

AN OVERVIEW OF ALGAE BIODIESEL

Abstract

The main energy sources include coal, petroleum, crude oil, nuclear energy and hydroelectricity. Due to the growing population and industrialization, there is an increasing demand for energy. Fossil fuels, such as petroleum, contribute to carbon dioxide emissions that cause global warming, hence their usage cannot be sustained owing to supply shortages. Research has focused on using biofuels to reduce greenhouse gas emissions. Macroalgae are particularly promising as they have a high level of lipid content, making up to 80% of their dry weight. Microalgae are also considered viable sources of raw materials for biofuels and other products. Choosing the right algae species is crucial for biodiesel synthesis, considering high biomass productivity and tolerance to local conditions. The yield of important chemicals during biomass generation, such as carbohydrates and lipids, affects the quality of biofuels. In Stress conditions, like salt or light stress, can influence lipid synthesis and other metabolic changes in microalgae. Light intensity and wavelengths also impact microalgae's biomass lipid production. To develop and use algae-derived biofuels effectively, further research and development are needed at every level of the production chain. Researchers are working on processes to accurately identify algal biomass components to fully utilise algal bioresources in biofuel production.

Key Words: Bold basal medium (BBM), Blue green medium (BG11), Horizontal tubular photobioreactor (HTB), Hydraulically integrated serial turbidostar algal reactor (HISTAR), Algal turf scrubber (ATF), Nanoparticles (NPs), Compressed natural gas (CNG)

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I. INTRODUCTION

Petroleum, crude oil, hydroelectricity, coal and nuclear energy are the main energy sources. Due to the increasing population and industrialization, there is a constant rise in energy demand. It is widely acknowledged that the continued use of fuels derived from petroleum is currently unsustainable due to the depletion of these resources and their contribution to the environmental accumulation of carbon dioxide, which contributes to the phenomenon known as global warming. In the past ten years, a substantial amount of research has been conducted on the use of biofuels to replace fossil fuels and reduce greenhouse gas emissions [1]. In terms of lipid content, macroalgae are superior to all other commercial oil-producing crops and can make up as much as 80% of the dry weight of algal biomass. In comparison to microalgae, agricultural oil crops like soybean and palm oil, which are widely used to make biodiesel, have comparatively low oil contents (5% of total biomass) [2]. Particularly, producing biofuels from renewable biological resources is highly desired to meet the needs of aircraft and other forms of global transportation [3].

Recent studies have included both existing renewable sources from aquatic systems and land plants. Almost all ecosystems contain the vast and extremely diverse group of creatures known as algae [4]. Microalgae are unicellular or multicellular photosynthetic organisms capable of converting light, water and carbon dioxide into oxygen and biomass through photosynthesis. Mainly, microalgal growth goes through four phases: the lag phase, the exponential phase, the stationary phase, and the lysis phase. Most importantly, microalgae consume carbon, which is helpful in the global carbon cycle, and fix nitrogen, which can be used as a biofertilizer [5]. Algae, both macro and micro, have been considered as a residual biomass available for use in energy production. Although the concept of using algae as a fuel source is not new [6,7], it is now being seriously considered in light of the rising price of petroleum and, more importantly, the growing concern over global warming linked to the burning of fossil fuels [1]. Microalgae are a viable source of raw materials for the production of biofuels [8] and other products, including biogas generation, wastewater purification [9], and the extraction of additives for use in food and pharmaceuticals [10]. The choice of an appropriate algae species is an essential component of biodiesel synthesis [11].

High biomass productivity and tolerance to local climatic conditions should be two essential traits of the chosen strains. Much focus has been placed on the yield of important chemicals, such as carbohydrates, lipids, pigments, or other metabolites, during the biomass production stage. Under stressful circumstances, such as nutritional deficits, several of these chemicals are biosynthesized as a means of survival for microalgae [12]. These substances also act as energy transporters when biomass is turned into biofuels. The quality and yield of the biofuel are determined by these biochemical parameters of the feedstock in conjunction with the reaction conditions of the process [13]. As a result, further along in the process, biomass has a significant influence because it can change depending on metabolic capabilities, growing methods, and non-biological factors like light and nutrient delivery [14]. The algal strains were researched under nutrient-deficient circumstances to possibly produce biomass with high lipid content after the microalgae had consumed the nutrients from the media. Both reduce greenhouse gas emissions and store CO₂ through photosynthesis; however, unlike land plants, microalgae may be grown in brackish or highly salinized water and can use wastewater as a source of nutrients [15]. The growth of microalgae in photobioreactors can be accelerated by using downstream processes that are designed to extract the bioproducts so that higher biomass production can be achieved [16]. Stress refers

to disturbances in equilibrium caused by a stressor's application. Stress responses are metabolic adjustments made by cells in an attempt to restore balance by adapting to changes in the environment. The stress response is divided into four phases: the alarm phase, the conditioning phase, the adaptation phase, and the adaptation phase [17]. Various stress strategies have been implemented to improve the production of high-value compounds by utilizing a single stress factor, such as nutritional factors (e.g., carbon sources, nitrogen, phosphorus), environmental factors (high light intensities, temperature, pH, salinity, reactor configurations, and operating conditions)[18]. Exposure to salt stress triggers a cascade of intricate biochemical and bioenergetic transformations within microalgae. This includes a surge in the synthesis of lipids, heightened biopolymer production, and an upsurge in energy generation. Concurrently, the delicate balance of membrane permeability is disrupted, leading to perturbations in ion equilibrium, while reactive oxygen species (ROS) levels surge. Remarkably, microalgae display an adaptive prowess by amassing antioxidant compounds like polyphenols, flavonoids, and carotenoids in response to the heightened ROS levels. This strategic response acts as a formidable defense, thwarting the harmful influence of free radicals.

The nitrogen-deprived conditions, conversely, induce a shift in lipid synthesis pathways among certain algae species. This results in a deceleration of cell division while steering lipid biosynthesis toward a preference for neutral lipids over membrane-associated variants [19]. Leveraging the profound influence of light, both in terms of appropriate wavelengths and intensity, significantly molds microalgae's capacity to yield biomass and lipids [20]. The light regime's dynamic modulation prompts alterations in the range and concentration of secondary metabolites such as phenolics and flavonoids. This cascade of transformations subsequently exerts an impact on antioxidant potency, creating an intricate interplay.

The correlation between the antioxidant efficacy and the cumulative content of phenolic and flavonoid compounds is undeniably direct, as established by rigorous research [16]. Thus, by devising a judicious cultivation strategy, the augmentation of biomass production intertwined with the generation of high-value products becomes an attainable endeavor.

Numerous microalgae exhibit the remarkable ability to synthesize triacylglycerols (TAG), storing them as lipid reserves up to half of their dry cell weight, especially when confronted with photooxidative stress or unfavorable environmental conditions [21]. The allure of algae as a promising biodiesel feedstock has surged due to recent investigations [22] highlighting their intrinsic advantages: swift biomass proliferation, elevated lipid content, and resilience in challenging surroundings. A paramount advantage of cultivating algae-based biofuels is the superior oil production efficiency compared to traditional fuel crops [23]. An ideal microalgae contender for biodiesel production must not only boast high lipid productivity but also possess a fitting fatty acid (FA) composition. These attributes—embracing kinematic viscosity, specific gravity, cetane number (CN), cloud point, iodine value (IV), long-chain saturated factor (LCFF), and cold filter plugging point (CFPP)—bear significant influence, as they shape key fuel characteristics. Nevertheless, existing gaps in research, regulatory frameworks, and strategic planning across the biofuel production continuum hinder the full realization of algal bioresources' potential [24]. Acknowledging the urgency of precise biomass component quantification, we have embarked on devising a series

of methodologies to profile algal biomass constituents, designed for versatile implementation in diverse laboratory settings [25].

II. CLASSIFICATION OF ALGAE

Although most algae (which can be found in unicellular or multicellular forms) are photosynthetic, others are heterotrophic. Nearly 300,000 different kinds of algae may be found in freshwater, marine water, and wastewater all around the world. Algae are simple and resemble plants, but they don't have leaves, stalks or roots [26].

1. Based on Habitat: Seven kinds of algae are classified based on their environment.

- Aquatic, free-floating, or totally submerged algae are known as hydrophilus algae.
- Terrestrial algae are referred to as edaphic algae. They occupy the earth's surface or its interior. Edaphic algae are divided into two groups:
 - Saprophytes, like *Mesotaemium* and *Botrydium*,
 - cryptophytes, like *Nostoc* and
- **Aerial algae:** This category includes aerial algae. The trunks of trees, buildings, fence wire, rocks, and animals all have them. There are four different varieties of aerial algae. These include: Epiphyllphytes (such as *Trentepohlia*), Epiphloephytes, Epizooephytes (such as *Chaetophorales*) and Lithophytes (such as *Sctonema*, *Vaucheria*, and *Nostoc*).
- **Cryophytic algae:** Cryophytes, or cryophytic algae, such as *Chlamydomonas*, *Ankistrodesmus*, and *Mesotaenium*, are algae that live on ice and snow.
- **Symbionts or Endophytes:** Symbionts are algae that coexist in a symbiotic relationship with other plants. There are three kinds: those that live within the pteridophyte *Azolla*, such as *Anabaena azollae*; those that dwell inside the corolloid roots of *Cycas*, such as *Anabaena cicadae*; and those that are symbiotic with fungi, such as *Chroococcus*, *Nostoc*, *Chlorella*, and *Palmella*.
- Endozoic algae are those that reside inside the bodies of animals. Examples include: inside freshwater sponges; inside *Hydra*.
- **Parasites:** Algae, such as *Cephaleuros virescens*, are parasites that live on other plants.

2. Based on Chlorophyll Content: Despite the fact that all algae contain chlorophyll, the main divisions may mostly be differentiated based on the dominant apparent colour since other photosynthetic pigments cover the chlorophyll green in most algae.

The primary groups or lines of algae are:

- *Chromista*, which comprises diatoms and brown algae as well as other types of golden brown algae. These algae have chlorophyll A and C in their plastids.
- The Red Line is a primitive branch of marine algae that only contains Chlorophyll A. A common sight on wave-washed boulders is red algae. Red algae are distinguished by the fact that only chlorophyll A is present in their plastids. Unlike green algae and plants, which include both chlorophyll a and b,
- Dinoflagellates are a whole other evolutionary line that, interestingly, includes ciliated protists.

- The Euglenids – This group of single celled organism include both photosynthetic and non-photosynthetic organisms.
- Plants are connected to The Green Line. Chlorophylls A and B are present in green algae and plants.

Among the various types of algae, the ones most commonly recognized are green algae, red algae, and brown algae (Chromista). Green algae stand out with their intricate and complex structures. Interestingly, the lineage of higher land plants can be traced back to the origins of green algae. A widely held belief is that the presence of protective cell layers around reproductive organs, a characteristic distinct from other types of algae, serves as a defining factor that separates non-algal plants from the realm of algae. This protective feature is the boundary that sets these two groups apart.

3. Based on Metabolism:

- Photoautotrophically, that is, relying only on photosynthesis to transform light energy into chemical energy.
- Heterotrophically, that is, solely using organic compounds as a source of energy and carbon since they depend on complex organic components for sustenance and feed on other species to get their food.
- Mixotrophically, in which photosynthesis is used as the primary energy source despite the fact that both organic molecules and CO₂ are necessary [27].

4. Based on Cellular Organization:

Instead of simply following their biological evolutionary connections, the term "algae" is employed to group together eight distinct divisions of organisms that are not closely related [28]. Among these, microalgae stand as solitary cells, while macroalgae take on a multi-cellular form. The smaller microalgae are often known as microphytes, while the larger macroalgae are commonly recognized as seaweeds [29].

- Microalgae, also referred to as microphytes, lack the conventional features of roots, stems, and leaves. Despite their small size, they can grow to heights of several hundred micrometers and inhabit both freshwater and saltwater environments.
- In contrast, macroalgae exhibit a more complex body structure and thrive in marine environments, often extending to considerable lengths, even hundreds of meters along the sea floor. Their primary purpose revolves around storing and converting energy, without progressing beyond their cellular organization. This uncomplicated growth pattern and development make them an exceptionally sustainable source of renewable energy, surpassing other alternatives [30].

III. BIOFUEL PRODUCTION FROM ALGAL BIOMASS

Microalgae are photosynthetic unicellular microorganisms that can store CO₂ and use it to produce energy-dense molecules such as starch and fatty acids [31]. They can be used to produce a variety of biofuels, including biomethane (through anaerobic digestion), biodiesel (using microalgal oil), and biohydrogen (via photobiological synthesis), which are more efficient than those derived from standard biofuel crops [23]. Microalgae are believed to be

10–20 times more productive than common biofuel crops like palm oil and soybeans. They can double their biomass in a day and complete a growth cycle in just a few days [32]. While the lipid content per unit dry biomass weight of different species of microalgae may vary, the species volumetric productivity also needs to be taken into account when determining the viability and optimum microalgae to use for biofuel generation [24]. The making of biofuel from microalgae involves several stages, including microalgae cultivation, recovery or harvesting, and downstream processing to separate the metabolites from the biomass [33].

- 1. Selection of Microalgae:** The effectiveness of producing algal biodiesel critically depends on choosing the right algae species. The ideal species should possess the following desired traits, among others: rapid growth, high oil content, wide environmental tolerance, large cell size, and ease of disruption [34]. Water samples with visible microalgal populations were taken from ponds, lakes, and rivers, both from the surface and bottom layers. All field samples were collected in 50 ml tubes and kept chilled during transportation to the lab [35].
- 2. Isolation:** Single microalgal species were extracted from field water samples using standard plating methods. Various medium mixtures, such as Guillard's f/2 and Bold basal medium with vitamins, were used to isolate colonies. Initially, the field samples were diluted, and the diluted samples were spread over sterile plastic petri dishes (100 x 15 mm) containing approximately 40 ml of agarized media. Afterward, the algae were left to grow in the lab on culture racks for roughly 14 days. To attain pure cultures, grown algal cultures were streaked onto fresh nutrient medium plates using sterile techniques. This streaking process was repeated until the desired unialgal cultures were obtained [35].
- 3. Morphological Identification:** Microalgal and cyanobacterial cultures were initially differentiated based on morphological analysis of the colonies on an agar nutrient medium. This approach of generic categorization only distinguished the most fundamental differences between isolates. Microscopic analysis of individual cell appearances was used to identify these isolates to the genus level [36].

IV. NUTRIENT AND INPUT REQUIREMENT FOR ALGAL GROWTH

Microalgae are found both independently and in association with other organisms in both terrestrial and aquatic settings. They efficiently obtain biomass and rapid growth rate by utilizing light energy, water and CO₂ through photosynthesis. The demand for sustainable biofuel production and certain medicinal proteins from microalgae is significantly increasing [37]. The availability and intensity of light, color, and temperature conditions all impact the extensive manufacturing of microalgae on a significant scale [38]. The specific amounts of small and large nutrients needed vary for different types of algal growth solutions. Research emphasizes the significance of chemical building blocks such as N, K, Ca, Cu, Fe, Mg, Mn, P, S, and Zn, supplied in the form of salts, to support the growth of microalgae [39, 40]. Selecting the right growth solution is crucial and relies on the chemical composition of the solution itself, which directly impacts the growth of algae biomass. The commonly utilized growth solutions for cultivating microalgae include Bold's basal medium (BBM), slightly acidic Bold's basic solution, Chu10 solution, BG 11 solution, and adjusted Hoagland's solution [41].

1. **Bold's Basal Medium (BBM):** Bold's basal media is created by combining 10 ml of every individual stock solution listed in Table 1 (specifically items 1-6), along with 1 ml of items 7-10. This mixture is then diluted with 1 L of distilled water using a volumetric flask.

Table 1: Composition of Bold's Basal Medium [41]

| S. No | Stocks of Chemicals | g/L |
|-------|--|-------|
| 1 | NaNO ₃ | 25.00 |
| 2 | KH ₂ PO ₄ | 17.50 |
| 3 | MgSO ₄ . 7H ₂ O | 7.50 |
| 4 | K ₂ HPO ₄ | 7.50 |
| 5 | NaCl | 2.50 |
| 6 | CaCl ₂ . 2H ₂ O | 2.50 |
| 7 | Trace elements: | |
| | • ZnSO ₄ .7H ₂ O | 4.42 |
| | • CuSO ₄ .5H ₂ O | 1.57 |
| | • MnCl ₂ . 4H ₂ O | 1.44 |
| | • Co (NO ₃) ₂ .6H ₂ O | 0.49 |
| | • MoO ₃ | 0.71 |
| 8 | H ₃ BO ₃ | 11.40 |
| 9 | EDTA and KOH solution: | |
| | EDTA Na ₂ | 50.00 |
| | KOH | 30.00 |
| 10 | FeSO ₄ . 7 H ₂ O with 1.0 mL concentrated H ₂ SO ₄ | 4.98 |

2. **BG11 (Blue-Green Medium):** Make up to 1 L of deionized water, and then use 1 M NaOH to get the pH to 7.1.[41] [42].

Table 2: Composition of blue-green media BG11 [41]

| S. No | Chemical composition | Stock solutions (in mL) per 1 L final medium | |
|-------|---|--|--------------------|
| | | Stock solution | Per 500 mL |
| 1. | NaNO ₃ | | 75.0 g |
| 2. | MgSO ₄ .7H ₂ O | | 3.75 g |
| 3. | K ₂ HPO ₄ | | 2.0 g |
| 4. | CaCl ₂ . 2H ₂ O | | 1.80 g |
| 5. | Ammonium ferric citrate | | 0.30 g |
| 6. | Citric acid | | 0.30 g |
| 7. | Na ₂ CO ₃ | | 1.00 g |
| 8. | EDTA Na ₂ | | 0.05 g |
| 9. | Trace metal solution | | Per 1000 mL |
| 10. | • ZnSO ₄ . 7H ₂ O | | 0.22 g |
| 11. | • MnCl ₂ . 4H ₂ O | | 1.81 g |
| 12. | • H ₃ BO ₃ | | 2.86 g |
| 13. | • Na ₂ MoO ₄ .2H ₂ O | | 0.39 g |

| | | | |
|-----|--|--------|--|
| 14. | • $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ | 0.05 g | |
| 15. | • $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ | 0.08 g | |

V. CULTIVATION OF ALGAE

Algae cultivation requires water, carbon dioxide, sunlight and essential nutrients like phosphorus, nitrogen, potassium, zinc, and calcium. These nutrients are crucial for the growth of algae through photosynthesis, a process that converts solar energy into stored chemical energy within the algae cells. There are four main methods of cultivating algae based on specific conditions required for their growth: photoautotrophic, heterotrophic, photoheterotrophic, and mixotrophic cultures [43]. In the photoautotrophic method, microalgae use light as an energy source and inorganic carbon for photosynthesis, producing chemical energy. This process is most productive in an environment rich in CO_2 [44]. In the heterotrophic method, microalgae use organic carbon sources for energy and growth stimulation [46]. Photoheterotrophic culture, also known as photometabolism, relies on light to utilize organic carbon sources for growth [45]. In mixotrophic cultures, microalgae can switch between photoautotrophy and heterotrophy, utilizing both organic and inorganic carbon sources.

Photoautotrophic production is commonly used for generating large amounts of algal biomass. There are three main ways to grow algae: open, closed, and hybrid systems. Hybrid systems combine the benefits of both open and closed setups, aiming for high biomass production. Open systems are affordable, closed systems are excellent at nutrient removal, and hybrid systems strike a balance between the two [47].

1. Open Pond Systems: The raceway pond, closed pond, shallow huge pond, and circular pond tank are widely used strategies for researching and industrializing algal cultivation [48]. Greenhouses can protect open pond cultures from harsh environmental conditions like rainfall, temperature changes, and sunlight. To cultivate resilient microalgae in challenging conditions, such as basic or highly saline mediums, it's important to allow for axenic culture production [49]. Circular or gravity-driven open ponds are common in wastewater treatment facilities [50]. Choosing the right location for the pond is crucial, ensuring adequate sunlight and optimal conditions for the algal strains. Despite their lack of stirring units, these systems offer cost-effective management and monitoring of the cultivation process.

Natural ponds are usually shallow, around half a meter deep, allowing light to penetrate and nourish many algae cells. Various algae strains, notably *Dunaliella salina*, can be grown profitably in these open systems [51]. A central spinning arm resembling a clock dial, similar to a raceway pond's design, helps mix algae cells and culture media, increasing efficiency compared to unstirred pools. However, exposure to the environment can lead to contamination. Circular pond efficiencies typically range from 8.5 to 21 g/(m² d) [52].

Raceway ponds have been used for algae cultivation since the 1950s, initially for *Spirulina* cultures. They can be oval or racetrack-shaped, often constructed with a concrete slab [53]. Raceway ponds provide recirculation of algal culture along with a steady supply of nutrients and carbon dioxide. A paddle wheel ensures gentle mixing to

prevent sedimentation, and an aerator can enhance air flow and carbon dioxide utilization [54]. While open ponds are cost-effective, they face challenges due to land demand, contamination risks, and weather constraints, making them harder to control. Their sensitivity to seasons, weather, and temperature is a drawback. Open pond systems struggle to achieve monocultures due to native algae and grazers causing pollution. Pond temperature and light levels rely on natural variations, and while evaporation cools ponds, it also leads to significant water loss [55].

- 2. Horizontal Tubular Photobioreactor:** Algal growth is often conducted in horizontal tubular photobioreactors (HTB) on a commercial scale. These bioreactors consist of long tubes made from see-through materials like silicone, glass, or plastic. These tubes are positioned horizontally to maximize light exposure, and they are kept at a moderate diameter. The reason tubular photobioreactors are favored for growing algae is due to their extensive surface area for light absorption. To circulate the algae cells within the tubes, a centrifugal pump or an airlift technique known as quantum fracturing can be employed.

Modern designs for tubular PBRs have been refined to create a thin, uncontaminated culture suspension with excellent light exposure and low energy consumption. However, using larger tube diameters might result in a lower surface-area-to-volume ratio and reduced light availability. Additionally, wider tubes could lead to uneven solar radiation distribution for algae cells at different levels within the tube. Longer tubes might also hinder an algal strain's ability to capture photons due to oxygen accumulation. These challenges could impede the scalability of tubular photobioreactors. Another concern is temperature regulation in these systems, which can be tricky. Options like cooling tubes and thermostats are available, but they come with high installation costs. Scaling up HTBs can be achieved by either stacking tubes vertically or using coiled tubes.

- 3. Vertical Column Photobioreactor:** This type of photobioreactor is constructed of vertically positioned acrylic or glass tubes that let light pass through. The small gas sparger system is used to introduce bubbles of the incoming gas into the reactor, which facilitates mass transfer of carbon dioxide, effective mixing and oxygen evacuation. A vertical column photobioreactor typically does not have a physical agitation mechanism. Based on arrays of liquid flow, vertical PBRs may be divided into airlift reactors and bubble columns [56].
- 4. Bubble Column Reactors:** The height of a bubble column reactor is more than twice that of its vessel diameter. These reactors are cost-effective and provide a large, well-lit surface area. Instead of using moving parts, they rely on an efficient sparger for mixing and mass transfer. The design of this sparger significantly affects how the photobioreactor works. Generally, plates with holes are used to break apart and distribute aggregated bubbles. Light comes in from the outside, creating liquid circulation that varies the gas flow rate. This circulation is crucial for efficient photosynthesis.

However, the size of bubbles also matters to prevent damage to cells from shear forces [57]. Some bubble column photobioreactors use a rubber membrane diffuser or two spargers to boost gas mass transfer, including carbon dioxide supply and oxygen

removal. This approach is favoured due to its strong mass transfer, low energy costs, and minimal physical stress. When twin spargers are employed instead of the usual method, CO₂ transfer efficiency increases fourfold. The membrane diffuser acts like a one-way valve, preventing liquid from flowing back [58].

In an airlift reactor, a gas mixture rises through the riser and downcomer, moving from the sparger to the surface in the first cylinder (the gas riser). The second cylinder (downcomer) guides the medium downward towards the base. It's important to consider that the time gas spends in a zone affects gas-liquid mass transfer, heat exchange, mixing, and turbulence. One drawback of vertical bubble column PBRs is that certain algae strains, like *S. costatum* and *C. muelleri*, experience shear stress in these setups, and some cells can't withstand the pump-induced pressure, leading to cell damage [59]. Recently, a novel zigzag-flow column photobioreactor (ZZ-flow PBR) was tested for cultivating *A. platensis* with high biomass efficiency. Enhanced zigzag beam structures were integrated into the outer part (riser) of the ZZ-flow PBR. Compared to traditional column PBRs, this design accelerated intracellular photosynthesis and electron transport, enhancing biomass production and CO₂ fixation [60].

- 5. Helical-type Photobioreactor:** A detachable degassing device can be used with a flexible, transparent tube composed of helical photobioreactor (PBR) units. The system relies on a centrifugal pump to transport the culture through the long tube to the degassing unit. However, the energy demands of the centrifugal pump and the resulting shear stress impose limitations on the practical application of this PBR design. Scaling up the system can be achieved by incorporating a light-harvesting device. One challenge with this setup is the potential for contamination within the reactor [61].

A modification was introduced by shaping the helical PBR into a conical form with a 60° cone angle. The dimensions of the conical helical system, including height and angle, were thoughtfully selected. This reactor was constructed using coiled polyvinyl chloride tubing arranged in a conical configuration. An air pump facilitated liquid circulation, and the system featured a heat exchanger for temperature control along with a degassing mechanism. Opting for a 60° angle led to a twofold increase in the photoreceptor area and thus elevated photosynthetic productivity. This design achieved a photosynthetic efficiency of 6.84%, the highest among the various cone angles studied. The advantage of the conical shape lies in its improved light-capturing efficiency without altering the basal area. Furthermore, this design is more energy-efficient and places less mechanical strain on the algae cells. The possibility of upscaling is limited by the defined angle and size and is primarily achievable by adding more light-capturing units, although with potential energy losses in the complex flow networks [62].

Despite its benefits, this type of reactor has drawbacks, such as limited gas exchange, high shear stress, biomass accumulation within the tubes, and the need for substantial energy input [63].

- 6. Stirred Tank Photobioreactor (STR):** The stirred tank reactor is a highly valuable type of reactor widely employed in both industries and laboratories. It operates by creating mechanical stirring through various impellers of different sizes and shapes. Baffles are often incorporated to reduce the swirling motion within the reactor. At the reactor's base, a mixture of CO₂-enhanced air is bubbled, providing the algae with a carbon source that

fosters their growth. To convert this reactor into a photobioreactor, external illumination is essential, achieved either through fluorescent lights or optical fibers. A key feature of this reactor is the significant separation between the unused sparged gas and the oxygen produced during photosynthesis. This separation occurs within a disengagement zone, allowing the oxygen to transition from the liquid phase to the gas phase.

One notable application of this reactor type is the Hydraulically Integrated Serial Turbidostat Algal Reactor (HISTAR) system. This system, with a capacity of 3.6 cubic meters, successfully cultivated *Selenastrum capricornutum*. HISTAR employed closed turbidostats and a series of interconnected continuous flow stirred tank reactors (CFSTRs). The utilization of CFSTRs notably boosted the biomass of the introduced culture. Although its future deployment remains uncertain, efforts are ongoing to develop a deterministic system for predicting microalgal yield. This is a crucial step in evaluating the practical viability of this technology for broader use [64].

However, the primary drawback of this system is its relatively small surface-to-volume ratio, which limits its ability to efficiently capture light. Attempts have also been made to implement optical fibers for lighting purposes, but this approach has the drawback of potentially obstructing the mixing patterns within the reactor [65].

- 7. Advanced Systems:** Hybrid frameworks are superbly designed systems that combine two different types of development frameworks. The conservative aspect is employed and intended for extensive green growth development. These frameworks overcome the drawbacks of open ponds and the high initial cost of closed systems. Algal growths are first refrigerated in a PBR (photobioreactor) to create high-density inoculants, and then they are transferred to an open framework to help achieve optimal biomass generation. Placing algae in an open framework significantly reduces the likelihood of contamination as algae take over and successfully compete with other microorganisms. However, hybrid systems require sizable infrastructure, expensive maintenance and constant oversight [66].

Professor Walter Adey first introduced an altered cultivation method known as an algal Turf Scrubber (ATS) at the start of the 1980s. An ATS culture system promotes the development of macroalgae by providing a downward-sloping surface that allows water or effluent to flow across it periodically or constantly [67].

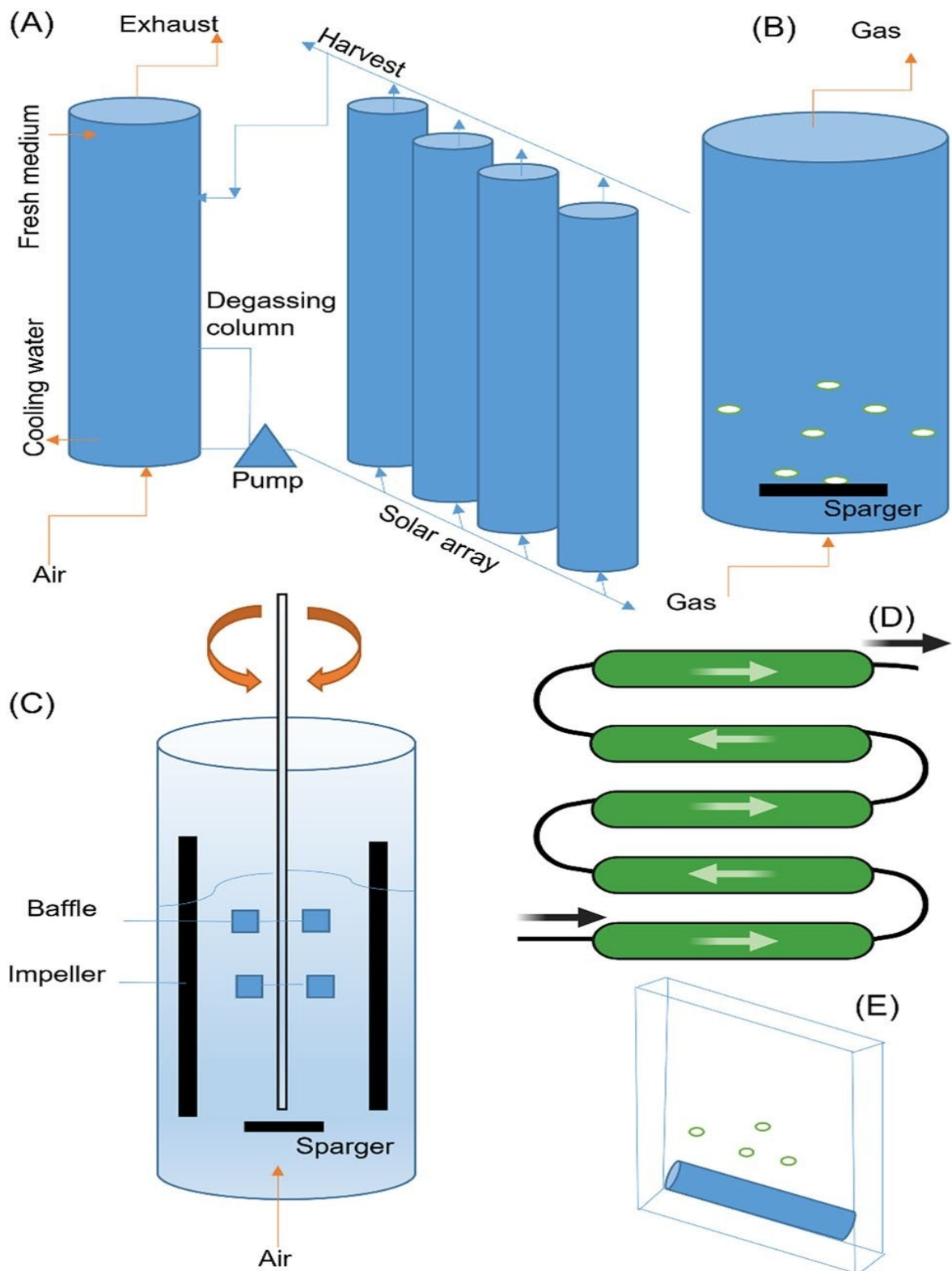


Figure 1: Various setups for photobioreactors include options such as a tubular photobioreactor (A), a bubble column photobioreactor (B), a flat panel photobioreactor (C), a helical tubular photobioreactor (D), and a stirred tank photobioreactor (E) [47].

VI. VARIABLES INFLUENCING THE GROWTH OF ALGAE AND THE SUBSEQUENT PRODUCTION OF BIOFUELS.

Several factors affect algal cultivation and biofuel production, including both biotic and abiotic variables. Abiotic factors include carbon dioxide, light, temperature, pH and nutrients, while biotic factors such as the type of algae being grown can also impact productivity and production.

- 1. Light:** Both the wavelength and intensity of light have a significant impact on the capacity of algae species to develop and accumulate biomass during culture. Some studies have shown that lipid content can increase when light intensity is higher [68]. The selection of algae as a raw material is often based on its high photosynthetic capacity, and light is crucial for regulating lipid formation and increase as it is necessary for photosynthesis and development. Researchers have also observed the shading effect of light, which hinders the growth of specific algal strains but resumes when the shading materials are removed. Fluorescent light sources have been found to improve growth compared to other light sources [69].
- 2. Temperature:** Temperature is a significant factor that affects biofuel production, lipid buildup, and algae growth. Most algae species thrive in the 20 to 35 degree Celsius temperature range, while certain species are mesophilic and prefer around 40 degrees Celsius. Overheating or heating below the necessary temperature can result in reduced yield and cell damage, respectively. Both extremely high and extremely low temperatures have been shown to reduce the amount of lipids in algal biomass [65].
- 3. Carbon Dioxide:** Various sources contribute significantly to the presence of carbon dioxide, including the atmosphere, emissions from vehicles and factories, and certain soluble compounds [70]. To create more algal lipids, it's necessary to have higher levels of carbon dioxide available [71]. Different types of writing provide diverse evidence about how carbon dioxide impacts the creation of lipids. When carbon dioxide levels are higher, the production of fatty acids is enhanced, while lower carbon dioxide concentrations hinder algae growth and the synthesis of fatty acids. However, a rise in carbon dioxide concentration can heavily influence the carbon cycle. Research on *Chlorella pyrenoidosa* SJTU-2 and *Scenedesmus obliquus* SJTU-3 demonstrated that growth improved when exposed to 10% carbon dioxide, and using 30–50% CO₂ led to increased production of fatty acids and lipid accumulation [72].
- 4. pH:** The pH is a critical parameter that significantly impacts lipid accumulation, oil production, and the enzymatic activity required for algae development. A neutral or slightly acidic environment promotes algae growth, but the presence of carbonic acids in the nutritional medium may lead to a lower pH, making conditions unfavourable for algae development. Lower pH also has a detrimental effect on carbon integration for lipid formation, as bicarbonate Concentration decreases [73].
- 5. Nutrients:** The availability of various nutrients affects the diversification of biochemical substances in algae. The rate of algae growth depends on the rate at which the most limiting nutrient is absorbed, assuming that pH and temperature are kept within the ideal range. Phosphate and nitrogen are the two macronutrients most important for healthy algae growth and development. Additionally, carbon, oxygen, and hydrogen are essential

nutrients for algae development, but their abundance does not pose a threat to the growth and metabolic processes of algae [74].

VII. HARVESTING OF MICROALGAE

Algae harvesting involves separating algae from their growth medium. The characteristics of the chosen microalgae, cell size, density, and end-product requirements all influence this process. Microalgae growth includes a crucial step—harvesting—that demands significant energy, constituting around 20–30% of total production costs, as per some studies [75]. Diverse methods like filtration, centrifugation, flocculation, and flotation, supported by mechanical, chemical, biological, and electrical approaches, have been employed for biomass collection [76]. Occasionally, a combination of techniques might be needed to enhance harvesting efficiency.

- 1. Filtration:** Microalgae may be present in the semi-permeable membrane that is utilised in the filtering procedure, which permits liquid media to flow through while leaving the microalgal biomass behind [77]. This method allows for the extraction of high cell concentrations from the medium, and the various opening diameters of the filter membrane make it simpler to work with more delicate organisms that are sensitive to compressive damage. This approach, however, is prone to fouling and clogging, demanding regular membrane or fresh filter replacements, which might dramatically increase operating costs. Given this challenge, the filtration membrane was created using low-cost and readily available substances. Bejor et al. therefore succeeded in fabricating a stretch cotton filter membrane with a 66–93% harvesting efficiency.
- 2. Centrifugation:** Reworded according to the density of the segment and the dimensions of its particles, the centrifugation procedure employs centrifugal force to separate microalgae cells from growth fluid [78]. Although the efficiency of cell harvesting is higher using this method, the technique is time- and energy-intensive. Additionally, the greater gravitational force involved in centrifugation may harm the cells, making it unsuitable for particular applications since the delicate nutrients may vanish. Different types of centrifugal systems, such as imperforated basket centrifuges, decanters, and hydro-cyclones, disc stack centrifuges have been employed by the industry [76].
- 3. Flocculation:** Using a flocculating mediator to reduce the surface charge of the cells results in the accumulation of free-floating, single-celled microalgae cells into a larger particle known as a floc. Chemical and biological flocculants are the two main categories into which flocculating agents can be divided. Chemical flocculants that are affordable and often useful, such as salts of iron and aluminium, have been widely used in the sector. According to a study by Chatsungnoen and Chisti, under normal circumstances, metal salts like iron chloride and aluminium sulphate may eliminate around 95% of the microalgae biomass. Due to their high toxicity, these compounds are not environmentally friendly and require extra processing steps, which increases manufacturing costs [79]. In contrast, bioflocculants are a safer and more ecologically conscious alternative to their chemical counterparts. They are also cost-effective and often eliminate the need for pre-treatment before reusing culture medium and processing microalgae downstream [80]. Most bioflocculants used are biopolymers like acrylic acid and chitosan, which can be produced chemically or organically [81]. Chitosan is said to achieve 90% cell recovery at

lower doses than chemical flocculants like aluminium sulphate, which need larger concentrations to get identical results [82].

4. **Flotation:** Flotation is a technique that uses tiny bubbles to attach to microalgae cells, helping them float on the surface of the liquid culture. This makes it easy to collect the cells. The benefits of flotation are better harvesting, simple upkeep, and efficient processing at a low cost. There are three main types of flotation methods, each creating air bubbles differently. One method involves pressurizing the culture with gas and then releasing it at normal pressure to create bubbles. Another method uses a sparger to make bubbles, needing less energy. The third method, called electro-flotation, uses electrolysis to make small bubbles from an electrode, catching the microalgae. This method can disrupt cells and harvest them at once using alternating current. However, it uses a lot of energy due to fouling and frequent electrode replacement, which raises production costs.
5. **Magnetic Separation:** Traditional methods of collecting microalgae, such as flotation, filtration, electrolysis, and centrifugation, are constrained by higher costs, higher energy requirements, and more difficult procedures. Along with these standard techniques, magnetic separation was used as a powerful tool for microalgal harvesting. The main advantages of magnetic separation technologies are high performance, cheap running costs, and quick and speedy processing [84].

Nanoparticles (NPs) are molecular aggregates or atoms having at least one dimension between 1 and 100 nm with drastically different physical and chemical properties from the bulk material. Because magnetic separation enables automated, rapid and scalable processing with increased harvesting efficiency and reduced contamination, magnetic nanoparticles (MNPs) are primarily employed for microalgae harvesting [85]. Magnetic nanoparticles also come in small sizes, have unique physicochemical properties, and are produced at lower prices. For the purpose of collecting algae, the three main types of magnetic particles are coated, surface-modified and bare (naked) [86].

- **Naked (bare) Magnetic Particles:** Researchers have used naked iron oxide nanoparticles, which are often considered to be low-cost and easy to use, to study how microalgae detach. When the mass ratio of nanoparticles to microalgae is properly chosen, effective detachment is achievable under changing ecological parameters, and the effects of pH and ionic strength are not particularly significant. This use of magnetic nanoparticles is an illustration of the biomass utilisation predictions made by nanobiotechnology. The higher affinity of the cell walls for the inorganic surface allows for harvesting efficiency of greater than 95% for *Scenedesmus ovalternus* and *Chlorella vulgaris* [87].

Botryococcus braunii and *Chlorella ellipsoidea* were successfully harvested using the Fe₃O₄ particles produced by chemical co-precipitation. In a different study, *Nannochloropsis maritima* marine microalgae were successfully processed using Fe₃O₄ nanoparticles. The recovery efficiency of cells from the culture medium was greater than 95% at a particle concentration of 120 mg/L within 4 minutes. According to reports, naked magnetite exhibits ion exchange characteristics, and the detachment is mostly caused by electrostatic contacts between the magnetite and the algal cells. Additionally, Fe ions that are produced from the surface of bare iron oxide magnetic

microparticles can function as flocculants and aid in the harvesting process, but the released toxins were found in the collected algal cells. Ions can increase the metal content, which may have an impact on how algal biomass is processed later. Fe, for instance, might contaminate the catalysts used in the desulfurization process and lower gasoline production. Therefore, while selecting a magnetic adsorbent, the potential impact on the downstream process should be taken into account [88]. The effectiveness of harvesting substantially increases as the pH value falls.

As a result, the low pH value attributed to the protonation of the nanoparticles surface is advantageous for adsorption between the nanoparticles and algal cells. A key factor in increasing the efficiency of microalgae harvesting is the addition of a stirring mechanism to aid in the interaction of iron oxide nanoparticles with the cell surface of the tiny algae before exposure to the extrinsic magnetic field. This action is anticipated to considerably increase harvest production [86].

- **Coated and Surface-Modified Nanoparticles:** A tiny organism tends to stick to solid surfaces in general to lower its energy. When microalgae attach to magnetic particles in water, we need to figure out all the interactions involved, like weak van der Waals forces, electric forces, and acid-base interactions. To avoid issues with collecting them, we switch the magnetic particles. Since most microalgae carry a negative charge, we often use positive polymers to coat them [89].

Around forty years ago, people first talked about using magnets to take out algae. This method can get over 90% of the cells in less than 5 minutes. By combining clumping and magnetism, this magnetic way is fast, efficient, and doesn't cost a lot. Plus, a magnetic field helps get rid of more liquid quickly and makes a dense mix of magnetic cells. Both plain and changed magnetic particles work well for this, but we need more research to know how good they are for bigger use [90].

VIII. PROCESSING OF ALGAE OIL EXTRACTION

There are a variety of techniques that have been used to process microalgae, and some of the more popular ones are described below:-

Table 3: Benefits, Limitations, and Financial Implications Associated with Diverse Approaches to Microalgae Processing [91].

| Method | Advantages | Drawbacks | Cost Involvement |
|--------------------------------|---|---|---|
| Expeller Press | No solvent is required. Easy operation. | Mechanical methods are energy-intensive. | High cost |
| Bead-beating /mill | No solvent required | Mechanical methods are energy-intensive | Cost-effective |
| Pressurized solvent extraction | Solvent use is relatively inexpensive | Energy-intensive (distillation is needed for the extraction of lipid from the solvents). The solvent may be | High cost due to the cumulative expenses incurred by the use of solvent and |

| | | | |
|--------------------------------|--|--|---|
| | | toxic | pressurized nitrogen |
| Soxhlet extraction | Solvent use is relatively inexpensive | Time-consuming and not suitable for large scale. | High cost |
| Ultrasonic extraction | Minimize the chemical conversion time by up to 90%. Eco friendly. Can replace the solvents with GRAS solvents | The solvent is needed to improve the recovery of lipid. The decline of power with time. No uniform distribution of ultra-sound energy. | Initial investment and maintenance costs high |
| Osmotic shock | No costly steps and requirement of solvents like other methods. | Longer duration of treatment time. | Low-cost method |
| Supercritical fluid extraction | Usage of less toxic solvents for the extraction. Suitable for thermo-labile compounds. Environmentally friendly. | Greater power consumption and complications in scaling up. High capital investment. No polar substances are extracted | High cost |
| Microwave-assisted extraction | Reduced solvent usage. Higher extraction rate and yield | High capital cost | Initial investment and maintenance costs high |
| Pyrolysis | Cost-effective. Ease of storage, transport, and preparation of bio-fuels by upgrading the bio-oil | Highly viscid, harsh, and no thermal stability. It shows low calorific values resemblance to the reactant oil as it has predominant oxygenated molecules. Catalyst deactivation will occur | Low-cost method |
| Direct Bio photolysis | Direct production of hydrogen from water and sunlight | It needs high intensity of light, low photochemical efficiency, and O ₂ is inhibitory. | Economically feasible. |
| Indirect bio photolysis | Blue-green algae can produce hydrogen from water and able to fix N ₂ from the atmosphere. | Removal of uptake hydrogenates is needed | Economically feasible |
| Enzyme-Assisted Extraction | It is an environmentally friendly and nontoxic process. High yield. Comparatively low-cost process due to the use of foodgrade enzymes | It is troubled by the lipid class composition and type of microalgae. It needs operation at reduced temperatures with high | High cost |

| | | | |
|---|---|--|--|
| | | specificity/selectivity for better efficiency. Cost intensive | |
| Pulsed Electric Field Assisted Extraction | High efficiency. Less energy requirement | Initial investment and maintenance costs are high | Initial investment and maintenance costs are greater, but can be operated at comparatively lower costs |

IX. TYPES OF ALGAE BASED BIOFUEL

At present, the world is exploring the potential of using algal biomass for bioenergy as a response to global warming and diminishing biofuel reserves. Additionally, crucial tactics to alleviate poverty involve improving energy access and security. The utilisation of algal biomass for producing biofuels stands as the primary alternative to reducing reliance on fossil fuels. Microalgae can be harnessed to produce various types of biofuels, such as biodiesel, bio-oil, bio-methane, bio-hydrogen, bio-gas, and bioethanol [55].

- 1. Bio-Oil:** A thermochemical process that runs at extremely high temperatures and without oxygen transforms biomass into oil, carbon, and gas to create bio-oil. Bio-oils can be used in place of petroleum oils despite being somewhat connected to them. The two main methods for producing bio-oil are thermochemical liquefaction and pyrolysis. In contrast to algal lipids, several organic composites are gathered as lipids, proteins, and carbohydrates in bio-oil, and the output is significant. *Spirulina*, *Scenedesmus*, *Dunaliella*, and *Desmodesmus*, in that order, produced biooil yields that varied from 24% to 45%, 37% to 49%, and as much as 41% [92].
- 2. Bio-Hydrogen:** The top three recommended ways to produce hydrogen are: direct photolysis, a process driven by ATP, and a path involving anaerobic conditions. Direct photolysis can work, but it requires continuous removal of the hydrogen and oxygen produced. However, this can lead to safety concerns and increased costs for separating the gases. This is due to the interconnected processes of photosynthesis and water splitting, which cause the simultaneous production of hydrogen and oxygen. Moreover, the hydrogenase enzyme used in this process is sensitive to oxygen. Hence, indirect methods are favoured to address these issues. In environments with low oxygen and limited sulphur, the starch in algal cell walls can be mostly converted into hydrogen.

The bulk of studies have shown that cyanobacteria are the primary biological producers of biohydrogen, and that the enzymes hydrogenase and nitrogenase act as catalysts in this process [92].

Due to its ability to produce photo-biological hydrogen, the single-celled green alga *Chlamydomonas reinhardtii* has been the subject of substantial research [90].

- 3. Bioethanol:** Algal bioethanol production has become very profitable as a result of these species increasing biomass yield, variety, unique chemical compositions, and improved

photosynthetic rates. Due to their abundance in carbs and polysaccharides as well as their weak cellulose walls, algae are the perfect source for the production of bioethanol [93]. As a major source of carbohydrates, microalgae rely heavily on polysaccharides like starch and cellulose. Microalgae are a desirable source of raw materials for the synthesis of bioethanol because they may accumulate significant amounts of polysaccharides in their intricate, multilayered cell walls [94].

- 4. Biodiesel:** The cost-effectiveness of producing biodiesel relies heavily on the choice of raw materials, which contributes to around 50 to 85% of the final fuel cost. To ensure economical biodiesel production, it's vital to assess the raw materials in terms of their quality, efficiency, and potential for utilizing by-products. Biodiesel is created through a process called transesterification, which converts lipids, mainly triacylglycerols and free fatty acids, from the raw materials into environmentally friendly biodiesel. The transesterification process involves reacting crude oil and alcohol in the presence of a catalyst, typically producing methyl esters of fatty acids (FAME) and glycerol as by-products. Although acid catalysts are advantageous due to their fast reaction rates, alkali catalysts are more commonly used in the industry due to their efficiency, often outnumbering acid catalyst usage by up to 400 times.

Research has particularly focused on two key microalgae species, *Chlorella vulgaris* and *Chlorella protothecoides*, for biodiesel production due to their high oil content [93]. Microalgal biodiesel primarily consists of unsaturated fatty acids. Since wastewater-derived algal biomass comprises various algae types, it yields different fatty acid profiles. The economic feasibility of microalgae-based biofuels depends on effectively regulating both biomass and lipid content. Another significant challenge in biofuel production is efficiently separating microalgal biomass from the culture broth. Various methods such as magnetic separation, pH-induced flocculation, and ultrafiltration have been explored to reduce microalgae harvesting costs and biodiesel manufacturing expenses [95].

Microalgae hold promise as a renewable resource for diverse industrial applications, including biofuel production, human and animal nutrition, cosmetics, pharmaceuticals, and aquaculture. Furthermore, microalgae are rich sources of natural compounds with various bioactive properties [96].

- **Transesterification of Algae Oil for Biodiesel Production:** The procedure of base catalysed transesterification with alcohol is one of many well-known commercial methods that may be used to create biodiesel from microalgae. A triglyceride-containing oil or fat reacts reversibly with an alcohol to produce glycerol and fatty acid alkyl ester through a process known as transesterification. According to stoichiometry, the reaction needs a 3:1 molar ratio of alcohol to oil, however extra alcohol is added (often methyl alcohol) to shift the equilibrium in favour of the product side [97]. This significant excess of methyl alcohol makes sure that the process is pushed towards methyl esters, or biodiesel.

On a weight basis, the yield of methyl esters surpasses 98% [98]. Triglycerides are first transformed into diglycerides, then into monoglycerides, and lastly into glycerol as part of the process [99]. (Fig. 2)

| | | | | | | |
|--|---|-------------------------------------|----------|---|---|--|
| Step 1: CH ₂ -COOR ₁ CH- COOR ₂ CH ₂ - COOR ₃ | + | CH ₃ -OH | Catalyst | CH ₂ - OH CH- COOR ₂ CH ₂ - COOR ₃ | + | CH ₃ -COOR ₁ |
| Triglyceride | | Methanol | | Diglyceride | | Methyl ester |
| (Parental oil) | | (Alcohol) | | | | (Biodiesel) |
| Step 2: | | | | | | |
| CH ₂ -OH CH- COOR ₂ CH ₂ - COOR ₃ Diglyceride | + | CH ₃ -OH Methanol | Catalyst | CH ₂ - OH CH- COOR 2 CH ₂ - OH Monoglyceride | + | CH ₃ - COOR ₂ Methyl ester |
| | | | | | | (Biodiesel) |
| Step 3: | | | | | | |
| CH ₂ -OH CH- COOR ₂ CH ₂ -OH Monoglyceride | + | CH ₃ -OH Methanol | Catalyst | CH ₂ - OH CH- OH CH ₂ - OH Glycerol | + | CH ₃ - COOR ₃ Methyl ester |
| | | | | | | (Biodiesel) |

Figure 2: Transesterification of algal oil to biodiesel

Transesterification may be done using a variety of catalysts, including alkali, acid, enzyme, heterogeneous, or alcohol in its supercritical state. However, enzyme catalysts are rarely used since they are less effective [100]. Alkali-accelerated transesterification happens around 4000 times more quickly than an acid-accelerated transesterification reaction. As a result, sodium and potassium hydroxides as well as other alkalis with a concentration of around 1% by weight of oil are frequently utilised as industrial catalysts. Alkoxides are utilised more commonly because they make better catalysts than sodium hydroxide. Sodium methoxide is one illustration. Utilising lipases has a number of benefits [99]. Alkali-catalyzed transesterification

occurs when methanol boils off at 65 °C under atmospheric pressure, which occurs at a temperature of roughly 60 °C. Higher pressures and temperatures can be employed; under these circumstances, the reaction occurs in around 90 minutes. The reaction mixture contains two liquid phases because methanol and oil do not mix. The cheapest alcohol is methanol, however other forms can also be used. The oil and alcohol need to be dry, and the oil should have a low content of free fatty acids to limit yield loss caused by saponification processes (the making of soap).

Biodiesel may be recovered by continuously washing it with water to get rid of glycerol and methanol. This technique of making biodiesel is found to be the most efficient and least corrosive of all the ways due to the relatively high reaction rate even at a low temperature of 60°C.

- **Steps Followed in Transesterification Reaction:** First, either wet algal biomass weighing 10 grams in terms of dry weight or freeze-dried algal biomass of 10 grams is placed into a glass vial. This mixture is treated with n-hexane and methanol after blending for 5 minutes. The resulting mixture is then transferred to centrifuge tubes. The glass is washed twice with distilled water and solvent. The tubes also receive the same treatment.

Next, the mixture in the tubes is centrifuged at 4,000 rpm for 15 minutes. The organic part containing algae oil is isolated and put into a pre-weighed glass vial. The algal oil is heated for 5 minutes at 75 °C to remove water. The algal oil, methanol, sulfuric acid, and n-hexane are mixed for 35 minutes at 75 °C. Once the reaction is done, the samples are cooled to room temperature, and the upper crude ester layer is separated from the glycerol layer using a separating funnel.

The raw ester layer contains methyl ester, possibly non-reactive oil, methanol, and glycerol. To remove the methanol, the organic layer is washed twice with distilled water in a separating funnel until the washings are neutral. The layer with FAMES (Fatty Acid Methyl Esters) is dried using a solution of NaCl. The FAMES-containing top layer is poured into a pre-weighed glass test tube. Using a Rotary Evaporator at 320 mbar and 36 °C, the solvent is evaporated, including the removal of n-hexane. Finally, the FAME content of the crude biodiesel fuel is examined through gas chromatography [101].

X. QUALITY ANALYSIS OF BIODIESEL

1. Fatty Acid Estimation by Gas Chromatography

- The fatty acid composition of the oils will be determined by gas chromatography (GC) as fatty acid methyl esters (FAME).
- Helium is used as a carrier gas which is operated at a flow rate of 1.00ml/min. The column temperature was isothermal at 190°C where in the injector and detector temperatures were 230°C and 240°C, respectively. FAME was identified by comparison of their retention times with those of the reference standards.

2. Acid no Test:

- **Apparatus:** Stirrer, 250 ml beaker, mass scale, and titration bulb are the apparatus. Biodiesel, phenolphthalein indicator, isopropanol/water solution 90/10 by volume, and isopropanol/KOH solution 0.1 N are among the chemicals.

- **Procedure:**

- Select the proper biodiesel sample size (using a prescribed methodology).
- Weigh the material into a 250 ml beaker to the closest 0.1 mg.
- Include 110 cc of the 90/10 blend.
- 4-6 drops of phenolphthalein indicator should be added.
- Use a solution of 0.1 N alcohol, isopropanol, and KOH to titrate till the phenolphthalein end point (pink).
- To check for errors, repeat the previous three times.
- The acid number (AN) is calculated using the equation shown below:

$$AN = (V_{\text{KOH}} - a) * N * 56.1 / W$$

Where:

V_{KOH} = Volume of potassium hydroxide

a = Volume of potassium hydroxide for blank solution

N = Concentration of alcohol (isopropanol) KOH solution

W = mass of sample (g)

3. Copper Strip Corrosion Test:

- **Apparatus:** Hot plate, copper strips, beaker, and thermometer are the apparatus.
- **Chemicals:** volatile sulfate-free hydrocarbon solvent and biodiesel.
- **Procedure:**

- Fill a beaker with biodiesel, then set it on a hot plate.
- Heat to a constant temperature of 50 °C.
- Insert a copper strip into the beaker.
- After three hours, wash with acetone or another solvent.
- Contrast the strip with the classification statistics for the Copper strip test that were received from NREL (2004).
- Label the strip as shown in the table with a number and a letter.
- To replicate the pH impact of fatty acids, add a little known amount of sulphuric acid to the biodiesel. Then, repeat the previous stages.

4. Soap and Catalyst Test:

- **Apparatus:** Scale and test tube
- **Chemicals:** Acetone, hydrochloric acid, phenolphthalein indicator, distilled water, and bromophenol blue indicator.
- **Procedure:** Obtained from NREL (2004) [102]

- Mix 100 ml of acetone with 2% distilled water to dissolve the sample. The sample size will depend on the anticipated catalyst and soap levels. Use 0.5 g of sample for unwashed methyl esters, 5 g for washed methyl esters, and 100 g of sample for crude glycerol.
- Add 2 ml of 1% isopropyl alcohol-based phenolphthalein indicator.

- Continue to titrate with 0.05 N HCl until the colour of the phenolphthalein shifts from red to clear. Identify this quantity of the solution as "A"
- Include 1 ml of water with 0.4% bromophenol blue indicator.
- At a pH of roughly 4.5, this indicator changes colour.
- Titrate until the bromophenol becomes yellow instead of blue. Assign the letter "B" to this amount of solution.

XI. CURRENT RESEARCH STATUS IN INDIA

India's fast growing economy has led to a high level of industrialisation in the country. The United States uses nearly five times as much diesel fuel as it does petrol, compared to almost all other countries in the globe. Finding alternative energy sources is very important for India, and using biofuel has a far greater impact on us than it does on other countries. According to specialists working on the New Millennium India Technology Leadership programme, one car was powered by B-20 biodiesel generated from marine microalgae. The effort was started by the CSIR and the Ministry of Earth Sciences along with researchers from nine different universities, including CSMCRI, IIT-Kharagpur, IICT-Hyderabad, NIOT-Chennai, and NIO-Goa. Microalgae mats that CSMCRI observed naturally existing in West Coast India were used to make the biodiesel.

Mysore has pioneered a technique to grow *Scenedesmus acutus*, a type of green algae, and *Spirulina platensis*, a form of blue-green algae, tailored to Indian conditions. The standout among these is Spirulina due to its ability to thrive with basic methods. The Indian Institute of Chemical Technology aims to cut the cost of producing algae-derived oils from INR 500 per liter to around INR 20. Notably, World Health Energy Holdings, Inc. has allocated \$100 million to establish a 250-acre algae biodiesel farm in Karnataka. Meanwhile, the Dr. MGR Algae Biofuel Research Institute is conducting a biodiesel experiment with microalgae in Sivakasi, a hotbed of both heat and CO₂ in Tamil Nadu [103].

XII. RECENT ADVANCES IN PRODUCTION TECHNOLOGY

Transesterification, biohydrogen production, and biomass hydrolysis can employ biochar as a catalyst. The best way to effectively collect and harvest the whole biomass is to co-cultivate filamentous fungi with selected microalgae. The production of lipid content in microalgae can be increased by the application of ultraviolet mutagenesis; however, the screening procedure is labour- and time-intensive.

The use of molecular biology techniques like CRISPR/Cas9 with guided RNA for genetic editing in algae has created new opportunities to fulfil the world's growing energy needs. Due to the exceptional structural characteristics, biodegradability, adaptability to different types of microalgae species, and environmental friendliness of chitosan-based flocculants, there has been considerable attention directed towards the field of microalgae harvesting. During the manufacturing of biofuels, the effects of weather and market changes should also be taken into account [103].

XIII. ALGAL BIODIESEL OPPORTUNITIES IN INDIA

An intelligence agency assessed India's population to be 1,166,079,217 as of July 2009. Despite occupying only 2.4% of the planet's total surface area, India is home to more

than 15% of the world's people. Demographics predict a growth in population since more than 40% of Indians under the age of 15 are anticipated to have children [104]. By 2050, India will have a total population of more than 1.5 billion people, an increase of 530 million, predict UN demographers. If the projected demographic trend for India's population is accurate, India would overtake China in terms of population by 2045. Rising energy consumption is now associated with economic growth in India, just like it is in many other emerging and developed countries.

The pressing environmental concerns in India are consistently addressed in policy discussions due to the dual factors of energy-intensive industries and growth hotspots within the country. Despite over 70% of Indians residing in rural areas across around 550,000 communities, urbanization has steadily surged since 1971. Notably, there are now 23 Indian cities, including Mumbai, Calcutta, Delhi, Chennai, and Bangalore, each harboring over a million inhabitants—a significant increase from just 12 in 1981 [104]. This urban boom has led to heightened vehicle ownership, with Delhi's registered cars soaring from 841,000 in 1985 to approximately 3.5 million in 2001.

India's rapid economic expansion has unfortunately given rise to severe air and water pollution, deforestation, water scarcity, and increased carbon emissions. The burgeoning urban and industrial sectors have propelled the use of nonrenewable energy sources, potentially endangering future energy security. Consequently, an urgent shift to alternative energy sources, like microalgae, has become imperative for India's sustainability. Recent studies propose that microalgae could offer a promising avenue for renewable energy in the nation.

There are additional advantageous aspects linked to biodiesel production in India. Diesel fuel enjoys government-subsidized prices, aimed at reducing transportation costs and boosting GDP. This has incentivized significant investment in diesel vehicles by Indian manufacturers. With a substantial portion of vehicles running on diesel, the need for expensive retrofits to switch to compressed natural gas (CNG) is minimized. Moreover, India's tropical climate provides a unique edge for algal biodiesel production due to its suitability for cultivating various microalgae species [105]. Unlike traditional high-oil crops like palm that yield 2000 to 2500 liters per acre, algae can produce an impressive 19,000 to 57,000 liters of biodiesel per acre. Embracing large-scale biodiesel production can help India lessen its dependence on foreign oil, enhance air quality in major cities like Delhi, Kolkata, Bengaluru, and Chennai, reclaim unproductive lands, provide employment, and align with the nation's targeted annual GDP growth of 8 to 10%, as per the 11th Five-Year Plan [28].

XIV. CURRENT STATUS AND FUTURE PROSPECTS

Although algae fuels are not currently commercially available, they have a bright economic future [106]. Although theoretically feasible, producing liquid fuels from algae is still more expensive than using petroleum-based fuels. The petroleum price's sensitivity to significant and unpredictably fluctuating swings is a significant barrier to investing in fuels made from algae. In a situation where crude oil sells for P100 per barrel, oil from algae is anticipated to be commercially feasible [32]. Algal fuels, as was previously discussed, have both good and bad aspects. On the negative side, resource needs may be burdensome and energy benefits may be uncertain. The production of oil may be economical, renewable, and sustainable. Algal fuels may outperform fossil fuels in terms of life-cycle analysis, although

this area of research is still in its infancy. Open ponds, one of the two main categories of large-scale algae cultivation systems [107], are less productive than photobioreactors. Although photobioreactors need a large upfront investment, they sometimes seem to be able to produce biomass at a cheaper ultimate cost.

In comparison to open ponds, photobioreactors produce a more concentrated algal solution, leading to substantial reductions in dewatering costs. Utilizing tubular photobioreactors, there is potential to achieve dewatered algal biomass at around €4 per kilogram of dry weight [107] [108]. Numerous startup companies are actively pursuing the commercialization of algal fuels, a field that holds great promise. Considering the overall environmental impact of these alternative fuel sources, they might already be competitive with conventional petroleum-based fuels. Climate change-driven concerns might necessitate a shift away from petroleum usage long before its depletion. Over the past four decades, significant progress has been made in understanding algae growth [108].

This involves a deeper comprehension of how nutrient availability and environmental conditions affect the composition and division of algal cells. However, the impacts of interactions brought on by several factors working together have traditionally received less attention. Growing, concentrating, separating, and converting microalgae biomass—some of which can be genetically modified—are steps in the process of making biofuel from algae. A sizeable amount of byproduct is left over after the targeted biofuel product or products are separated from the microalgae biomass. For the process to be economically viable and environmentally sustainable, it is crucial that the leftover byproducts serve a function that is both safe and productive. A deeper comprehension of the fundamental biology of algal cells will result from ongoing study, which will also help in the creation of more precise forecasting models for algae growth. To manage algal growth in large-scale production systems, predictive models may be utilised to create automated, optimum control systems.

REFERENCE

- [1] A. Abd El-Moneim M.R, S. Emad A, and S. Sanaa M.M, “Enhancement of biodiesel production from different species of algae,” *Grasasy Aceites*, vol. 61 (4), pp. 416-422, 2010.
- [2] S. Balijeet S, S. Deepansh, S. Ranjit, S. Ozlem, W. Alan, “Towards Algal Biofuel Production: A Concept of Green Bio Energy Development,” *Innovative Romanian Food Biotechnology*, vol. 12, 1- 2, 2013.
- [3] S.Val H, S. Belinda.S.M, de N. Frank.J, B. Sharon A, “The ecology of algal biodiesel production,” *Trends in Ecology and Evolution*, Vol.25 No.5, pp. 301-309, 2010.
- [4] B. Jeroen, “Future of Algae Based Biodiesel Production in The Netherlands,” july 2013
- [5] T. Sakshi, S. Harshita, A. Kumar, and Rai. P.K, “ Biodiesel Production From Algae: Principles And Processes,” *Invertis Journal of Renewable Energy*, Vol. 11 No. 1, January-March, pp. 10-16, 2021.
- [6] N. Nick, L. Pete, “Production of methyl ester fuel from microalgae,” *Appl. Biochem. Biotechnol*, vol. 24, 335-61,1990.
- [7] S. Shigeki, I. Seiichi, D. Yutaka, and Y. Shin-ya, “Co2 fixation and oil production through microalga,” *Energy Convers. Mgmt*, Vol. 36 No. 6-9, pp.729-731, 1995.
- [8] S. Stuart A, D. Matthew P, D. John S, H. Irmtraud, H. Christopher J, Lea-Smith. David.J et al, “Biodiesel from algae: Challenges and prospects,” *Curr. Opin. Biotechnol*, 21, pp. 277–286, 2010.
- [9] Lundquist. T.J, Woertz. I, Quinn. Nigle W.T, B. John, “ A Realistic Technology and Engineering Assessment of Algae Biofuel Production,” University of California, Berkeley, CA, USA, 2010
- [10] S. Pauline, Joannis-cassan. C, D. Elie, I. Arsene, “Commercial Applications of Microalgae,” *J. Biosci. Bioeng*, vol.101, pp. 87–96, 2006.
- [11] S. Ramganes, F. Tamás, T. Tamás, S. Elumalai, S. Timothy, and T. Memory, “Screening and Evaluation of Some Green Algal Strains (Chlorophyceae) Isolated from Freshwater and Soda Lakes for Biofuel Production,” *Energies*, vol. 8, pp.7502-7521, 2015.

- [12] Ramírez-Romero. Adriana, M. Bruno Da Costa , Dimitriades-Lemaire. Alexandra, S. Jean-Francois , D. Florian, and S. Jean-Philippe, “Chlorellaceae Feedstock Selection under Balanced Nutrient Limitation,” *Fermentation*, pp. 8, 554, 2022.
- [13] N. Thanh Tien, L. Man Kee, U. Yoshimitsu, Mansora. Nurlidia, L. Jun.Wei, S. Pau Loke et al, “High biodiesel yield from wet microalgae paste via in-situ transesterification: Effect of reaction parameters towards the selectivity of fatty acid esters,” *Fuel*, vol. 272, pp. 117718, July 2020.
- [14] M. Giorgos, and N. Elias, “Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions” *Biotechnology Advances*, vol. 31, pp. 1532–1542, 2013.
- [15] K. Muhammad Imran, S. Jin Hyuk, and Kim. Jong Deong, “The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed and other products,” *Microb Cell Fact*, 17:36, 2018.
- [16] A. Hamdy E.A, El-fayoumy. Eman A, R. Wessam E, S. Ramadan M, A. Mohd A, “Two stage cultivation of *Chlorella vulgaris* using light and salt stress conditions for simultaneous production of lipid, carotenoids, and antioxidants,” *Journal of Applied Phycology*, vol. 33, pp. 227–239, 2021.
- [17] B. Michael A, “The ‘stress’ concept in microalgal biology—homeostasis, acclimation and adaptation,” *Journal of Applied Phycology*, 2018.
- [18] S. Syed M.U, and A. Mohd A, “Effects of macro/micronutrients on green and brown microalgal cell growth and fatty acids in photobioreactor and open-tank systems,” *Biocatalysis and Agricultural Biotechnology*, vol.14, pp. 10–17, 2018.
- [19] V. Milada, B. Kateřina, K. Shigeyuki, Z. Vilém, “Accumulation of energy reserves in algae: from cell cycles to biotechnological applications,” *Biotechnological advances*, Volume. 33, pp. 1204-1218, 2015.
- [20] S. Baharak , C. Wei Chen, R. Abdul Aziz Abdul , I. Shaliza, “Microalgae Lipid and Biomass for Biofuel Production: A Comprehensive Review on Lipid Enhancement Strategies and Their Effects on Fatty Acid Composition,” Elsevier, 2018.
- [21] Shanab-Abou. Reda, M. Ibrahim A, K. Su-Nam, O. You-Kwan, C. Jaeyoung, J. Byong- Hun, “Characterization and identification of lipid- producing microalgae species isolated from a freshwater lake
- [22] Y. Hyun-Shik, L. Hongkyun, P. Young-Tae, Ji. Min-Kyu, K. Akhil N, J. Chung et al, “Isolation of novel microalgae from acid mine drainage and its potential application for biodiesel production,” *Appl. Biochem. Biotechnol*, vol.173, pp. 2054–2064, June 2014.
- [23] C. Yusuf, “Biodiesel from microalgae,” *Biotechnol Adv*, vol. 25, pp. 294–306, 2007.
- [24] M. Teresa M, M. Antonio A, C. Nidia S, “Microalgae for biodiesel production and other applications: A review,” *Renew Sustain Energy Rev*, vol. 14, pp. 217–232, 2010.
- [25] L. Lieve M. L, D. Thomas A, Jones. Howland D. T, W. Edward J, W. Stefanie V, McA. Jordan S. P et al, “Algal Biomass Constituent Analysis: Method Uncertainties and Investigation of the Underlying Measuring Chemistries,” *Anal. Chem*, vol. 84, pp. 1879–1887, 2012.
- [26] Oilgae. “Oilgae Guide to Fuels from Macroalgae. Tamilnadu, India”.
- [27] B. Rachna, & D. Renu, “Algae as biofuel,” Taylor & Francis, 2015.
- [28] B. Amit Kumar, M. Sachin K, S. MR, T. Kiran, R. Shirish, “Algal biodiesel: the next generation biofuel for India,” *Soc Appl Sci*, vol. 1, pp.728–39, 2010.
- [29] Suganya. T, Varman. M, Masjuki. HH, Renganathan. S, “Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: a biorefinery approach,” *Renew Sustain Energy Rev*, vol. 55, pp. 909–41, March 2016.
- [30] A. Oladapo Martins, A. Ulugbek , B. Alexey, “Algae biofuel: Current status and future applications,” *Renewable and Sustainable Energy*, pp. 316-335, 2018.
- [31] G. Danilo, F. Alberti, F. Solfanelli, D. Vairo, and R. Zanolì, “Third generation algae biofuels in Italy by 2030: A scenario analysis using Bayesian networks,” *Energy Policy*, vol. 103, pp. 165–178, 2017.
- [32] C. Yusuf, “Biodiesel from microalgae beats bio-ethanol,” *Trends in Biotechnol*, vol. 26, pp. 126-31, 2008.
- [33] G. E Molina, Belarbi. E-H, F. F G. Acien, M. A. Robles, and C. Yusuf, “Recovery of microalgal biomass and metabolites: Process options and economics,” *Biotechnology Advances*, vol. 20, pp. 491–515, Jan 2003.
- [34] L. Jin, H. Junchao, C. Feng, “Microalgae as Feedstocks for Biodiesel Production,” *Stoytcheva M.C, Ed.; INTECH: Rijeka, Croatia*, pp. 58–78, 2011.
- [35] L. Keesoo, E. Megan L, R. Rindi, P. Swaminathan, N. Paul K, “Isolation and screening of microalgae from natural habitats in the midwestern United States of America for biomass and biodiesel sources,” *Journal of Natural Science, Biology and Medicine*, Vol. 5, Issue 2, 2014.
- [36] D. Wehr John, and G. Sheath Robert, *Freshwater algae of north america: ecology and classification*,” Academic Press, San Diego, 2003.

- [37] Abdeshahian. P, Dashti. MG, Kalil. MS, Yusoff. W.M.W, “ Production of biofuel using biomass as a sustainable biological resource,” *Biotechnology*, vol. 9(3), pp. 274–282, 2010.
- [38] X. Marguerite A, Frost. Paul C, Elser. James J, “Joint effects of UV radiation and phosphorus supply on algal growth rate and elemental composition,” *Ecology*, vol. 83(2), pp. 423–435, Feb 2002.
- [39] Kaplan. D, Richmond. A E, Dubinsky. Z, Aaronson. S, “CRC handbook of microalgal mass culture,” CRC Press, Boca Raton, FL, 1986.
- [40] S. Osama H., El- Shahed. Ahmed M, “Growth, photosynthesis and circadian pattern in *Chlorella vulgaris* (Chlorophyta) in response to growth temperature,” *Cryptogamic Algologie*, vol. 21, Issue 3, pp. 283-290, 2000.
- [41] I. Asokaraja, M. Davoodbasha, R. Praveen K, E. Baldev, T. Nooruddin, “Optimization of various growth media to freshwater microalgae for biomass production,” *Biotechnology*, vol.10 (6), 540–545, 2011.
- [42] V. Manisha, and M. Vishal, “An Introduction to Algal Biofuels,” *Clean Energy Production Technologies*, vol. 1, pp. 1-34, 2020.
- [43] W. Jinghan, Y. Haizhen, W. Feng, “Mixotrophic cultivation of microalgae for biodiesel production: status and prospects,” *Appl Biochem Biotechnol*, vol. 172(7), pp. 3307–3329, 2014.
- [44] C. Sheng-Yi, K. Chien-Ya, C. Chiun-Hsun, K. Tang-Ching, O. Seow-Chin, L. Chih-Sheng, “Reduction of CO₂ by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor,” *Bioresour Technol*, vol. 99(9), pp. 3389– 3396, 2008.
- [45] Y. Jin Shui, R. Ehsan, T. Prapakorn, S. Kate M, Y. HongLi, H. Krassimira R, “Mathematical model of *Chlorella minutissima* UTEX2341 growth and lipid production under photoheterotrophic fermentation conditions,” *Biores Technol*, vol. 102(3), pp. 3077– 3082, 2011.
- [46] M. S Venkata, Rohit. MV, Chiranjeevi. P, C. Rashmi, Navaneeth. B, “Heterotrophic microalgae cultivation to synergize biodiesel production with waste remediation: progress and perspectives,” *Biores Technol*, vol. 184, pp. 169–178, 2015.
- [47] M. Tehreem, H. Nazim, S. Areej, M. Sikandar I, I. Hafz M.N, B. Muhammad, “Sustainable production of biofuels from the algae-derived biomass,” *Bioprocess and Biosystems Engineering*, 2022.
- [48] T. Archana, K. Thomas, P. Anjana, “Algal cultivation for biofuel production,” Elsevier, New York, pp. 383-403, 2019.
- [49] C. Jorge A.V, de M. Michele G, “An open pond system for microalgal cultivation. In: *Biofuels from algae*,” Elsevier, New York, pp. 1-22, 2014.
- [50] N. Rakesh R, G. Sourabh, S. Kalpesh K, Thomas-Hall. Skye R, D. Miklos, L. Yan, S. Peer M, “Comparison of microalgae cultivation in photobioreactor, open raceway pond, and a two-stage hybrid system,” *Front Energy Research*, vol. 4, pp. 1–10, 2016.
- [51] B. Liam, O. Philip, “Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products,” *Renew Sustain Energy Rev*, vol. 14(2), pp. 557–577, 2010.
- [52] T. Han, H. Lu, S. Ma, Z. Yuanhui, Z. Liu, Na. Duan, “Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: a review,” *Int J Agric Biolog Eng*, vol. 10(1), pp. 1–29, 2017.
- [53] Sun. Z, Liu. J, Zhou. ZG, “Algae for biofuels: an emerging feedstock,” Elsevier, New York, pp. 673-698, 2016.
- [54] Y. Abu, “Fundamentals of microalgae cultivation,” Elsevier, New York, pp. 1-9, 2020.
- [55] M. Judy , G. Chandima, “Development of Microalgae-based Biofuels as a Viable Green Energy Source: Challenges and Future Perspectives,” *Biointerface Research in Applied Chemistry*, vol. 12 Issue 3, pp. 3849 – 3882, 2021.
- [56] C. Ana P, M. Luis A, M. F Xavier, “Microalgal reactors: a review of enclosed system designs and performances” *Biotechnol Prog*, vol. 22(6), pp. 1490–1506, 2006.
- [57] L. Florian, P. Clemens, “Closed photo-bioreactors as tools for biofuel production,” *Curr Opin Biotechnol*, vol. 20(3), pp. 280–285, 2009.
- [58] Poulsen. BR, Iversen. JJ, “Characterization of gas transfer and mixing in a bubble column equipped with a rubber membrane diffuser,” *Biotechnol Bioeng*, vol. 58(6), pp. 633–641, 1998.
- [59] M. Asterio S, C. Francisco G, G. Antonio C, G. Emilio M, C. Yusuf, “Bubble-column and airlift photobioreactors for algal culture,” *AIChE J*, vol. 46(9), pp. 1872–1887, 2000.
- [60] Kubar. AA., Cheng. J, Kumar. S, Liu. S, Tian. J, “Developing a Zigzag-bafed column photobioreactor to increase mass-transfer, CO₂ fixation and biomass yield during *A. platensis* cultivation,” *Journal of CO₂ Util*, vol. 63, 102126, 2022.
- [61] S. RN, S. Shaishav, “Development of suitable photobioreactor for algae production—a review,” *Renew Sustain Energy Rev*, vol. 16(4), pp. 2347–2353, 2012.

- [62] Morita. M, Watanabe. Y, Saiki. H, “Investigation of photobioreactor design for enhancing the photosynthetic productivity of microalgae,” *Biotechnol Bioeng*, vol. 69(6), pp. 693–698, 2000.
- [63] S. Kari, A. Uno, H. Thorsten, B. Stig A, “Design and construction of a photobioreactor for hydrogen production, including status in the field,” *J Appl Phycol*, vol. 28(4), pp. 2205–2223, 2016.
- [64] X. Ling, W. Pamela J, X. Xue R, L. Chun Z. “Microalgal bioreactors: challenges and opportunities,” *Eng Life Sci*, vol. 9(3), pp. 178–189, 2009.
- [65] Singh. S.P, S. Priyanka, “Effect of temperature and light on the growth of algae species: a review,” *Renew Sustain Energy Rev*, vol. 50, pp. 431–444, 2015.
- [66] M. Sanjeet, G. Rahul Kumar, V. Pradeep, L. Roberto, Z. Antonio, “Integrated approach for wastewater treatment and biofuel production in microalgal biorefineries,” *Energies*, vol. 14(8), pp. 2282, 2021.
- [67] S. EL-Sayed, K. Mayur B, Abou-Shanab. Reda A.I, El-Dalatony. Marwa M, Y. iL-Seung, M. Booki et al, “Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation,” *Renew Sustain Energy Rev*, vol. 79(May), pp. 1189–121, 2017.
- [68] M. Maria N, P. George, K. Ioannis T, K. Nikolaos, “Effect of light intensity and quality on growth rate and composition of *Chlorella vulgaris*,” *Plants*, vol. 9(1), pp. 1–17, 2020.
- [69] S. Sutamas, S. Kritsana, K. Pongpatai, N. Narin, A. Somkid, T. Wannapong, “Modeling the effects of light sources on the growth of algae,” *Adv Difer Equ*, 2019.
- [70] Y. Chan, J. So-Young, L. Jae-Yon, A. Chi-Yong, O. Hee-Mock, “Selection of microalgae for lipid production under high levels carbon dioxide,” *Biores Technol*, vol. 101 (1 Suppl.), pp. S71–S74, 2010.
- [71] Zhu. L.D, L. ZH, Hiltunen. E, “Strategies for lipid production improvement in microalgae as a biodiesel feedstock,” *Biomed Res Int*, pp. 7–9, 2016.
- [72] T. Dahai, H. Wei, L. Penglin, M. Xiaoling, Z. Jianjiang, “CO₂ biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO₂ levels,” *Biores Technol*, vol. 102(3), pp. 3071–3076, 2011.
- [73] C. Yoon Young, J. Jae Min, L. Jeewon, H. Min Eui, P. Hoang-Minh, C. Won Seok et al, “Development of large-scale and economic pH control system for outdoor cultivation of microalgae *Haematococcus pluvialis* using industrial flue gas,” *Biores Technol*, vol. 244, pp. 1235–1244, 2017.
- [74] J. Ankita, C. Ruben M, M. Ganti S, “Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review,” *Energies*, vol. 6(9), 4607–4638, 2013.
- [75] T. Jia Sen, L. Sze Ying, C. Kit Wayne, L. Man Kee, L. Jun Wei, H. Shih-Hsin, S. Pau Loke, “A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids,” *Bioengineered*, vol. 11, pp. 116-129, 2020.
- [76] S. Gulab, Patidar. S.K, “Microalgae harvesting techniques: A review,” *Journal of Environmental Management*, vol. 217, pp. 499-508, 2018.
- [77] H. Mariam, G. Abdel, H. Amal, “Microalgae Harvesting Methods for Industrial Production of Biodiesel: Critical Review and Comparative Analysis,” *Journal of Fundamentals of Renewable Energy and Applications*, vol. 5(2), 2015.
- [78] S. Rizwan, N. Theoneste, Z. Xianhai, L. Yinghua.; L. Lu.; D. Michael K. “Development of a Two-Stage Microalgae Dewatering Process – A Life Cycle Assessment Approach,” *Frontiers in Plant Science*, vol. 7, 2016.
- [79] Rinanti. A, Purwadi. R, “Harvesting of freshwater microalgae biomass by *Scenedesmus* sp. as bioflocculant,” *IOP Conference Series: Earth and Environmental Science*, vol. 106, 2018.
- [80] N. Phuong T.D, L. Thi V.A, S. Pau- Loke, N. Thanh Thuy, T. Minh H, T. Thu T.N et al, “Bio flocculation formation of microalgae-bacteria in enhancing microalgae harvesting and nutrient removal from wastewater effluent,” *Bio resource Technology*, vol. 272, pp. 34-39, 2019.
- [81] P. Arivalagan, S. Sutha, B. Peter, N. Nandor, X. Ao, J. Rajesh Banu, K. Gopalakrishnan, “A review on chemical mechanism of microalgae flocculation via polymers,” *Biotechnology Reports*, vol. 21, 2019.
- [82] Z. Yuan, L. Wenyan, Liu. Lijun, Li. Feizhen, F. Qianlong, S. Xiaoli, “Harvesting *Chlorella vulgaris* by magnetic flocculation using Fe₃O₄ coating with poly aluminium chloride and polyacrylamide,” *Bio resource Technology*, vol. 198, pp. 789-796, 2015.
- [83] N. Theoneste, C. Jingyu, X. Zongyuan, S. Wenyao, Z. Xianhai, N. I-Son et al, “Flotation: A promising microalgae harvesting and dewatering technology for biofuels production,” *Biotechnology Journal*, vol. 11, pp. 315-326, 2016.
- [84] K. Alexandra, G. Kristina, “Microalgae Harvesting: A Review,” vol. 27 No 44, pp. 129-143, 2019.
- [85] S. Jung Y, K. Minjeong G. L. Kyubock, L. Young-Chul, N. Jeong-Goel, J. Sang Goo, P. Seung B et al, “Multifunctional Nanoparticle Applications to Micro algal Bio refinery,” *Nanotechnology for Bioenergy and Biofuel Production*, pp.59-87, 2017.

- [86] B. Supriya, D. Krutika, "Harvesting Chlorella species using magnetic iron oxide nanoparticles," *Phycological Research*, vol. 67, pp. 128-133, 2018.
- [87] F-G. Paul, K. Peter, B. Markus, S. Sebastian, B. Sonja, "Bare Iron Oxide Nanoparticles for Magnetic Harvesting of Microalgae: From Interaction Behaviour to Process Realization," *Nanomaterials*, vol. 8(5), pp. 292, 2018.
- [88] W. Shi-Kai, S. Amanda R, G. Chen, L. Chun-Zhao, "Harvesting microalgae by magnetic separation: A review," *Algal Research*, vol. 9, pp. 178-185, 2015
- [89] M.Thangavel, M. Nirupama, "Comprehensive review on harvesting of microalgae for biodiesel – Key challenges and future directions," *Renewable and Sustainable Energy Reviews*, vol. 91, pp.1103-1120, 2018.
- [90] Z. Jiao, R. Junfeng, W. Qiang, " Mixotrophic cultivation, a preferable microalgae cultivation mode for biomass/bioenergy production, and bioremediation, advances and prospect," *International Journal of Hydrogen Energy*, vol. 42, pp. 8505-8517, 2016.
- [91] G. Ramya, Manigandan. S, S. Melvin S, S. Rajshree, B. Kathirvel, C. Nguyen T.L et al, "A review on prospective production of biofuel from microalgae," *Biotechnology Reports*, vol. 27, 2020.
- [92] C. Hui, W. Xinyang, Wang. Qiang, "Micro algal biofuels in China: The past, progress and prospects," *GCB Bioenergy*, vol. 12, 1044-1065, 2020.
- [93] S. Poonam, S. Nivedita, " Industrial and Biotechnological Applications of Algae: A Review," *Journal of Advances in Plant Biology*, vol. 1, pp. 1-25, 2017.
- [94] G. Mario, W. Qiang, "Microalgae for Industrial Purposes," In: Vaz Jr. S. (eds) *Biomass and Green Chemistry*, Springer, Cham, pp. 133-167, 2018.
- [95] C. Hui, W. Xinyang, W. Qiang, " Microalgal biofuels in china: The past, progress and prospects," *GCB Bioenergy* , vol. 12, pp 1044-1056, 2020.
- [96] A. Maria Bueno, C. Thalita Marcilio, J. Ana L.M, V. Maria V.R, de C. Joao C.M, B. Andre Rolim, "Cosmetic attributes of algae - A review," *Algal Research*, vol. 25, pp. 483-487, 2017.
- [97] Fukuda. H, Kondo. A, and Noda. H, "Biodiesel fuel production by transesterification of oils," pp. 405-16, 2001.
- [98] M. Fanguri and H. Milford A, "Biodiesel production: a review," vol. 70 Issue 1, pp. 1-15, 1999.
- [99] B. Anirban, S. Rohit, C. Yusuf, and Banerjee. U. C, "A renewable source of hydrocarbons and other chemicals," 245-79, 2002.
- [100] Y. Yeoung-Sang, L. Sun Bok, P. Jong Moon, L. Choong-II, Y. Ji-Won, "Carbon dioxide fixation by algal cultivation using wastewater nutrients," *J. Chem. Technol. Biotechnol*, vol. 69, pp. 451-515, 1999.
- [101] T. Nguyen T.P, N. Thanh T, & T. Bui Xuan, "Biodiesel Production from Microalgae by Extraction – Transesterification Method," *Waste Technology*, Vol. 1(1), pp. 6-9, 2013
- [102] G. J Van, Shanks. B, and Pruszko. R, " Biodiesel Analytical Methods," NREL/SR, 510-36240, july 2004.
- [103] G. Aachal, J. PROF. J. P, "Biofuel From Algae: A Sustainable Third-Generation Biofuel," *IRE Journals*, vol .5 Issue 7, Jan 2022.
- [104] <http://delhiplanning.nic.in/Economic%20Survey/Ecosur2003-04/ch12tablefinal2.pdf>
- [105] B. Laura L, B. Eric S, P. John W, and P. Matthew C, "Engineering algae for biohydrogen and biofuels production," vol. 20 Issue 3, pp. 264-271, 2009.
- [106] M. Amanda Leigh, "Gold rush for algae," *Nature*, vol. 461, pp. 460–461, 2009.
- [107] S. Evan, Ross Ian L, K. Zachary, M. Jan H, K. Olaf, P. Clemens et al, "An economic and technical evaluation of microalgal biofuels," *Nat Biotechnol*, vol. 28, pp. 126–128, 2010.
- [108] C. Ana P, M. Luis A, M. F Xavier, "Microalgal reactors: a review of enclosed system designs and performances," *Biotechnol Prog.*, vol. 22, pp. 1490–1506, 2006.