Farghali, M., Ihara, I., Al-Muhtaseb, A.H., Rooney, D.W., Yap, P.S., Sillanpää, M., 2023. Biofuel production, hydrogen production and water remediation by photocatalysis, biocatalysis and electrocatalysis, *Environmental Chemistry Letters*. Springer International Publishing. <https://doi.org/10.1007/s10311-023-01581-7>

103. Pandit, P.R., Fulekar, M.H., 2019. Biodiesel production from microalgal biomass using CaO catalyst synthesized from natural waste material. *Renew. Energy* 136, 837–845. <https://doi.org/10.1016/j.renene.2019.01.047>

104. Pandit, P.R., Fulekar, M.H., 2017. Egg shell waste as heterogeneous nanocatalyst for biodiesel production: Optimized by response surface methodology. *J. Environ. Manage.* 198,

319–329. <https://doi.org/10.1016/j.jenvman.2017.04.100>

105. Parmentier, D., Manhaeghe, D., Baccini, L., Van Meirhaeghe, R., Rousseau, D.P.L., Van Hulle, S., 2020. A new reactor design for harvesting algae through electrocoagulation-flotation in a continuous mode. *Algal Res.* 47, 101828. <https://doi.org/10.1016/j.algal.2020.101828>

106. Ponce, M., 2015. Design and Performance of Waste Stabilization Ponds Hamzeh Ramadan and Victor 1–37.

107. Putri, D.S., Astuti, S.P., Alaa, S., 2019. The growth of microalgae *Chlorococcum* sp. isolated from Ampenan estuary of Lombok Island in Walne's medium. *AIP Conf. Proc.* 2199. <https://doi.org/10.1063/1.5141301>

108. Rajkumari, K., Rokhum, L., 2020. A sustainable protocol for production of biodiesel by transesterification of soybean oil using banana trunk ash as a heterogeneous catalyst. *Biomass Convers. Biorefinery* 10, 839–848. <https://doi.org/10.1007/s13399-020-00647-8>

109. Rangabhashiyam, S., Behera, B., Aly, N., Balasubramanian, P., 2017. Biodiesel from microalgae as a promising strategy for renewable bioenergy production - A review. *J. Environ. Biotechnol. Res.* 6, 260–269.

110. Rao, N.R.H., Tamburic, B., Doan, Y.T.T., Nguyen, B.D., Henderson, R.K., 2021. Algal biotechnology in Australia and Vietnam: Opportunities and challenges. *Algal Res.* 56, 102335. <https://doi.org/10.1016/j.algal.2021.102335>

111. Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Appl. Energy* 103, 444–467. <https://doi.org/10.1016/j.apenergy.2012.10.004>

112. Rendón, 2013. Effect of Carbon Dioxide Concentration on the Growth Response of *Chlorella vulgaris* Under Four Different Led Illumination. *Int. J. Biotechnol. Wellness Ind.* <https://doi.org/10.6000/1927-3037.2013.02.03.3>

113. Research and Markets, 2022. Global Algae Biofuel Market Report (2022 to 2027).

Globe Newswire 2022, 6–8.

114. Rice, E.W., Baird, a B., Eaton, A.D., Clesceri, L.S., 2017. Standard Methods for the Examination of Water and Wastewater. Am. Public Heal. Assoc. 1496.

115. Rosenberg, J.N., Oyler, G.A., Wilkinson, L., Betenbaugh, M.J., 2008. A green light for engineered algae : redirecting metabolism to fuel a biotechnology revolution 430–436. <https://doi.org/10.1016/j.copbio.2008.07.008>

116. Saad, M.G., Dosoky, N.S., Zoromba, M.S., Shafik, H.M., 2019. Algal biofuels: Current status and key challenges. *Energies*. <https://doi.org/10.3390/en12101920>

117. Safi, C., Zebib, B., Merah, O., Pontalier, P.Y., Vaca-Garcia, C., 2014. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. *Renew. Sustain. Energy Rev.* 35, 265–278. <https://doi.org/10.1016/j.rser.2014.04.007>

118. Saikia, M., Bikash, B., Bora, D.K., Kalita, K., 2019. Thermal Performances of Neat Bio-Diesels in Compression Ignition Engine for Diesel Fuel Substitution. 2nd Int. Conf. Energy, Power Environ. Towar. Smart Technol. ICEPE 2018 1–6. <https://doi.org/10.1109/EPETSG.2018.8659194>

119. Saleem, M., Jamil, F., Qamar, O.A., Akhter, P., Hussain, M., Khurram, M.S., Al-Muhtaseb, A.H., Inayat, A., Shah, N.S., 2022. Enhancing the Catalytic Activity of Eggshell-Derived CaO Catalyst and Its Application in Biodiesel Production from Waste Chicken Fat. *Catalysts* 12. <https://doi.org/10.3390/catal12121627>

120. Salim, S., Bosma, R., Vermuë, M.H., Wijffels, R.H., 2011. Harvesting of microalgae by bio-flocculation. *J. Appl. Phycol.* 23, 849–855. <https://doi.org/10.1007/s10811-010-9591-x>

121. Sander, K., Murthy, G.S., 2010. Life cycle analysis of algae biodiesel. *Int. J. Life Cycle Assess.* 15, 704–714. <https://doi.org/10.1007/s11367-010-0194-1>

122. Saranya, G., Ramachandra, T. V., 2020. Novel biocatalyst for optimal biodiesel production from diatoms. *Renew. Energy* 153, 919–934.

<https://doi.org/10.1016/j.renene.2020.02.053>

123. Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussnug, J.H., Posten, C., Kruse, O., Hankamer, B., 2008. Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *BioEnergy Res.* 1, 20–43. <https://doi.org/10.1007/s12155-008-9008-8>

124. Senthil, M., Visagavel, K., Saravanan, C.G., Rajendran, K., 2016. Investigations of red mud as a catalyst in Mahua oil biodiesel production and its engine performance. *Fuel Process. Technol.* 149, 7–14. <https://doi.org/10.1016/j.fuproc.2016.03.027>

125. Sharma, P., Dhar, A., 2018. Effect of hydrogen supplementation on engine performance and emissions. *Int. J. Hydrogen Energy* 43, 7570–7580. <https://doi.org/10.1016/j.ijhydene.2018.02.181>

126. Shi, R., Handler, R.M., Shonnard, D.R., 2019. Life cycle assessment of novel technologies for algae harvesting and oil extraction in the renewable diesel pathway. *Algal Res.* 37, 248–259. <https://doi.org/10.1016/j.algal.2018.12.005>

127. Show, K.Y., Lee, D.J., Mujumdar, A.S., 2015. Advances and Challenges on Algae Harvesting and Drying. *Dry. Technol.* 33, 386–394. <https://doi.org/10.1080/07373937.2014.948554>

128. Shuba, E.S., Kifle, D., 2018. Microalgae to biofuels: ‘Promising’ alternative and renewable energy, review. *Renew. Sustain. Energy Rev.* 81, 743–755. <https://doi.org/10.1016/j.rser.2017.08.042>

129. Silva, F. de S., Costa, A.P., Carantino, M., Colzi Lopes, A., Fares Abdala Neto, E., Carrhá Leitão, R., Mota, C.R., Bezerra dos Santos, A., 2014. Comparison of pretreatment methods for total lipids extraction from mixed microalgae. *Renew. Energy* 63, 762–766. <https://doi.org/10.1016/j.renene.2013.10.038>

130. Silva, F.J.A., Souza, R.O., Castro, F.J.F., 2009. Strategies for upgrading primary facultative ponds in Fortaleza, Northeast Brazil. *Rev. Tecnol.* 30, 104–113.

131. Singh, A., Srivastava, S., Rathore, D., Pant, D., Health, E., 2020. Environmental Microbiology and Biotechnology. Springer.
132. Singh, S., Garg, A., 2021. Conversion of Waste Materials into Heterogeneous Oxidation Catalysts for Treatment of Chlorinated Organics. *J. Environ. Eng.* 147. [https://doi.org/10.1061/\(asce\)ee.1943-7870.0001837](https://doi.org/10.1061/(asce)ee.1943-7870.0001837)
133. Singh, T.S., Verma, T.N., 2019a. Taguchi design approach for extraction of methyl ester from waste cooking oil using synthesized CaO as heterogeneous catalyst: Response surface methodology optimization. *Energy Convers. Manag.* 182, 383–397. <https://doi.org/10.1016/j.enconman.2018.12.077>
134. Singh, T.S., Verma, T.N., 2019b. Biodiesel production from *Momordica Charantia* (L.): Extraction and engine characteristics. *Energy* 189, 116198. <https://doi.org/10.1016/j.energy.2019.116198>
135. Skorupskaite, V., Makareviciene, V., Gumbyte, M., 2016. Opportunities for simultaneous oil extraction and transesterification during biodiesel fuel production from microalgae: A review. *Fuel Process. Technol.* 150, 78–87. <https://doi.org/10.1016/j.fuproc.2016.05.002>
136. Solmaz, H., Halis, S., 2017. The Effects of Hazelnut Oil Methyl Ester on Engine Performance and Exhaust Emissions, in: 8th International Advanced Technologies Symposium (IATS'17), 19-22 October 2017, Elazığ, Turkey. pp. 19–22.
137. Sooch, S.S., Saini, J.S., Singh, H., 2016. Tomorrow ' s Fuels : Renewable Energy Sources. *Int. J Ext Res.* 3, 61–71.
138. Sooch S S and Gautam A, 2013. Present status of renewable energy sources in Punjab. *Int. J. Agric. Environ. Technol.* 6, 317–33.
139. Stančin, H., Mikulčić, H., Wang, X., Duić, N., 2020. A review on alternative fuels in future energy system. *Renew. Sustain. Energy Rev.* 128. <https://doi.org/10.1016/j.rser.2020.109927>

140. Subramanian, K.G., Dhanushkodi, M., Satyapriyan, A., 2023. An Intensive Study on Algal Diversity in the Ancient Man - Made Aquatic Ecosystem of Tiruvallur , South India : Exploration for Sustainable Development. *Mol. Biotechnol.* <https://doi.org/10.1007/s12033-023-00817-9>
141. Syafiuddin, A., Chong, J.H., Yuniarto, A., Hadibarata, T., 2020. The current scenario and challenges of biodiesel production in Asian countries: A review. *Bioresour. Technol. Reports* 12, 100608. <https://doi.org/10.1016/j.biteb.2020.100608>
142. Tabatabaei, M., Aghbashlo, M., Dehghani, M., Panahi, H.K.S., Mollahosseini, A., Hosseini, M., Soufiyan, M.M., 2019. Reactor technologies for biodiesel production and processing: A review. *Prog. Energy Combust. Sci.* 74, 239–303. <https://doi.org/10.1016/j.pecs.2019.06.001>
143. Taipabu, M.I., Viswanathan, K., Wu, W., Hattu, N., Atabani, A.E., 2022. A critical review of the hydrogen production from biomass-based feedstocks: Challenge, solution, and future prospect. *Process Saf. Environ. Prot.* 164, 384–407. <https://doi.org/10.1016/j.psep.2022.06.006>
144. Talapatra, N., Gautam, R., Mittal, V., Ghosh, U.K., 2023. A comparative study of the growth of microalgae-bacteria symbiotic consortium with the axenic culture of microalgae in dairy wastewater through extraction and quantification of chlorophyll. *Mater. Today Proc.* 80, 2268–2273. <https://doi.org/10.1016/j.matpr.2021.06.227>
145. Talebian-Kiakalaieh, A., Amin, N.A.S., Mazaheri, H., 2013. A review on novel processes of biodiesel production from waste cooking oil. *Appl. Energy* 104, 683–710. <https://doi.org/10.1016/j.apenergy.2012.11.061>
146. Thangaraj, B., Solomon, P.R., Muniyandi, B., Ranganathan, S., Lin, L., 2019. Catalysis in biodiesel production - A review. *Clean Energy* 3, 2–23. <https://doi.org/10.1093/ce/zky020>
147. Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A., 2010. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. *J. Renew. Sustain. Energy* 2.

<https://doi.org/10.1063/1.3294480>

148. Ummu Kalsum, Ahmad Roesyadi, Mahfud Mahfud, Mahmuddin, Lamatinulu, 2022. The effect of n-hexane as co-solvent in direct trans esterification of *Spirulina platensis* using microwave. *World J. Adv. Res. Rev.* 16, 741–746. <https://doi.org/10.30574/wjarr.2022.16.3.1407>

149. Uyumaz, A., Aydoğan, B., Yılmaz, E., Solmaz, H., Aksoy, F., Mutlu, İ., İpci, D., Calam, A., 2020. Experimental investigation on the combustion, performance and exhaust emission characteristics of poppy oil biodiesel-diesel dual fuel combustion in a CI engine. *Fuel* 280. <https://doi.org/10.1016/j.fuel.2020.118588>

150. Vagheei, R., 2021. Upgrading of waste stabilization ponds using a low-cost small-scale fine bubble diffused aeration system. *Water Sci. Technol.* 84, 3104–3121. <https://doi.org/10.2166/wst.2021.330>

151. van den Oever, A.E.M., Costa, D., Cardellini, G., Messagie, M., 2022. Systematic review on the energy conversion efficiency of biomass-based Fischer-Tropsch plants. *Fuel* 324, 124478. <https://doi.org/10.1016/j.fuel.2022.124478>

152. Verma, S., Das, L.M., Kaushik, S.C., 2017. Effects of varying composition of biogas on performance and emission characteristics of compression ignition engine using exergy analysis. *Energy Convers. Manag.* 138, 346–359. <https://doi.org/10.1016/j.enconman.2017.01.066>

153. Viikari, L., Vehmaanperä, J., Koivula, A., 2012. Lignocellulosic ethanol: From science to industry. *Biomass and Bioenergy* 46, 13–24. <https://doi.org/10.1016/j.biombioe.2012.05.008>

154. Wahlen, B.D., Willis, R.M., Seefeldt, L.C., 2011. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresour. Technol.* 102, 2724–2730. <https://doi.org/10.1016/j.biortech.2010.11.026>

155. Wang, Y.T., Cong, W.J., Zeng, Y.N., Zhang, Y.Q., Liang, J.L., Li, J.G., Jiang, L.Q., Fang, Z., 2021. Direct production of biodiesel via simultaneous esterification and transesterification of renewable oils using calcined blast furnace dust. *Renew. Energy* 175, 1001–1011. <https://doi.org/10.1016/j.renene.2021.05.013>
156. Whangchai, K., Souvannasouk, V., Bhuyar, P., Ramaraj, R., Unpaprom, Y., 2021. Biomass generation and biodiesel production from macroalgae grown in the irrigation canal wastewater. *Water Sci. Technol.* 84, 2695–2702. <https://doi.org/10.2166/wst.2021.195>
157. Xie, W., Han, Y., Wang, H., 2018. Magnetic Fe₃O₄/MCM-41 composite-supported sodium silicate as heterogeneous catalysts for biodiesel production. *Renew. Energy* 125, 675–681. <https://doi.org/10.1016/j.renene.2018.03.010>
158. Xie, W., Huang, M., 2018. Immobilization of *Candida rugosa* lipase onto graphene oxide Fe₃O₄ nanocomposite: Characterization and application for biodiesel production. *Energy Convers. Manag.* 159, 42–53. <https://doi.org/10.1016/j.enconman.2018.01.021>
159. Xie, W., Wan, F., 2019. Immobilization of polyoxometalate-based sulfonated ionic liquids on UiO-66-2COOH metal-organic frameworks for biodiesel production via one-pot transesterification-esterification of acidic vegetable oils. *Chem. Eng. J.* 365, 40–50. <https://doi.org/10.1016/j.cej.2019.02.016>
160. Xie, W., Wang, H., 2021. Grafting copolymerization of dual acidic ionic liquid on core-shell structured magnetic silica: A magnetically recyclable Brønsted acid catalyst for biodiesel production by one-pot transformation of low-quality oils. *Fuel* 283, 118893. <https://doi.org/10.1016/j.fuel.2020.118893>
161. Xie, W., Wang, H., 2020. Immobilized polymeric sulfonated ionic liquid on core-shell structured Fe₃O₄/SiO₂ composites: A magnetically recyclable catalyst for simultaneous transesterification and esterifications of low-cost oils to biodiesel. *Renew. Energy* 145, 1709–1719. <https://doi.org/10.1016/j.renene.2019.07.092>
162. Xie, W., Wang, J., 2014. Enzymatic production of biodiesel from soybean oil by using

immobilized lipase on Fe₃O₄/Poly(styrene-methacrylic acid) magnetic microsphere as a biocatalyst. *Energy and Fuels* 28, 2624–2631. <https://doi.org/10.1021/ef500131s>

163. Xu, W., Duan, L., Wen, X., Li, H., Li, D., Zhang, Y., Zhang, H., 2022. Effects of Seasonal Variation on Water Quality Parameters and Eutrophication in Lake Yangzong. *Water (Switzerland)* 14. <https://doi.org/10.3390/w14172732>

164. Yadav, M., Balan, V., Varjani, S., Tyagi, V.K., Chaudhary, G., Pareek, N., Vivekanand, V., 2022. Multidisciplinary Pretreatment Approaches to Improve the Bio-methane Production from Lignocellulosic Biomass. *Bioenergy Res.* <https://doi.org/10.1007/s12155-022-10489-z>

165. Yen, H., Yang, S., Chen, C., Chang, J., 2014. Supercritical fluid extraction of valuable compounds from microalgal biomass. *Bioresour. Technol.* <https://doi.org/10.1016/j.biortech.2014.10.030>

166. Young, J., Jung, J., Jung, S., Park, Y., Fai, Y., Lin, K.A., Choi, Y., Kwon, E.E., 2022. Biodiesel from microalgae : Recent progress and key challenges. *Prog. Energy Combust. Sci.* 93, 101020. <https://doi.org/10.1016/j.peccs.2022.101020>

167. Zhou, W., Min, M., Hu, B., Ma, X., Liu, Y., Wang, Q., Shi, J., Chen, P., Ruan, R., 2013. Filamentous fungi assisted bio-flocculation: A novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells. *Sep. Purif. Technol.* 107, 158–165. <https://doi.org/10.1016/j.seppur.2013.01.030>

168. Zhu, L.D., Xu, Z.B., Qin, L., Wang, Z.M., Hiltunen, E., Li, Z.H., 2016. Oil production from pilot-scale microalgae cultivation: An economics evaluation. *Energy Sources, Part B Econ. Plan. Policy* 11, 11–17. <https://doi.org/10.1080/15567249.2015.1052594>