

# ARGO-WASTE CONVERSION FOR THE PRODUCTION OF BIOFUEL USING MICROALGAE

## Abstract

Microalgae are photosynthetic organisms that grow rapidly and have the potential to be used as an alternative source of liquid fuels to address the world's rising energy needs. However, microalgae culture must be enhanced to lower the cost of the biomass generated. Nutrient expenditures such as CO<sub>2</sub>, nitrogen, and phosphorus are among the key costs associated with algae culture. In this study, distinct microalgae strains were cultured with the derived agro-waste treatment facilities as nutritional sources, and the study gives insight into the perception of distinct lipid content enhancements reported in different strains of microalgae.

**Keywords:** Microalgae, Agro-waste, Transesterification, Alternative energy, lipid.

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## I. INTRODUCTION

Microalgae are autotrophic eukaryotic organisms found in marine and freshwater ecosystems which produce lipids, carbohydrates, and proteins through their metabolic processes. Because of their high lipid content, quick growth rate, and utilization of solar energy, microalgae hold a lot of potential for contributing to sustainable bioenergy [1]. Although Microalgae offer a wide range of uses such as dietary supplements, lipids, enzymes, biomass polymers, and pigments termed "Green energy". Several energy experts believe that algae fuel can drastically reduce reliance on fossil fuels while also lowering greenhouse gas (GHG) emissions [2]. They can generate a significant number of lipids that can be readily turned into biofuels using bio-/thermochemical processes. The neutral lipids-tri-glycerides that serve as energy storage for microalgae are specifically transformed into biofuel through a transesterification process [3]. Proteins, carbohydrates, lipids, and nucleic acids are microalgae's most abundant biochemical components. The quantity of the components varies depending on the species and is heavily impacted by environmental variables like light intensity, temperature, pH, and nutrient availability [4]. Global demand for sustainable products, especially energy, is increasing as human civilization and industry progress. The rapid depletion of petroleum resources due to overuse necessitates using sustainable raw materials. Agricultural waste refers to undesirable refuse originating solely from agricultural activities aimed at cultivating crops or tending to animals for the purpose of profit or sustenance [5]. Converting agricultural waste to biofuel is not harming food security; it helps with waste management, reduces environmental damage, and assures energy security [6]. Biofuels are categorized into primary and secondary types. Primary biofuels encompass raw, natural sources like firewood, plants, forest materials, animal waste, and crop residue, which are directly used for power and heat generation. On the other hand, secondary biofuels are obtained from processed biomass and are classified into three categories: first-generation, second-generation, and third-generation biofuels. First-generation biofuels are primarily produced using feedstock derived directly from food crops, which leads to limitations in food production by exploiting arable land. On the contrary, second-generation biofuels focus on harnessing the potential of lignocellulose biomass, its by-products, and non-edible plant materials. These biofuels offer significant benefits, as they are not derived from food sources, are highly cost-effective, and make efficient use of waste biomass [7]. The utilization of lignocellulose biomass serves as a crucial fuel source for combustion, heat, and power generation. Additionally, it can undergo processing to produce various biofuels, including bioethanol, biogas, bio-hydrogen, and biodiesel. Examples of lignocellulose biomass encompass whole plant agricultural residues such as leaves, Stover, straws, husks, pods, seeds, bagasse, roots, cobs, and seed pods, as well as agro-waste like solid cattle manure and forest biomass. Forest trash, industrial waste (such as chemical pulps and primary wastewater solids), and municipal solid waste (including food waste, newspaper, Kraft paper, and sorted rubbish) also contribute to the diverse sources of lignocellulose biomass. Among these, the current global focus of study lies in exploiting agricultural residue for the generation of biofuels [8]. Reports indicate that the annual global production of plant biomass amounts to about  $200 \times 10^9$  tons, with an estimated  $8 \times 10^9 - 20 \times 10^9$  tons potentially suitable for biofuel utilization. Additionally, agricultural waste contributes approximately  $1.5 \times 10^{11}$  tons of lignocellulose biomass each year, which unfortunately tends to be abandoned, left in fields, or incinerated, leading to additional environmental degradation [9]. Consequently, employing these wastes as a resource for biofuel production proves to be an environmentally beneficial choice. Another positive aspect of utilizing agricultural waste as a biofuel source is the reduced dependence on woody biomass from forests, thereby leading to a decrease in

deforestation. Moreover, the regularity of crop residue supply is enhanced owing to their short harvest time [10].

## II. MICROALGAE

**1. General Characteristics:** Microalgae are a diverse group of photosynthetic organisms categorized as Protista. Aquatic ecosystems heavily rely on them as they play a crucial role and serve as a significant component of the marine food chain. Microalgae are mostly found in aquatic environments such as oceans, lakes, ponds, and rivers. They are responsible for primary productivity, which means they convert carbon dioxide and sunlight into organic matter. In contrast to other microbes, Inside the cells, there is an abundance of chlorophyll, as well as a well-defined nucleus, cell wall, and colors. Microalgae encompass a diverse array of photosynthetic and heterotrophic organisms, belonging to various evolutionary and taxonomic groups[11].Microalgae can be found globally, predominantly inhabiting freshwater and saltwater environments. Their ability to adapt to changing environmental conditions is evident in the wide variety of lipids and other synthesized compounds they possess. Many algae accumulate a significant quantity of non-polar lipids, often in the form of TAG or hydrocarbons, with amounts reaching up to 10% of the total 20-50% of the overall dry cell weight because of their rapid growth rates and ability to produce critical compounds, they are being studied as a sustainable source of food, feed, and biofuels. Some microalgae species are abundant in proteins, omega-3 fatty acids, vitamins, and other essential components, making them ideal candidates for a substitute supply [12].

Microalgae present a promising alternative source of lipids, containing anywhere between 15% to 75% of their dry weight, depending on their type and the conditions of their growth [13]. In instances where the lipid content exceeds 75% of their dry mass, cellular proliferation slows down, as seen in the case of *Botryococcusbraunii* sp. However, other microalgae with oil levels ranging from 20% to 50%, such as *Chlorella* sp., *Bulaliella* sp., *Isochrysis* sp., *Nannochloris* sp., *Nannochloropsis* sp., and *Tetraselmis* sp., have shown higher growth rates [14]as mentioned in the below Table.1.

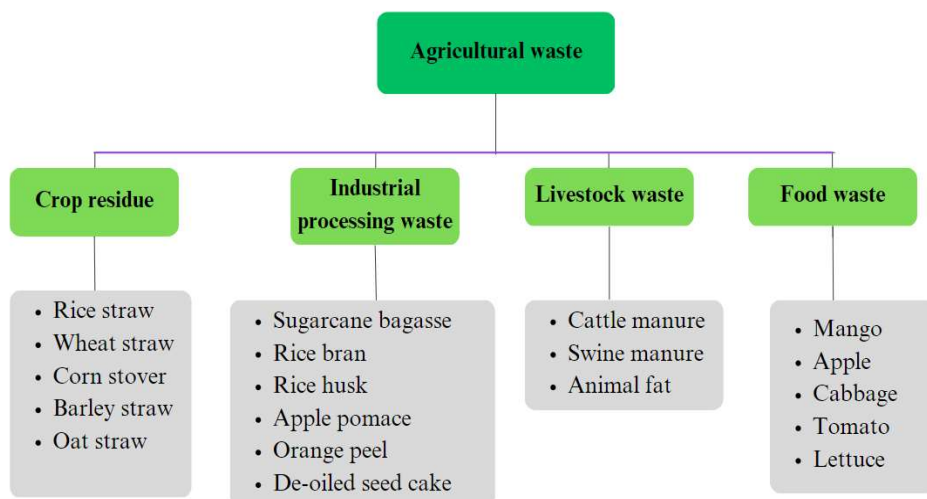
**Table 1: Lipid Content of Microalgae**

Microalgae	Lipid content(% dry weight)	Reference
<i>Botryococcusbraunii</i>	25-80	[15]
<i>Chlorellaminutissima</i>	57	[16]
<i>Chlorella vulgaris</i>	14-20	[17]
<i>Euglena gracilis</i>	14-20	[18]
<i>Hormidium</i> sp.	38	[19]
<i>Isochrysis</i> sp.	25-33	[20]
<i>Nannochloris</i> sp.	30-50	[21]
<i>Nannochloropsis</i> sp.	31-68	[22]
<i>Nitzschia</i> sp.	45-47	[23]
<i>Phaeodacetylumtricornutum</i>	20-30	[24]
<i>Pleurochrysis</i> carterae	30-50	[25]
<i>Porphyridium</i> cruentum	9-14	[26]
<i>Prymnesium</i> Parvum	22-38	[18]
<i>Scenedesmus</i> Dimorphus	16-40	[27]

- 2. Algal Metabolism:** Microalgae possess a lipid content ranging from 10% to 50% by dry weight, varying with the species and growth conditions. Their significant lipid accumulation makes them an excellent source for biofuel through fatty acid transesterification [28]. Moreover, subjecting microalgae to abiotic stress conditions, such as adjusting light intensity, pH, and temperature, or altering the growth medium composition, can lead to an increase in their lipid content. In particular, cultivating microalgae in agro-waste as a substrate has shown promise in enhancing lipid content and contributing to a more environmentally and economically friendly biofuel production process [29]. The metabolic reaction pathway in all photosynthetic organisms exhibits significant similarity. The vital component involves the absorption of nutrients from the environment through various metabolic and transport pathways. For photosynthetic metabolic activities, carbon (C) and nitrogen (N) are two essential elements. Throughout these metabolic processes, there are crucial changes in cell mass, volume, density, protein, chlorophyll, RNA, and vitamin content [30]. Microalgae may synthesize lipids by several methods. Acyl-CoA molecules and glycerol-3-phosphate combine to make diacylglycerol (DAG), which is then converted into triacylglycerol (TAG), the most common type of storage lipid in microalgae. This occurs in lipid droplets, which are specialized cellular compartments [31].

### III. AGRO WASTE

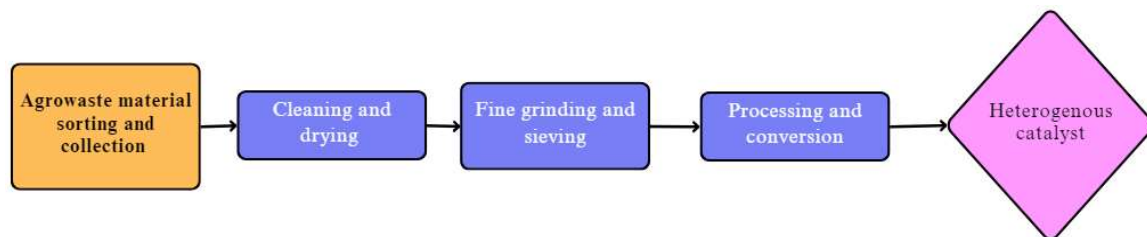
Agricultural wastes are residues of agricultural product manufacturing and processing that contain lignocellulose components, mainly cellulose, hemicellulose, and lignin. Because lignocellulose material is poorly degradable, numerous treatments are required to enhance hydrolysis [32]. The figure. 1 shows the agricultural wastes originating from diverse sources, primarily arising from farming, livestock, and aquaculture activities. The composition of these wastes varies based on the specific agricultural system and type of activity involved, and they can exist in the forms of liquids, slurries, or solids [33]. Agricultural waste encompasses a wide range of materials, including animal waste like manure and animal carcasses, food processing waste (where only 20% of maize is canned and the remaining 80% becomes waste), crop waste such as corn stalks, sugarcane bagasse, and discarded fruits and vegetables, as well as hazardous and toxic substances like pesticides, insecticides, and herbicides [34].



**Figure 1:** Classification of Agricultural waste

With the exception of a few regions, agriculture stands as the largest consumer of water worldwide. In this context, the body of water responsible for transporting agricultural waste, including leaves, plant stalks, and animal dung, plays a crucial role. [35]. Point source agriculture waste would be greatly impacted by large livestock and poultry operations [36]. Livestock businesses have expanded quickly over the past few decades, from small to large-scale. N and P make up the majority of the wastewater produced by animal farms. Ammonium and organic nitrogen make up the majority of agricultural wastewater. Ammonium, which constitutes over half of the total nitrogen source, is the primary component of nitrogen waste found in animal feces. Because agricultural operations including animal nutrition, consumption, production, and placement will result in much more animal wastewater [37].

Various feedstocks from Fig. 1 have been successfully converted into biofuel using heterogeneous catalysts. Commercially available metal oxides and mixed metal oxides such as calcium oxide (CaO), calcium methoxide, calcium diglyceride, magnesium oxide, magnesium zirconate ( $Mg_2Zr_5O_{12}$ ), aluminum oxide-supported molybdenum oxide, magnesium pyrophosphate, sulfated zirconia, and sodium molybdate have been employed for this purpose[38]. While certain catalysts have shown remarkable conversion efficiency, those derived from agricultural waste stand out as cost-effective, non-corrosive, and readily available options. They generate minimal waste, exhibit high conversion rates, and are biodegradable, making them appealing for biodiesel producers seeking to manage agricultural waste. In recent years, numerous agricultural wastes have been harnessed, processed, and utilized as low-cost and environmentally friendly catalysts for synthesizing biodiesel from various low-grade feedstock[39]. Figure.2 heterogeneous catalysts with substantial catalytic activities can be derived from a variety of agricultural wastes, including eggshells, shrimp shells, animal bones, oyster shells, chicken bones, banana peels, ash from banana trunks, and *Musa balbisiana* peels, cocoa pod husk ash, coconut husk ash, pineapple (*Ananas comosus*) leaves ash, waste fish (*Labeorohita*) scale, waste fish bones, and more [40].



**Figure 2:** Method of Preparing Agro Waste for use as a Heterogeneous Catalyst

There are several methods for converting agricultural waste into a heterogeneous catalyst that may be utilized as a substrate for the growth of microalgae.

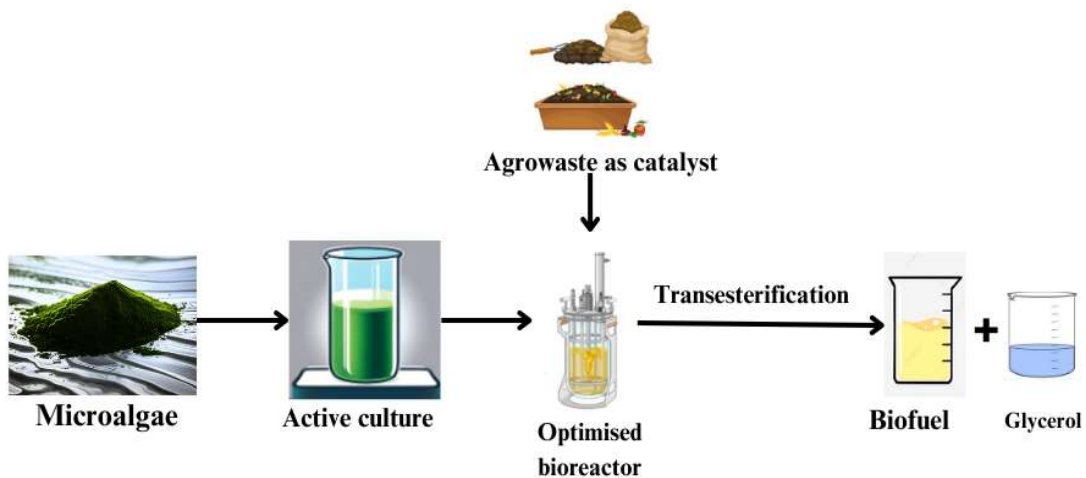
1. The Physical Mixing method involves the immediate combination or blending of two or more powdered components. This process takes place either before, during, or after pulverizing the components in a mortar or grinder. As a result, the resulting blend may exhibit enhanced compositional, structural characteristics, and physicochemical attributes derived from the mixture's ingredients[41].

2. During the process of calcination, solid catalysts, typically in powdered form, undergo high-temperature treatment in an oven or furnace. The objective is to enhance the materials' physicochemical properties and catalytic activity. Calcination leads to improved catalytic effectiveness by increasing specific surface area, pore size, and volume, as well as the concentration of active sites [39].
3. The aim of the wet impregnation technique is to enhance the catalytic activity of solid catalysts derived from agricultural waste, thereby increasing yield, product quality, and purity. In this method, the catalyst is mixed thoroughly with supporting ingredients using a stirrer until a slurry is formed [42]. After forming the resulting slurry, it is subjected to baking in an oven at an appropriate temperature for a specific duration until it solidifies into a dry cake. Subsequently, the dry cake is crushed and sieved using a fine screen, followed by calcination at high temperatures [43].
4. The bifunctional modification method represents a novel approach in the development of efficient green catalysts, driven by technological advancements. This innovative technique produces amphoteric catalysts with both acidic and basic properties, resulting in a higher number of acidic and basic sites, thereby enhancing overall catalytic performance[44].By allowing the combination of the physicochemical attributes lacking in each catalyst, this method offers cost-effectiveness, addresses challenges related to the transesterification of feedstock with high free fatty acid (FFA) content, and improves overall efficiency[45].
5. In the Co-precipitation method for catalyst modification, catalyst precursors are dissolved in distilled water and vigorously mixed at high speeds. Subsequently, drops of base precipitating agents, such as potassium carbonate ( $K_2CO_3$ ), ammonium hydroxide ( $NH_4OH$ ), KOH, and NaOH, are added to the solution while stirring for 3 hours to maintain the pH at 10 [46].After preparing the resultant mixture, it is allowed to coagulate before undergoing dehydration for 24 hours in an oven set at 100-120°C. The dried sample is then calcined at the appropriate temperature in a furnace, and the resulting powder serves as a catalyst for biodiesel synthesis[47].
6. Physical mixing, calcination, wet impregnation, bifunctionality, and co-precipitation represent valuable methods for enhancing the activity of heterogeneous catalysts derived from waste materials. Combining any of these modification procedures is recommended to further improve the catalysts' performance. Overall, biocatalysts obtained from agricultural waste exhibit high activity, reusability, and ease of preparation. These waste-derived catalysts eliminate the need for expensive commercial catalysts, reduce catalyst costs, and are environmentally friendly. Additionally, the use of agricultural waste-derived heterogeneous catalysts not only simplifies biofuel purification but also allows for discarded catalysts to be used as manure [48].

#### IV. METHODOLOGY

Microalgae cultivation is commonly utilized to generate huge quantities of biomass, taking advantage of this feedstock's rapid growth. Furthermore, wide culture will make microalgal biofuel economically viable by cutting growing costs by utilizing low-cost nutrients such as agro-waste. Deep research into large-scale microalgae production using agro-waste, on the other hand, is necessary for long-term, contamination-free cultivation

without periodic re-inoculation [49]. To achieve cost-effective biofuel production, microalgal cultivation involves various factors such as strain selection, maintaining cultural integrity, retaining monoculture, optimizing growing conditions, managing medium composition, and ensuring proper gaseous exchange. Microalgae constitute distinct groups of photosynthetic organisms that flourish in diverse environments, including freshwater, seawater, and wastewater [50]. Specific alkaliphile (high pH) and halophile (high salinity) strains have been identified as suitable for outdoor cultivation due to their ability to prevent cross-contamination with other microalgal strains. Additionally, to ensure long-term protection against contamination, seed culture should be consistently propagated as the dominant culture [51]. Microalgae are often cultivated both outdoors (in open ponds) and indoors (in tubular photobioreactors). Light source and light intensity govern both cultural techniques. Microalgae can thrive in stressful environments, according to current research, and might be employed in large-scale production. An optimized bioreactor is a closed, lit culture tank that produces regulated biomass [52]. Bioreactors are closed systems with no direct gas or pollutant interaction with the surrounding environment. Bioreactors have multiple significant benefits over open systems, notwithstanding their high-cost bioreactors reduce contamination and enable the development of selective axenic microalgae. Bioreactors provide more control over variables such as pH, temperature, light, CO<sub>2</sub> concentration, and so on. aid to decrease CO<sub>2</sub> emissions. Keep water from evaporating. Allow for greater cell concentrations. Allow for the synthesis of biofuels [53].



**Figure 3:** Schematic Representation of Lipid Extraction from Microalgae.

To increase active culture development, heterogeneous catalysts of agricultural waste can serve as a substrate in a bioreactor that provides a microenvironment and improves high-yield lipid synthesis. The figure. 3. shows the selection of a heterogeneous catalyst is often dependent not only on the amount of lipid available in the feedstock but also on the fatty acid content (FFA) in those feedstocks since they determine the qualities of generated biofuel [54]. Due to its capability to achieve substantial yields at affordable expenses, the transesterification of triglycerides (TG) with short-chain alcohol, facilitated by a catalyst, is commonly employed for biodiesel synthesis. The efficiency of transesterification is influenced by several variables, including reaction temperature, methanol-to-oil ratio, reaction duration, catalyst type and concentration, FFA content, and water content of the oil [55]. Non-catalytic transesterification procedures can also result in high biofuel

conversion, but they are frequently carried out under circumstances of high pressure (10 to 25 MPa) and temperature (200<sup>0</sup> to 400<sup>0</sup> C), which makes them less economically viable. Therefore, biofuel is frequently converted using a catalyst under less extreme circumstances. One of the most important variables to have a high biodiesel conversion at a quick reaction time is choosing an effective catalyst with the right concentration [56]. For the transesterification process, homogeneous catalysts like potassium hydroxide, sodium hydroxide, or sulfuric acid have been applied commercially. Commercial catalysts are very inexpensive and widely available, however, because of their dangers and unfriendly environmental effects, they are not environmentally sustainable[57]. It is an energy-intensive procedure since these catalysts produce a lot of effluent during the postprocessing processes that cannot be recycled. Additionally, because the catalyst is only partly miscible with biofuel, there are issues with product separation from the reactant mixture[58].

A vital step in the creation of biofuel a sustainable and environmentally benign substitute for fossil fuels, is transesterification. Due to their high lipid content and quick growth rates, microalgae, a kind of unicellular photosynthetic organism, have become a viable feedstock for the generation of biofuel[59]. The sustainability and cost-effectiveness of this method, however, continue to be major obstacles. Utilizing hetero-catalysts made from agricultural waste is one method that may be used to increase the effectiveness and decrease the environmental impact of microalgae transesterification. In Fig.3. transesterification process, microalgal lipids are transformed into fatty acid methyl esters (FAMES), which are the main ingredients in biofuel[60]. Heterocatalysts made from agro-waste speed up the conversion rate by supplying active sites for the interaction of the reactants and decreasing the activation energy. The catalyst also improves selectivity towards FAME synthesis and lowers the generation of undesirable byproducts. As efficient hetero-catalysts for microalgae transesterification, a number of agricultural waste products have demonstrated potential. Examples include used fruit peels, maize cobs, and rice husks[61]. These components are easily obtainable from the agricultural sectors and may be converted into catalysts utilizing easy and affordable processes. It reduces the requirement for traditional homogeneous catalysts, whose manufacture could be expensive and harmful to the environment. The green chemistry principles of renewable, non-toxic, and biodegradables are upheld by agro-waste catalysts[62].

Transesterification involves combining triglycerides from microalgae oil with alcohol (commonly methanol or ethanol) in the presence of a heterogeneous catalyst to produce glycerol and fatty acid methyl esters (FAMES). Due to the lower density of FAMES compared to glycerol, the former floats to the top while the latter sinks [63]. The top layer, which contains biodiesel, and the bottom layer, which contains glycerol, may be collected as a result of this natural phase separation. Next, the two layers are carefully decanted or pumped into separate containers. However, traces of glycerol may remain in the biodiesel phase, affecting its quality[64]. To remove the remaining glycerol, a washing step is employed. Water is often used to wash the biodiesel, as it helps to extract the glycerol from the FAMES due to their differing solubilities. The mixture is agitated, and after some time, the water-glycerol phase is separated from the biodiesel phase[65]. While the washing step removes a considerable amount of glycerol, it may not be sufficient for commercial-grade biodiesel production. Therefore, an additional purification process called "drying" or "polishing" is conducted[66]. In this step, small amounts of solid desiccants (such as silica gel) or ion exchange resins are added to the biofuel. These materials absorb any remaining water and glycerol, further improving the quality and stability of the biofuel[67].



## V. CONCLUSION

“Agro waste Conversion for the Production of Biofuels using Microalgae” gives a possible route for solving the combined concerns of waste management and renewable energy generation in the search for sustainable energy solutions[68]. Agro waste, a common result of agricultural operations and food processing, has long been a source of environmental concern owing to inappropriate disposal. However, by viewing agro-waste as a resource rather than a burden, researchers have discovered its enormous potential as a nutrient-rich substrate for microalgae cultivation[69]. This synergy enables the conversion of organic waste into biofuels via a process known as heterotrophic culture. One advantage of microalgae is their adaptability in consuming diverse agro-waste substrates. Regardless of the waste, whether it is fruit peels, straw, husks, or even wastewater, microalgae demonstrate their ability to efficiently utilize these resources, lowering the environmental impact of agro-waste buildup[70]. Microalgae is positioned as a very adaptable and versatile solution to the global agro-waste problem as a result of these unique features. Another potential aspect is the development of a closed-loop system in which agro-waste is utilized to grow microalgae and the resulting biomass is converted into biofuels. The study “Agro waste Conversion for the Production of Biofuels using Microalgae” sheds light on an innovative and long-term solution to the essential issues of agro-waste management and renewable energy generation[71]. By exploiting the inherent potential of microalgae, we may convert garbage into valuable resources while reducing our reliance on fossil fuels. Adopting this biotechnological technique has the potential to adopt the way for a greener, more sustainable future in which Agro-waste is considered a source of opportunity and growth rather than a problem. We can realize the full potential of microalgae-based biofuels through continued research, investment, and collaboration, paving the path for a greener and more prosperous future[72].

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