Journal of Food Science and Technology (Iran)

Homepage: www.fsct.modares.ir

Scientific Research

Academic Review: Biodiesel Production from Vegetable Oils and Animal Fats with the Help of Microorganisms

Haider Falih Shamikh Al-Saedi[1], Ali Adel[2], Ola Kamal A. Alkadir[3], Heba Ahmed[4], Nekaa Kassim[5], Sadiq H. Al-shaikh[6], Kadhum Sultan [7]

1. Faculty of pharmacy/ department of pharmaceutics/ University of Al-Ameed/ Iraq.
2. Al-Turath University/ Iraq.
3. Al-Nisour University College, Nisour Seq. Karkh, Baghdad, Iraq.
4. Warka University College/ Iraq.
5. department of dentistry/ Al-Manara College For Medical Sciences/ (Maysan)/Iraq.
6. Department of Medical Laboratory Technics, Al-Zahrawi University College, Karbala, Iraq.
7. Muntadher Mazaya university college/ Iraq.

ARTICLE INFO

Article History:

Received: 2025/6/30
Accepted: 2025/8/2

Keywords:

Biodiesel,

Vegetable Oil,

Animal Fat,

Microorganisms

DOI: 10.22034/FSCT.22.164.86.

*Corresponding Author E-Mail:

haider.fs@yahoo.com

ABSTRACT

The growing global demand for sustainable and renewable energy sources has intensified research into biodiesel production as an alternative to fossil fuels. Traditionally, biodiesel is synthesized through the transesterification of vegetable oils and animal fats using chemical catalysts. However, chemical processes often involve harsh reaction conditions, high energy consumption, and challenges related to catalyst recovery and waste management. In recent years, microbial biocatalysts-particularly lipases derived from bacteria, fungi, and yeast have emerged as promising alternatives for catalyzing transesterification reactions under milder, environmentally benign conditions. This review critically examines the fundamentals of biodiesel production from vegetable oils and animal fats, with a particular emphasis on the mechanisms and kinetics of both conventional and enzymatic transesterification processes. The pivotal role of microorganisms in enhancing process efficiency, through strategies such as enzyme immobilization, genetic engineering, and reaction optimization, is systematically explored. Furthermore, comparative analyses of microbial and chemical methodologies are presented, highlighting advantages, limitations, and the economic feasibility of microbial-assisted production. The review concludes with a discussion on current challenges and future research prospects, underscoring the potential of microbial biotechnologies to advance sustainable biodiesel production and contribute to global bioenergy goals.

1-Introduction

Biodiesel Production from Vegetable Oils and Animal Fats The quest for sustainable energy sources has gained immense traction in recent years, especially considering the environmental concerns associated with fossil fuel consumption. Biodiesel, a renewable and biodegradable alternative to traditional diesel, is primarily derived from vegetable oils and animal fats [1]. Its production process, known as transesterification, has garnered significant attention, particularly in the context of optimizing this process through the utilization of microorganisms [2]. Biodiesel is a form of mono alkyl esters derived from natural oils or fats. It can be used as a direct substitute for petroleum diesel, making it compatible with existing diesel engines [3]. The significance of biodiesel lies in its renewable nature, lower greenhouse gas emissions, and potential to reduce reliance on fossil fuels. Furthermore, biodiesel can be produced from non-food biomass, which minimizes competition with food production, making it a more sustainable energy alternative [4]. The primary feedstocks for biodiesel production include various vegetable oils (such as soybean, sunflower, canola, and palm oils) and animal fats (such as tallow and lard). The choice of feedstock significantly influences the yield and quality of biodiesel produced. Vegetable oils generally yield higher biodiesel content, while the higher free fatty acid (FFA) content in animal fats can complicate the transesterification process. Thus, selecting appropriate feedstock based on availability, characteristics, and economic factors is vital in biodiesel production. The Transesterification Process Transesterification is the chemical reaction that converts triglycerides (the main component of fats and oils) into biodiesel (fatty acid methyl esters, or FAME) by reacting them with alcohol (usually methanol or ethanol) in the presence of a catalyst [5]. This process can be summarized in the following steps:

1. Feedstock Preparation: Raw oils and fats are preprocessed to remove impurities and water that can interfere with the reaction.

2. Catalyst Selection: Catalysts play a crucial role in transesterification. Both alkaline (sodium hydroxide, potassium hydroxide) and acid catalysts (sulfuric acid) are widely used, each with its own advantages and limitations.

3. Reaction Process: The mixture of oils and alcohol is subjected to appropriate temperature and pressure conditions to facilitate the transesterification reaction.

4. Separation and Purification: Following the reaction, the biodiesel is separated from glycerol, the byproduct of the process. Purification steps, such as washing and drying, are essential to obtain high quality biodiesel.

5. Characterization: The produced biodiesel must be characterized and tested against standards (such as ASTM D6751 or EN 14214) to ensure it meets the necessary performance criteria for use in engines [6].

Microorganisms, particularly bacteria, yeast, and fungi, have emerged as promising tools in enhancing biodiesel production processes. Their involvement in several stages of biodiesel production presents opportunities for cost reduction and improved efficiency. Enzymatic Transesterification Microbial lipases are enzymes that catalyze the transesterification process [7]. Using lipases, which can be produced by various microorganisms, can provide distinct advantages over traditional chemical catalysts: 1. Tolerance to High Free Fatty Acids (Lipases can effectively convert feedstocks with high FFA content without the need for pretreatment, making them suitable for lower quality feedstocks such as used cooking oils). 2. Milder Reaction Conditions (Enzymatic reactions typically occur under milder temperature and pressure conditions, leading to lower energy consumption and reduced production costs). 3. Environmental Benefits (The use of microorganisms minimizes the need for harsh chemicals, making the process more environmentally friendly. Microbial Fermentation Some microorganisms also facilitate fermentation processes to produce oils and fats that can be subsequently converted to biodiesel) [8].

For instance, specific strains of yeast and fungi can yield oils from agricultural waste or lignocellulosic materials, providing an innovative means of producing biodiesel feedstock. Optimization of the Transesterification Process Microorganisms can also play a crucial role in optimizing the transesterification process. Research has demonstrated that: Strain Improvement (Genetic engineering and selective breeding can enhance desirable characteristics in microbial strains, leading to improved lipase production), Co culture Systems (Employing consortia of microorganisms can yield synergistic effects, leading to more efficient substrate utilization and higher biodiesel yields), Bioreactor Innovations (Advances in bioreactor design facilitate improved microbial growth and enzyme production, further enhancing the overall biodiesel production process) [9]. As the world moves towards sustainable energy solutions, biodiesel from vegetable oils and animal fats presents significant promise. The transesterification process serves as the cornerstone for converting oils and fats into biodiesel. The integration of microorganisms into this process is at the forefront of research, providing innovative ways to optimize production while minimizing environmental impact [10]. This research promises to provide insights into the multifaceted nature of biodiesel production, showcasing the blend of traditional chemistry with cutting edge microbiological techniques. By focusing on the interplay between microorganisms and the transesterification process, we aim to inspire further research and innovation in the field of sustainable energy, ultimately contributing to a greener, more sustainable future. Join us on this journey as we unravel the complexities of biodiesel production and the transformative potential technology holds for our energy landscape.

Table 1

. Research conducted on biodiesel production using microbial enzymes

Researcher	Year	Process conditions	Resul
Watanabe et al	2001	Stabilized Enzyme *Candida antarctica* (Novozym 435) **Raw materials:** Waste cooking oil **Temperature:** 30-40°C **Time:** 50 hours **Ratio of methanol to oil:** 3:1 (added in a stepwise manner)	97% FAME yield High tolerance to FFA (free fatty acids) without soap formation Catalyst reusability for 100 cycles
Shimada et al	2002	Stabilized enzyme *Candida antarctica* **Raw materials: ** Soybean oil **Temperature: ** 30°C **Methanol to oil ratio: ** 3:1 **Additive: ** 4-5% water (by volume)	98% FAME yield in 10 hours - Optimal water to maintain enzyme activity - High purity glycerol was produced
Hama et al	2013	Whole cells of *Rhizopus oryzae* (immobilized in biopolymer) **Raw	92% FAME yield - Cost reduction by eliminating enzyme

		materials: ** Crude palm oil **Temperature: ** 45°C **Time: ** 60 hours	extraction - High FFA tolerance (up to 10%) without the need for pretreatment
Adewale et al	2015	Pseudomonas cepacia lipase (immobilized on silica) **Raw materials: ** Animal fat (beef tallow) **Temperature: ** 50°C **Time:** 24 hours	95% FAME yield - Excellent performance on saturated fats - 70% reduction in wastewater compared to chemical catalyst
Lee et al	2022	Engineered lipase *Thermomyces lanuginosus* (magnetic nanoparticles **Raw materials:** Soybean oil Waste cooking oil < br>;**Temperature:** 55° **Time:** 12 hours	96% FAME yield - Easy catalyst recovery with magnet - 85% activity retention after 15 cycles of - 40% reduction in operating costs

2. Fundamentals of Biodiesel Production

Biodiesel has emerged as a promising alternative to conventional diesel fuel, combining environmental sustainability with energy security. Produced from renewable resources such as vegetable oils and animal fats, biodiesel offers several advantages, including reduced greenhouse gas emissions and decreased reliance on fossil fuels [8]. Feedstock Selection Types of Feedstocks Biodiesel can be produced from a variety of feedstocks, primarily categorized into three groups:

1. Vegetable Oils: Common sources include soybean oil, rapeseed oil, palm oil, and sunflower oil. These oils are rich in triglycerides, which are chemically converted into biodiesel.

2. Animal Fats: Rendered fats from meat processing can also serve as effective feedstocks. Examples include tallow and lard, which can yield biodiesel similar in quality to that produced from vegetable oils.

3. Waste Oils: Used cooking oils and grease represent a cost effective and environmentally sound feedstock. Recycling these oils reduces waste and limits the need for virgin resources [11].

Factors Affecting Feedstock Choice The choice of feedstock is influenced by several factors: Availability (The local availability of feedstocks can dictate their use). Regions rich in certain crops may favor those oils. Cost Economic viability is critical. Feedstocks that are readily available and inexpensive will enhance the profitability of biodiesel production [12]. Fatty Acid Composition (The composition of fatty acids within the feedstock affects the quality of the final biodiesel). Higher amounts of unsaturated fats typically yield biodiesel with better low temperature properties. Biodiesel Production Processes Transesterification The primary method for producing biodiesel is transesterification, a chemical reaction that involves the conversion of triglycerides into biodiesel (fatty acid methyl esters FAME) and glycerol. Transesterification reacts triglycerides with alcohol (usually methanol or ethanol) in the presence of a catalyst commonly sodium hydroxide or potassium hydroxide [13]. The reaction proceeds through the following steps Triglycerides react with the alcohol, forming diglycerides and releasing glycerol. Diglycerides further react with alcohol, yielding monoglycerides and more glycerol. Finally, monoglycerides react with alcohol, producing biodiesel and glycerol. Batch Process often used for small scale production, this method processes fixed amounts of feedstock and alcohol in controlled conditions. Continuous Process suitable for large scale operations, this process continuously feeds reactants and draws off completed biodiesel and glycerol, increasing efficiency [14]. Other methods of biodiesel production include: Supercritical Fluid Processing (This method uses supercritical methanol, eliminating the need for a catalyst and allowing for faster reaction times). Enzymatic Transesterification (Utilizing enzymes as catalysts, this method can operate under milder conditions, making it suitable for feedstocks containing high levels of free fatty acids). Quality Standards Biodiesel Specifications To ensure the safety and performance of biodiesel, several quality standards have been established globally. The most prominent include ASTM D6751 in the United States EN 14214 in Europe These standards cover multiple parameters such as: Viscosity (Appropriate viscosity is crucial for fuel injection systems). Flash Point (A higher flash point enhances safety during handling). Cold Flow Properties (These dictate biodiesel's performance in colder temperatures) [11]. Testing and Certification Quality testing involves thorough analysis to ascertain that biodiesel meets the specified standards. Common tests include: Fuel Composition Analysis and Stability Tests. Market Demand The demand for biodiesel is driven by policy incentives, such as Renewable Fuel Standards (RFS) and mandates for biofuel usage. Increased interest in sustainable energy fuels has bolstered the biodiesel market, promoting growth and investments in production technologies. Biodiesel production presents a scalable, environmentally friendly alternative to fossil fuels. By understanding the intricacies of feedstock selection, production processes, quality standards, and economic considerations, stakeholders can enhance the sustainability and viability of biodiesel as a mainstream energy solution. As technologies continue to evolve, biodiesel has the potential to play a pivotal role in transitioning to a more sustainable energy landscape [16].

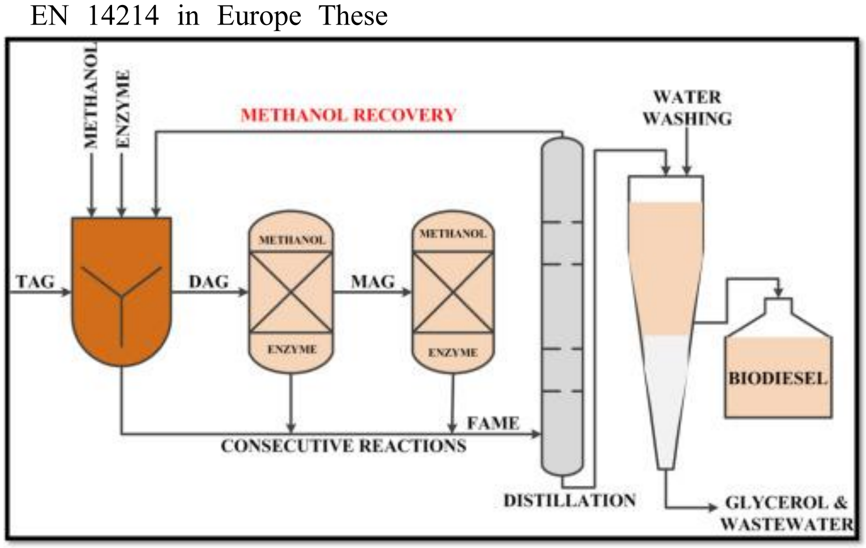

Figure 1: Feedstock Flexibility [17].

3. Conventional Transesterification Processes

Conventional Transesterification Processes Transesterification is a chemical process that transforms triglycerides (fats and oils) into fatty acid methyl esters (FAMEs) and glycerol. This reaction is pivotal in the production of biodiesel and is widely explored for its application in biofuels to reduce reliance on fossil fuels. Mechanism of Transesterification The transesterification process involves the reaction of triglycerides with alcohol, primarily methanol or ethanol, in the presence of a catalyst [18]. The mechanism can be divided into several steps:

1. Formation of Alkoxide Ion: The alcohol first reacts with a catalyst to form an alkoxide ion, which is a strong nucleophile.

2. Nucleophilic Attack: The alkoxide ion attacks the carbonyl carbon of the triglyceride, leading to the formation of a tetrahedral intermediate.

3. Formation of FAME and Glycerol: The tetrahedral intermediate rearranges, causing the cleavage of a fatty acid chain and producing FAME and glycerol.

4. Equilibrium Shift: The reaction is reversible; thus, it is crucial to remove glycerol or excess alcohol to drive the reaction toward the formation of FAME [19].

Catalysts Used in Transesterification typically employs two types of catalysts: alkaline (basic) and acidic catalysts. Each has distinct advantages and disadvantages, which determine their application in specific contexts. Alkaline Catalysts Alkaline catalysts, such as sodium hydroxide (NaOH) and potassium hydroxide (KOH), are widely favored due to their high reactivity and efficiency [20]. They are suitable for oils with low free fatty acid (FFA) content (usually less than 5%). The advantages include: Fast Reaction Rates: Alkaline catalysts achieve a higher rate of reaction due to the strong nucleophilicity of alkoxide ions [21].

Lower Energy Requirement: Their catalytic activity allows for milder reaction conditions and lower energy input. However, alkaline catalysts have their limitations, such as: Sensitivity to Water: Presence of water can hinder the reaction, making them unsuitable for high FFA feedstocks. Saponification Risk: They can lead to saponification, which produces soap and complicates the separation of products. Acidic Catalysts Acidic catalysts, including sulfuric acid (H_2SO_4) and phosphoric acid (H_3PO_4), are employed when oils with high FFA content are used. Tolerance to Water: Acidic catalysts are more tolerant of water and can handle feedstocks with FFAs greater than 5%. No Saponification: The risk of saponification is minimal compared to alkaline catalysts. However, the use of acidic catalysts comes with tradeoffs: Slower Reaction Rates: The reaction speed is considerably slower than with alkaline catalysts [22]. Higher Costs: Acidic catalysts are generally more expensive and require longer reaction times. Factors Influencing Transesterification Several factors affect the efficiency and yield of the transesterification process: Alcohol to Oil Ratio The alcohol to oil molar ratio significantly influences the reaction rate and yield. Typically, a higher ratio (methanol or ethanol) can enhance the conversion rate, usually ranging from 6:1 to 12:1. However, using excess alcohol can lead to increased costs and environmental concerns. Reaction Temperature and Time Temperature and reaction time are critical parameters [23]. Higher temperatures tend to accelerate the reaction, enhancing the yield. Optimal temperatures typically range from 50°C to 65°C. Reaction time generally varies from 1 to 8 hours, depending on the catalyst used and the nature of the feedstock. Mixing Intensity Adequate mixing is essential for successful transesterification. Poor mixing can lead to phase separation and reduced yields. Continuous agitation ensures the uniform interaction between reactants. Types of Feedstocks The selection of feedstock plays a crucial role in biodiesel production and impacts the overall economics of the process. Feedstocks can include: Vegetable Oils: Commonly used oils (e.g., soybean oil, canola oil, sunflower oil) typically have low FFA levels, making them ideal for alkaline catalysis [24].

Animal Fats: Tallow and lard can also serve as feedstocks; however, they may contain higher levels of saturated fats, impacting the biodiesel properties. Waste Cooking Oils: Used oils can be economically favorable but generally have higher FFA levels, necessitating acidic catalysis. Environmental Implications The use of transesterification in biodiesel production presents considerable environmental benefits. Biodiesel is biodegradable, reduces greenhouse gas emissions compared to fossil fuels, and helps to decrease reliance on non-renewable resources. However, issues related to land use changes for feedstock production, energy inputs, and the end life of byproducts like glycerol must also be carefully managed [25]. Conventional transesterification processes are vital for producing biodiesel and have a significant impact on reducing dependence on fossil fuels. The choice of catalyst, feedstock, and optimization of reaction conditions play crucial roles in determining the efficiency and sustainability of the process. As the demand for renewable energy sources grows, enhancing and refining transesterification methods will remain essential for advancing biodiesel production. The combined understanding of the chemical mechanisms, catalytic processes, and their environmental implications will facilitate the growth of this sector [26].

4. Role of Microorganisms in Biodiesel Production

Several types of organisms such as whole "An uncommon focus has been placed of green growth, organisms, yeast, and microbes are utilized to create biofuel which incorporate a few steps such as high-impact and anaerobic aging, transesterification, etc. for biofuel generation. Various microorganisms have been detailed to be included in biofuel preparations such as bioethanol/biobutanol, biogas, biohydrogen, and bioelectricity generation [27]. An uncommon focus has been placed on later microbial assets recognized for these purposes from saline and other natural conditions. Particular applications of microorganisms in pretreatment of strong squander and wastewater are moreover examined [28]. Saccharomyces sp., Kluyveromyces sp., Clostridium sp., and Trichoderma sp. have been broadly misused to get a tall abdicate of less difficult sugars, lower concentration of inhibitory compounds, and tall biofuel abdicate [29].

Algae, another fascinating group of microorganisms, can produce oils that are convertible into biodiesel. These microorganisms thrive in various environments, including freshwater and marine ecosystems, making them incredibly versatile. Researchers are exploring different strains of algae to optimize oil production, using sunlight and carbon dioxide in the process, which makes them a promising option for a more sustainable biofuel source [28]. The rapid growth and high yield of algae give them an edge in biofuel production compared to traditional crops. In addition to algae and yeast, certain bacteria have shown the ability to break down waste materials and transform them into renewable energy sources. This not only provides a sustainable way to generate biofuels but also helps in reducing waste by converting it into something useful [30]. Overall, the diverse capabilities of microorganisms in biofuel production highlight their potential to address energy needs while minimizing the environmental impact, making them integral to the future of renewable energy [31]. A few steps have been taken in later a long time to create hereditarily designed microorganisms to improve saccharification of lignocellulosic biomass, diminish the generation of inhibitory sugars, and increment the resilience level of the maturing microorganisms for alluring conclusion items [27].

Tiny living things, called microorganisms, are studied in many areas of science. They are found in many places on Earth, and using them is starting a small but important change in technology. Tiny living things called microbes are being used to make ethanol for biofuels [31]. This ethanol comes from lignocellulose, which is a mix of cellulose, hemicellulose, and lignin found in plant cell walls. The enzyme that breaks down cellulose is called cellulase. Scientists have been studying where this enzyme comes from in different kinds of tiny living things found in various places. Some of these places are strange, like the insides of termites' stomachs and the soil near volcanoes. Sulfurous solfataras' is a tiny living thing called an archaeon that lives in hot pools near Mount Vesuvius. Researchers are trying out genetic changes to help this microbe make the enzymes it needs better [32]. The fungus Trichoderma reesei is found in soil all around the world. It eats by releasing a lot of cellulase. This fungus was first found during World War II. It caused "jungle rot," which ruined the tents and uniforms of American soldiers by breaking down the material. A Canadian company has changed the fungus using science to make it produce more cellulase, which helps turn straw into sugar. This sugar can then be converted into ethanol. They have turned 75% of the straw into sugar. Another possible answer is algae [33]. They use sunlight to turn carbon dioxide into sugar, and then they use that sugar to make fats. Scientists are using small lab machines to turn oils into biodiesel and plant sugars from algae into bioethanol. If they can make it bigger for factories, using algae to make biofuel could be a big part of our energy sources. There is also the issue of trash. Since the plant material used, like straw, can't be eaten, it gets broken down. A system that uses tiny living things to make biofuel is better for the environment, fairer, and less expensive. It also greatly lowers the use and release of greenhouse gases [34].

5. Optimization of Transesterification by Microorganisms

Transesterification is a chemical reaction that converts triglycerides into fatty acid methyl esters (FAME) and glycerol, typically used for biodiesel production. The process can be significantly enhanced through the use of microorganisms that have the capability to catalyze the transesterification of lipids. Transesterification is a pivotal reaction in the biodiesel industry, where oils and fats, such as vegetable oils and animal fats, are converted into biodiesel and glycerol, a valuable by product. The reaction involves the exchange of ester groups between glycerides and alcohol (usually methanol or ethanol) in the presence of a catalyst. Traditionally, the process is catalyzed using chemical methods that require harsh conditions. However, the use of microorganisms provides a biocatalytic alternative that is environmentally friendly and potentially more efficiency [35].

Microorganisms, such as bacteria and yeast, can produce lipases, enzymes that hydrolyze triglycerides into free fatty acids and glycerol, and subsequently catalyze the esterification of these fatty acids with alcohol [36]. Microbial transesterification proceeds through several steps:

1. Hydrolysis of Triglycerides: Lipases catalyze the hydrolysis of triglycerides into free fatty acids and monoglycerides.

2. Esterification: Free fatty acids are converted into FAME through their reaction with an alcohol.

3. Formation of Biodiesel: The final product, FAME, constitutes biodiesel, which can be further processed and purified [37].

Factors Affecting the Rate of Transesterification Several factors influence the efficiency and output of transesterification by microorganisms, including:

1. Substrate Concentration The concentration of the oils or fats used in the reaction plays a crucial role in determining the reaction rate. Higher concentrations can lead to increased yields; however, excessively high concentrations can inhibit the activity of microbial lipases due to substrate inhibition.

2. Type of Microorganism Different microorganisms exhibit varying levels of lipase activity. Yeast, such as Candida, and bacteria, such as Pseudomonas and Bacillus, are known to produce efficient lipases. Selecting the appropriate microorganism based on the source and type of oil being used is critical for optimizing yield.

3. Alcohol to Oil Ratio The molar ratio of alcohol to oil is an important factor in transesterification. Optimal ratios generally range between 1:1 and 6:1, with higher ratios often required for high viscosity oils. The right alcohol to oil ratio ensures the complete conversion of triglycerides into biodiesel.

4. Reaction Conditions pH, temperature, and time significantly affect the performance of microbial lipases. Most organisms have specific conditions that maximize lipase activity. For example, a temperature range of 30-60°C and a neutral or slightly alkaline pH are commonly optimal for many lipase producing microbes.

5. Cell Density The concentration of microbial cells in the reaction medium, or cell density, also has a significant effect on the rate of the transesterification reaction [38]. Higher cell densities can lead to increased lipase availability, accelerating the transesterification.

Potential Microbial Candidates Numerous microorganisms show promise in optimizing transesterification:

1. Bacillus subtilis Bacillus subtilis is a gram-positive bacterium known for its robust lipase production. Its ability to thrive in various conditions makes it a potential candidate for biodiesel production.

2. Candida rugosa This yeast is widely studied for its efficient lipase, exhibiting substantial activity against a range of triglycerides and is often used in biodiesel production.

3. Pseudomonas fluorescens inconsistent pronoun reference, Pseudomonas fluorescens produces effective lipases that can be harnessed for transesterification [39].

Enhancing Microbial Efficiency To maximize the effectiveness of microorganisms in transesterification, several strategies can be employed:

1. Genetic Engineering Genetic modifications can enhance the lipase production and transesterification activity of microorganisms. Techniques such as CRISPR Cas9 can be utilized to optimize lipase genes.

2. Process Optimization Bioprocess engineering approaches, including continuous flow systems, can enhance the efficiency of microbial transesterification. By continuously feeding substrates and removing products, the overall yield can be increased.

3. Co culturing Utilizing a combination of different microbial species can lead to synergistic effects, boosting lipid degradation and esterification rates.

4. immobilization of Microorganisms Immobilizing microorganisms can enhance their

stability and reusability, allowing for easier recovery and implementation in industrial processes [37]. The optimization of transesterification by microorganisms holds significant promise for sustainable biodiesel production. With an understanding of the enzymatic mechanisms and the impact of various factors, researchers can improve the efficiency of microbial transesterification. Further exploration of microbial diversity and the application of advanced biotechnological methods will contribute to making biodiesel a viable alternative to fossil fuels. By addressing the key elements of substrate selection, environmental conditions, and biocatalyst optimization, the potential for microbial transesterification continues to grow, paving the way for a more sustainable energy future [40].

6. Comparative Analysis of Microbial and Conventional Methods

Microbial methods pertain to techniques that utilize microorganisms, such as bacteria, fungi, and viruses, for various applications. These methods exploit the natural properties of microbes, enabling processes such as fermentation, biodegradation, and bioremediation [41].

Conventional methods refer to traditional analytical and production techniques that do not rely on living organisms. These often include chemical synthesis, physical processing, and standard laboratory methods like chromatography, titration, and spectrometry. Principles Microbial Methods Microbial methods are grounded in the biological processes of microorganisms. By harnessing specific metabolic pathways, these methods can produce desired substances (e.g., antibiotics, enzymes) or degrade pollutants (e.g., oil spills, heavy metals). Conventional methods operate on chemical reactions and physical changes, often employing reagents to perform analyses or achieve specific outcomes [42]. They rely on standardized procedures to ensure reproducibility and accuracy. Applications Microbial Methods:

1. Pharmaceutical Industry: Microbial fermentation is crucial for producing antibiotics, hormones, and vaccines.

2. Agriculture: Biofertilizers and biopesticides derived from microbes improve crop yields and reduce chemical usage.

3. Environmental Remediation: Microbial methods are employed in bioremediation to detoxify contaminated environments, such as oil spills or heavy metal pollution.

4. Food Production: Microbial fermentation processes are essential in producing yogurt, cheese, and fermented beverages [43].

Conventional Methods:

1. Analytical Chemistry: Techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) are commonly used for detailed chemical analysis.

2. Quality Control: Conventional methods ensure product safety and consistency in industries like food and pharmaceuticals through methods like microbial quality testing.

3. Materials Science: Conventional synthesis routes are critical in manufacturing polymers, metals, and composites.

4. Food Safety: Traditional chemical tests like pH measurements and titrations are used to check food quality [44].

Advantages Microbial Methods:

1. Eco Friendly: Microbial processes are often more sustainable and environmentally friendly compared to chemical processes.

2. Efficiency: They can produce complex compounds that are challenging to synthesize chemically.

3. Cost Effective: Fermentation can be less expensive on a large scale than traditional chemical synthesis.

4. Biodegradability: Products derived from microbes are often biodegradable, thus reducing pollution [45].

Conventional Methods:

1. Precision and Control: Conventional methods often offer higher precision and reproducibility in analytical processes.

2. Established Protocols: Many conventional methods are standardized and widely accepted in laboratories, ensuring consistent results.

3. Speed: Conventional methods can sometimes provide faster results than microbial methods, especially in analytical contexts.

4. Scalability: Many conventional techniques can be scaled easily for mass production [46].

Limitations Microbial Methods:

1. Variability: Microbial processes can be influenced by environmental conditions, leading to variability in product yield.

2. Time Consuming: The fermentation period can be lengthy, delaying product availability.

3. Contamination Risk: Many processes are susceptible to contamination, which can compromise purity and safety.

4. Regulatory Hurdles: The use of live organisms raises bioethical and regulatory issues that can complicate product approval [47].

Conventional Methods:

1. Environmental Impact: Many conventional methods generate substantial chemical waste, posing an environmental hazard.

2. Limited Complexity: Some complex biomolecules cannot be synthesized using traditional synthesis routes.

3. Costly Reagents: The cost of reagents and chemicals in conventional methods can escalate production costs.

4. Health Risks: The use of hazardous chemicals in traditional methods can pose health risks to workers and consumers [48].

The future of microbial methods is promising, particularly with advancements in genetic engineering and synthetic biology. These innovations are expected to enhance the efficiency of microbial processes, reduce production times, and expand the range of applicable microorganisms. Conventional Methods While conventional methods will remain essential for many analytical applications, there is a growing trend toward integrating them with microbial methods [49]. Hybrid approaches may provide the best of both worlds, combining precision with sustainability. The comparative analysis of microbial and conventional methods reveals significant differences in their principles, applications, advantages, and limitations. Each method has its unique strengths and weaknesses, which will drive their evolution and integration in the future [50] As industries strive for greater sustainability, the synergy between microbial and conventional methods will likely lead to innovative solutions that address current and future challenges in various fields. Both methodologies have vital roles and will continue to coexist and evolve side by side, ultimately enhancing our ability to respond to complex global needs [51].

7. Future Prospects and Research Directions

Future Outlook and Research Direction and Animal Fats with Microorganisms The interest in biodiesel production has surged in recent years owing to the growing need for sustainable energy sources. Specifically, the conversion of vegetable oils and animal fats into biodiesel through microbial processes presents an innovative avenue for researchers [52]. Strain Selection and Engineering The choice of microorganism is critical to biodiesel production efficiency. Traditional microbial strains do not always yield high lipid content or have suboptimal growth rates. Furthermore, genetic modifications can lead to a lack of stability or unwanted by products. Process Optimization Microbial biodiesel production must balance various factors, including temperature, pH, and nutrient availability [53]. Often, suboptimal conditions lead to low lipid yields. Additionally, the kinetics of microbial growth and lipid accumulation are not fully understood, resulting in inefficiencies in production cycles. While laboratory scale experiments can show promising results, scaling up to industrial levels poses significant challenges. The production systems must be economically viable and easily manageable-issues that current research has struggled to address adequately [54]. The use of diverse feedstocks like waste vegetable oils, animal fats, and microalgae introduces variability in lipid composition. This variability affects both yield and quality of biodiesel, complicating the standardization of production methods. Concerns Questions regarding the environmental impacts of cultivating feedstocks are also critical. Regulatory frameworks for biodiesel production vary worldwide, necessitating comprehensive studies to evaluate compliance and sustainability [55]. To overcome the aforementioned challenges, future research should focus on the following key areas [56]:

1. Advancements in Synthetic Biology Synthetic biology offers a promising pathway for engineering microbial strains with high lipid accumulation capabilities. Genome editing techniques such as CRISPR/Cas9 could be leveraged to enhance microbial lipid biosynthesis pathways. Future research should focus on identifying and modifying genes responsible for lipid production to improve yield and stability.

2. Process Integration and Optimization Research should explore integrating different bioprocesses such as fermentation and lipid extraction. This could involve the use of co cultures of different microorganisms that can work synergistically to optimize lipid yields. Additionally, the application of machine learning and artificial intelligence in modeling and optimizing bioprocess parameters can lead to substantial improvements in production efficiency.

3. Experimenting with Cultivation Techniques Investigating innovative cultivation methods, such as photobioreactors for microalgae or continuous culture systems, could enhance lipid productivity. Future studies should assess the economic viability of these technologies in conjunction with microbial screening efforts.

4. Exploring Alternative Feedstocks Expanding the focus to less conventional and waste-based feedstocks would improve sustainability and reduce competition with food resources. Research into lignocellulosic materials or non-food crops could provide alternative sources for microbial biodiesel, thus broadening feedstock options while addressing environmental concerns.

5. Life Cycle Assessment (LCA) Studies To ensure that biodiesel production methods are sustainable, comprehensive life cycle assessments should be conducted. Future research must prioritize understanding the entire environmental impact, from feedstock cultivation to end use, thereby ensuring that microbial

biodiesel production is truly a sustainable alternative.

To foster the transition to microbial biodiesel production, it is crucial to align research with societal needs and market opportunities. Collaboration with industry stakeholders is essential for scaling up research findings to practical applications. Furthermore, educating the public about the benefits of biodiesel can enhance acceptance and potentially drive demand [57].

8. Conclusion

Biodiesel Production from Vegetable Oils and Animal Fats Utilizing Microorganisms The global quest for sustainable and renewable energy sources has led to increased interest in biodiesel production, particularly through the transesterification of vegetable oils and animal fats. This conclusion synthesizes the key findings of the study, emphasizing the significance of microorganisms in optimizing the transesterification process, the challenges faced, and future perspectives for biodiesel production. Traditional methods often employ chemical catalysts, which may have limitations such as energy consumption and environmental impact. Therefore, the utilization of microorganisms presents a promising alternative due to their potential to carry out transesterification efficiently and sustainably. Role of Microorganisms in Transesterification Microorganisms such as bacteria, yeast, and fungi have been shown to possess lipases, the enzymes responsible for catalyzing the transesterification reaction. Lipases can operate under mild conditions, which reduces energy requirements and minimizes by product formation compared to chemical catalysts. Furthermore, the adaptability of microorganisms allows them to utilize a wide range of feedstocks, including waste oils and fats that would otherwise be discarded. Research has indicated that certain strains of microorganisms can enhance biodiesel yield by optimizing reaction conditions, such as temperature, pH, and substrate concentration. For instance, lipase producing bacteria like Pseudomonas and Candida species have demonstrated significant efficiency in converting various lipid sources into biodiesel. These microorganisms not only facilitate the reaction but also contribute to the development of biotechnological processes that can lead to the production of biodiesel in a more environmentally friendly manner. The optimization of the transesterification process through microbial action requires an in depth understanding of the microorganisms involved. Factors such as the selection of microbial strains, enzyme activity, and the fermentation conditions can greatly influence biodiesel yield. Strategies such as genetic engineering, metabolic pathway optimization, and the use of co cultures have been explored to boost lipase production and activity. Moreover, the integration of waste management and biodiesel production processes can enhance sustainability. By utilizing agricultural and food waste as feedstocks, the overall carbon footprint of biodiesel production can be reduced, contributing to a circular economy. This approach not only maximizes resource efficiency but also addresses waste disposal challenges. Challenges and Limitations Despite the promising potential of microorganisms in biodiesel production, several challenges remain. The scalability of microbial processes is a critical consideration; while laboratory experiments often yield high efficiency, replicating these results in an industrial setting poses difficulties related to mass production of lipases and maintaining optimal growth conditions for microorganisms. Another challenge is the competition between microorganisms for substrates, which can lead to unpredictable outcomes in mixed cultures. Moreover, the sensitivity of microbial enzymes to environmental factors necessitates careful control and monitoring of production conditions. Future Perspectives Looking forward, the field of biodiesel production through microbial transesterification is ripe for innovation. Further research is essential to identify and engineer microbial strains that can withstand harsher industrial conditions and have higher lipid recovery efficiencies. Advanced genomic techniques, such as CRISPR and synthetic biology, hold great promise for tailoring microbial strains to optimize biodiesel production pathways. Additionally, ongoing studies into the synergistic effects of microbial consortia can lead to the development of more robust biodiesel production systems. Integrating bioprocessing with waste treatment and resource recovery could also enhance the overall sustainability and economic viability of biodiesel as an alternative energy source. In conclusion, the study of biodiesel production from vegetable oils and animal fats utilizing microorganisms highlights the critical role of biocatalysis in enhancing transesterification processes. By leveraging the natural capabilities of lipase producing microorganisms, it is possible to achieve efficient and sustainable biodiesel production. Although challenges in scaling up and optimizing microbial processes persist, the future remains optimistic with the advancement of biotechnological approaches and a growing commitment to sustainable energy practices. Through concerted efforts in research and development, the full potential of microbial biodiesel production can be realized, supporting global transitions toward renewable energy and cleaner environments.

9. References

[1] Ganesan, D., Rajendran, A., & Thangavelu, V. (2009). An overview on the recent advances in the transesterification of vegetable oils for biodiesel production using chemical and biocatalysts. *Reviews in Environmental Science and Bio/Technology*, *8*, 367-394.

[2] Parawira, W. (2009). Biotechnological production of biodiesel fuel using biocatalysed transesterification: a review. *Critical reviews in biotechnology*, *29*(2), 82-93.

[3] Bhan, C., & Singh, J. (2020). Role of microbial lipases in transesterification process for biodiesel production. *Environmental Sustainability*, *3*, 257-266.

[4] Patel, A., Karageorgou, D., Rova, E., Katapodis, P., Rova, U., Christakopoulos, P., & Matsakas, L. (2020). An overview of potential oleaginous microorganisms and their role in biodiesel and omega-3 fatty acid-based industries. *Microorganisms*, *8*(3), 434.

[5] Demirbas, A. (2008). Comparison of transesterification methods for production of biodiesel from vegetable oils and fats. *Energy conversion and management*, *49*(1), 125-130.

[6] Kayode, B., & Hart, A. (2019). An overview of transesterification methods for producing biodiesel from waste vegetable oils. *Biofuels*, *10*(3), 419-437.

[7] Kayode, B., & Hart, A. (2019). An overview of transesterification methods for producing biodiesel from waste vegetable oils. *Biofuels*, *10*(3), 419-437.

[8] Hájek, M., Vávra, A., de Paz Carmona, H., & Kocík, J. (2021). The catalysed transformation of vegetable oils or animal fats to biofuels and bio-lubricants: a review. *Catalysts*, *11*(9), 1118.

[9] Hymavathi, D., Prabhakar, G., & Babu, B. S. (2014). Biodiesel production from vegetable oils: an optimization process. *International Journal of Chemical & Petrochemical Technology (IJCPT)*, *4*(2), 21-30.

[10] Ghaly, A. E., Dave, D., Brooks, M. S., & Budge, S. (2010). Production of biodiesel by enzymatic transesterification. *Am J Biochem Biotechnol*, *6*(2), 54-76.

[11] Badday, A. S., Abdullah, A. Z., Lee, K. T., & Khayoon, M. S. (2012). Intensification of biodiesel production via ultrasonic-assisted process: A critical review on fundamentals and recent development. *Renewable and Sustainable Energy Reviews*, *16*(7), 4574-4587.

[12] Cako, E., Wang, Z., Castro-Muñoz, R., Rayaroth, M. P., & Boczkaj, G. (2022). Cavitation based cleaner technologies for biodiesel production and processing of hydrocarbon streams: A perspective on key fundamentals, missing process data and

economic feasibility–A review. *Ultrasonics sonochemistry*, *88*, 106081.
[13] De Blasio, C., & De Blasio, C. (2019). Biodiesel. *Fundamentals of Biofuels Engineering and Technology*, 253-265.
[14] Aqeel, A., Zafar, J., Mohammadi, P., Tabatabaei, M., Aghbashlo, M., Mahlia, T. I., & Nizami, A. S. (2023). Biodiesel: the fundamentals. In *Sustainable Biodiesel* (pp. 1-20). Academic Press.
[15] Li, H., Cann, A. F., & Liao, J. C. (2010). Biofuels: biomolecular engineering fundamentals and advances. *Annual review of chemical and biomolecular engineering*, *1*(1), 19-36.
[16] Ferreira Maluf Braga, A., & Zaiat, M. (2022). Fundamentals of biofuel production using anaerobic digestion: metabolic pathways and factors affecting the process. *Renewable Energy Technologies for Energy Efficient Sustainable Development*, 3-21.
[17] Adewale, P., Dumont, M. J., & Ngadi, M. (2015). Recent trends of biodiesel production from animal fat wastes and associated production techniques. *Renewable and Sustainable Energy Reviews*, *45*, 574-588.
[18] Montcho Papin, S., Christian, K. T., Pascal, A. D., Assou, S., & Sohounhloue, C. K. (2018). Comparative study of transesterification processes for biodiesel production (A review). *Elixir Applied Chemistry*, *120*, 51235-51242.
[19] Ideris, F., Zamri, M. F. M. A., Shamsuddin, A. H., Nomanbhay, S., Kusumo, F., Fattah, I. M. R., & Mahlia, T. M. I. (2022). Progress on conventional and advanced techniques of in situ transesterification of microalgae lipids for biodiesel production. *Energies*, *15*(19), 7190.
[20] Kafuku, G., & Mbarawa, M. (2013). Influence of fatty acid profiles during supercritical transesterification of conventional and non-conventional feedstocks: a review. *American Journal of analytical chemistry*, *2013*.
[21] Lee, J. C., Lee, B., Heo, J., Kim, H. W., & Lim, H. (2019). Techno-economic assessment of conventional and direct-transesterification processes for microalgal biomass to biodiesel conversion. *Bioresource Technology*, *294*, 122173.
[22] Haq, I. U., Akram, A., Nawaz, A., Abbas, S. Z., Xu, Y., & Rafatullah, M. (2021). Comparative analysis of various waste cooking oils for esterification and transesterification processes to produce biodiesel. *Green Chemistry Letters and Reviews*, *14*(3), 462-473.
[23] Patil, P., Gude, V. G., Pinappu, S., & Deng, S. (2011). Transesterification kinetics of Camelina sativa oil on metal oxide catalysts under conventional and microwave heating conditions. *Chemical Engineering Journal*, *168*(3), 1296-1300.
[24] Moazeni, F., Chen, Y. C., & Zhang, G. (2019). Enzymatic transesterification for biodiesel production from used cooking oil, a review. *Journal of cleaner production*, *216*, 117-128.
[25] Brask, J., Damstrup, M. L., Nielsen, P. M., Holm, H. C., Maes, J., & De Greyt, W. (2011). Combining enzymatic esterification with conventional alkaline transesterification in an integrated biodiesel process. *Applied biochemistry and biotechnology*, *163*, 918-927.
[26] Salaheldeen, M., Mariod, A. A., Aroua, M. K., Rahman, S. A., Soudagar, M. E. M., & Fattah, I. R. (2021). Current state and perspectives on transesterification of triglycerides for biodiesel production. *Catalysts*, *11*(9), 1121.
[27] Maleki, B., Talesh, S. A., & Mansouri, M. (2022). Comparison of catalysts types performance in the generation of sustainable biodiesel via transesterification of various oil sources: A review study. *Materials Today Sustainability*, *18*, 100157.
[28] Kumari, A., Kundu, P. K., Gupta, M. M., Bala, K., Chandra, S., Dutta, R., & Das, A. (2022). Role of microorganisms in production of biofuels. In *Novel feedstocks for biofuels production* (pp. 65-116). Singapore: Springer Nature Singapore.
[29] Meng, X., Yang, J., Xu, X., Zhang, L., Nie, Q., & Xian, M. (2009). Biodiesel production from oleaginous microorganisms. *Renewable energy*, *34*(1), 1-5.
[30] Yi, Y. (2021). Tiny bugs play big role: Microorganisms' contribution to biofuel production. In *Advances in 2nd generation of bioethanol production* (pp. 113-136). Woodhead Publishing.

[31] Basak, A. K., Basak, S. M., Strzałka, K., & Chatterjee, P. K. (2021). The role of microbes in biofuel production. In *Biofuel from Microbes and Plants* (pp. 65-127). CRC Press.
[32] Carreiro, S. C. (2024). The importance of microorganisms for biofuels production. In *Agroenergy* (pp. 253-263). Woodhead Publishing.
[33] Adetunji, C. O., Olaniyan, O. T., & Okeke, N. E. (2020). Production of next-generation biodiesel from high yielding strains of microorganisms: Recent advances. *Sustainable Green Chemical Processes and their Allied Applications*, 31-43.
[34] Zhang, L., Loh, K. C., Kuroki, A., Dai, Y., & Tong, Y. W. (2021). Microbial biodiesel production from industrial organic wastes by oleaginous microorganisms: current status and prospects. *Journal of Hazardous Materials*, *402*, 123543.
[35] Liu, Y., Li, C., Wang, S., & Chen, W. (2014). Solid-supported microorganism of Burkholderia cenocepacia cultured via solid state fermentation for biodiesel production: optimization and kinetics. *Applied energy*, *113*, 713-721.
[36] Louhasakul, Y., Cheirsilp, B., Maneerat, S., & Prasertsan, P. (2018). Direct transesterification of oleaginous yeast lipids into biodiesel: development of vigorously stirred tank reactor and process optimization. *Biochemical Engineering Journal*, *137*, 232-238.
[37] Elhussieny, N. I., El-Refai, H. A., Mohamed, S. S., Shetaia, Y. M., Amin, H. A., & Klöck, G. (2023). Rhizopus stolonifer biomass catalytic transesterification capability: optimization of cultivation conditions. *Microbial Cell Factories*, *22*(1), 154.
[38] Yousuf, A., Khan, M. R., Islam, M. A., Wahid, Z. A., & Pirozzi, D. (2017). Technical difficulties and solutions of direct transesterification process of microbial oil for biodiesel synthesis. *Biotechnology letters*, *39*, 13-23.
[39] Loures, C. C., Amaral, M. S., Da Rós, P. C., Zorn, S. M., de Castro, H. F., & Silva, M. B. (2018). Simultaneous esterification and transesterification of microbial oil from Chlorella minutissima by acid catalysis route: A comparison between homogeneous and heterogeneous catalysts. *Fuel*, *211*, 261-268.
[40] Nan, Y., Liu, J., Lin, R., & Tavlarides, L. L. (2015). Production of biodiesel from microalgae oil (Chlorella protothecoides) by non-catalytic transesterification in supercritical methanol and ethanol: Process optimization. *The Journal of Supercritical Fluids*, *97*, 174-182.
[41] Lopes, A. R., Faria, C., Prieto-Fernández, Á., Trasar-Cepeda, C., Manaia, C. M., & Nunes, O. C. (2011). Comparative study of the microbial diversity of bulk paddy soil of two rice fields subjected to organic and conventional farming. *Soil Biology and Biochemistry*, *43*(1), 115-125.
[42] Pershina, E., Valkonen, J., Kurki, P., Ivanova, E., Chirak, E., Korvigo, I., ... & Andronov, E. (2015). Comparative analysis of prokaryotic communities associated with organic and conventional farming systems. *PLoS One*, *10*(12), e0145072.
[43] Abayasekara, L. M., Perera, J., Chandrasekharan, V., Gnanam, V. S., Udunuwara, N. A., Liyanage, D. S., ... & Ilango, J. (2017). Detection of bacterial pathogens from clinical specimens using conventional microbial culture and 16S metagenomics: a comparative study. *BMC infectious diseases*, *17*, 1-11.
[44] Dohnalkova, A. C., Marshall, M. J., Arey, B. W., Williams, K. H., Buck, E. C., & Fredrickson, J. K. (2011). Imaging hydrated microbial extracellular polymers: comparative analysis by electron microscopy. *Applied and environmental microbiology*, *77*(4), 1254-1262.
[45] Wu, Y., & Gadsden, S. A. (2023). Machine learning algorithms in microbial classification: a comparative analysis. *Frontiers in Artificial Intelligence*, *6*, 1200994.
[46] Sakhno, N. G., & Gunar, O. V. (2016). Microbial identification methods in pharmaceutical analysis: Comparison and evaluation. *Mathews J Pharm Sci*, *1*(001).
[47] Takahashi, H., Matsushita, Y., Ito, T., Nakai, Y., Nanzyo, M., Kobayashi, T., ... & Ando, S. (2018). Comparative analysis of microbial diversity and bacterial seedling disease-suppressive activity in organic-farmed and standardized commercial conventional soils for rice nursery cultivation. *Journal of phytopathology*, *166*(4), 249-264.

[48] Yin, H. B., & Patel, J. (2018). Comparison of methods to determine the microbial quality of alternative irrigation waters. *Agricultural Water Management*, *201*, 38-45.

[49] Schang, C., Henry, R., Kolotelo, P. A., Prosser, T., Crosbie, N., Grant, T., ... & McCarthy, D. T. (2016). Evaluation of techniques for measuring microbial hazards in bathing waters: A comparative study. *PloS one*, *11*(5), e0155848.

[50] Wetzel, K., Lee, J., Lee, C. S., & Binkley, M. (2010). Comparison of microbial diversity of edible flowers and basil grown with organic versus conventional methods. *Canadian Journal of Microbiology*, *56*(11), 943-951.

[51] Al-Sahari, M., Al-Gheethi, A. A., Mohamed, R. M. S. R., Yashni, G., Vo, D. V. N., & Ismail, N. (2022). Microbial fuel cell systems; developments, designs, efficiencies, and trends: A comparative study between the conventional and innovative systems. *Chemosphere*, *298*, 134244.

[52] Sangkharak, K., Klomklao, S., Paichid, N., & Yunu, T. (2023). Statistical optimization for fatty acid reduction in waste cooking oil using a biological method and the continuous process for polyhydroxyalkanoate and biodiesel production. *Biomass Conversion and Biorefinery*, *13*(11), 9841-9854.

[53] Rocha-Meneses, L., Hari, A., Inayat, A., Yousef, L. A., Alarab, S., Abdallah, M., ... & Kikas, T. (2023). Recent advances on biodiesel production from waste cooking oil (WCO): A review of reactors, catalysts, and optimization techniques impacting the production. *Fuel*, *348*, 128514.

[54] Venkataraman, S., Rahul, R., Afrrin, M., Rajendran, D. S., Vaidyanathan, V. K., & Karthik, P. (2025). Harnessing cooking oil residue for volatile fatty acids production and its transformative role in sustainable bioenergy platforms. In *Biofuels and Bioenergy* (pp. 121-146). Elsevier Science Ltd.

[55] Soudagar, M. E. M., Shelare, S., Marghade, D., Belkhode, P., Nur-E-Alam, M., Kiong, T. S., ... & Fattah, I. M. R. (2024). Optimizing IC engine efficiency: A comprehensive review on biodiesel, nanofluid, and the role of artificial intelligence and machine learning. *Energy Conversion and Management*, *307*, 118337.

[56] Binhweel, F., Hossain, M. S., & Ahmad, M. I. (2023). Recent trends, potentials, and challenges of biodiesel production from discarded animal fats: a comprehensive review. *BioEnergy Research*, *16*(2), 778-800.

[57] Farouk, S. M., Tayeb, A. M., Abdel-Hamid, S. M., & Osman, R. M. (2024). Recent advances in transesterification for sustainable biodiesel production, challenges, and prospects: a comprehensive review. *Environmental Science and Pollution Research*, *31*(9), 12722-12747.