**An Overview of Algae Biodiesel**

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**Abstract**

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**The main energy sources include petroleum, natural gas, coal, hydroelectricity, and nuclear energy. Due to the growing population and industrialization, there is an increasing demand for energy. However, the use of fossil fuels like petroleum is unsustainable due to supply depletion and their contribution to carbon dioxide emissions, causing global warming. Research has focused on using biofuels to reduce greenhouse gas emissions. Macroalgae are particularly promising as they have a high 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 production, such as lipids and carbohydrates, affects the quality of biofuels. Stress conditions, like light or salt 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)

1. **INTRODUCTION**

Petroleum, natural gas, coal, hydroelectricity, and nuclear energy are the main energy sources. Due to the growing population and industrialization, there is a constant rise in the demand for energy. The continued use of fuels derived from petroleum is now widely acknowledged as unsustainable due to the depletion of supply and their contribution to the environmental accumulation of carbon dioxide, which contributes to global warming. Numerous studies on the use of biofuels to replace fossil fuels and reduce greenhouse gas emissions have been conducted in the past ten years [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 CO2, water, and light 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 lipids, carbohydrates, 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, the biomass has a significant influence because it can change depending on metabolic capabilities, growing methods, and abiotic factors like light and nutrient delivery [14].

For this investigation, we used a one-stage method with balanced nutrient limitation in which nutrients were supplied under appropriate circumstances to promote biomass accumulation while recovering all macronutrients from the medium. The algal strains were researched under nutrient-depleted 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]. By using downstream methods designed to extract the bioproducts, microalgae growth in photobioreactors can be accelerated for higher biomass production [16].Stress is the result of a stressor's application that disturbs equilibrium. The metabolic adjustments made by cells as they adapt and work to reestablish equilibrium are known as stress responses. The alarming stage, regulatory stage, acclimation stage, and adaptation stage are the various stress reaction stages [17]. Stress strategies, utilizing a single stress factor, such as nutritional factors (e.g., carbon source, nitrogen, phosphorus), environmental factors (high light intensities, temperature, pH, salinity, reactor configurations, and operating conditions), have been adopted to improve the production of high-value compounds [18]. Salt stress brings about numerous biochemical and bioenergetic changes, including increased rates of lipid biosynthesis, increased biopolymer and energy production, changes in membrane permeability with disruption of ion homeostasis, and increased levels of reactive oxygen species (ROS). Microalgae may build up antioxidant substances, including polyphenols, flavonoids, and carotenoids, in response to the elevated ROS levels to inhibit the free radicals. Nitrogen-limiting circumstances can cause lipid synthesis in some algae species by slowing cell division and changing the lipid biosynthetic pathways to produce more neutral lipids than membrane lipids [19]. Microalgae's ability to produce biomass and lipids can also be significantly influenced or controlled by light of the right wavelengths and intensity [20]. Light intensity changes the types and concentrations of secondary metabolites such as phenolics and flavonoids, which in turn affect antioxidant activity. There is a direct relationship between the antioxidant activity and the total phenolic and flavonoid contents [16]. Therefore, an appropriate farming strategy can be used to increase biomass output with high-value products.

Numerous microalgae have the capacity to manufacture triacylglycerols (TAG) as a stored lipid at levels of up to 50% of their dry cell weight when exposed to photo oxidative stress or other unfavorable environmental circumstances [21]. Algae have attracted much more interest as prospective feedstocks for biodiesel because of recent research [22] that demonstrates their inherent advantages, such as rapid biomass growth, high lipid content, and tolerance for harsh environments. One of the potential benefits of the development and use of algae-derived biofuels is the higher production efficiency of the necessary oils compared to other fuel crops [23]. To produce biodiesel, a microalgae candidate must not only have high lipid productivity but also have an appropriate fatty acid (FA) composition, as these properties—such as kinematic viscosity, specific gravity, cetane number (CN), cloud point, iodine value (IV), long-chain saturated factor (LCFF), and cold filter plugging point (CFPP) can be greatly influenced by the FA composition. However, there are still gaps in research and development, legislation, and strategies at every level of the biofuel production chain to fully utilize algal bioresources [24]. Since it's crucial to quickly quantify the components of the biomass, we set out to create a set of processes to identify algal biomass components in a way that can be used in a variety of laboratories [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].

**A**. **Based on habitat**

Seven kinds of algae are classified based on their environment.

(a) Aquatic, free-floating, or totally submerged algae are known as hydrophilus algae.

(b) 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 *Botryduium*,
* cryptyophytes, like *Nostoc* and

(c) 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: Epiphyllophytes (such as *Trentepohlia*), Epiphloephytes, Epizoophytes (such as *Chaetophorales*) and Lithophytes (such as *Sctonema, Vaucheria*, and *Nostoc*).

(d) Cryophytic algae: Cryophytes, or cryophytic algae, such as *Chlamydomonas*, *Ankistrodesmus*, and *Mesotaenium*, are algae that live on ice and snow.

(e) 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*.

(f) Endozoic algae are those that reside inside the bodies of animals. Examples include: inside freshwater sponges; inside Hydra.

(g) Parasites: Algae, such as *Cephaleuros virescens*, are parasites that live on other plants.

1. **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 of 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.

The three most well-known types of algae are green algae, red algae, and brown algae (Chromista), with green algae having some of the most complicated forms. The higher land plants eventually descend from this branch (green algae). It is generally accepted that the existence of reproductive organs with protective cell layers, a trait not found in other algal species, marks the boundary between these non-algal plants and algae.

1. **Based on metabolism**

(a) Photoautotrophically, that is, relying only on photosynthesis to transform light energy into chemical energy.

(b) Heterotrophically, that is, solely using organic compounds as a source of carbon and energy since they depend on complex organic components for sustenance and feed on other species to get their food.

(c) Mixotrophically, in which photosynthesis is used as the primary energy source despite the fact that both organic molecules and CO2 are necessary [27].

1. **Based on cellular organisation**

Instead of reflecting their biologically ordered evolutionary links, the term "algae" is used to refer to a set of eight divisions of distantly related organisms [28]. Microalgae are unicellular, while macroalgae are multicellular; the former are often referred to as microphytes, while the latter are seaweeds [29].

1) Microalgae (microphytes) lack roots, stems, and leaves; they may reach heights of several hundred micrometres and are found in both freshwater and saltwater environments.

2) Macroalgae, on the other hand, possess a body-like structure and can be found around sea beds growing up to hundreds of metres. Their structures are primarily for storing and converting energy without any development beyond their cells, and the simplicity of their growth and development has made them more sustainable than any other renewable source [30].

**III. Biofuel production from algal biomass**

Microalgae are typically unicellular photosynthetic microorganisms that can store CO2 and use it to produce energy-dense molecules such as fatty acids and starch [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 soybean and palm oil. 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 production of biofuel from microalgae involves several stages, including microalgae cultivation, recovery or harvesting, and downstream processing to separate the metabolites from the biomass [33].

**A. 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 discernible 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].

**B. Isolation**

Standard plating techniques were employed to isolate single microalgal species from the field water samples. The colonies were isolated using several medium formulations (Guillard’s f/2 medium, Bold basal medium with vitamins). To facilitate the isolation procedure, the field samples were first diluted. These diluted samples were placed on sterile plastic petri dishes (100 x 15 mm) with around 40 ml of agarized media. One milliliter of the diluted sample was uniformly distributed over the entire surface of the media plate. The algae were allowed to develop for approximately 14 days in the lab on culture racks. To isolate them, grown algal cultures were streaked onto new sets of nutrient medium plates using sterile procedures. This streaking technique was repeated until the desired unialgal cultures were achieved [35].

**C. 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. Nutrients and Growth Inputs 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, CO2, and water 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 large-scale production of microalgae [38]. The amount of micro and macronutrients required varies for each algal growth medium, and studies highlight the importance of chemical elements, including N, K, Ca, Cu, Fe, Mg, Mn, P, S, and Zn, for microalgae development in the form of salts [39,40]. The choice of growth medium is crucial and depends on the chemical makeup of the media, which influences the growth of biomass. The most commonly used growth media for cultivating microalgae are Bold’s basal medium (BBM), acidified Bold’s basal medium, Chu10 media, BG (Blue-green) 11 medium, and modified Hoagland's medium [41].

**A. Bold’s Basal Medium (BBM)**

Bold's basal media is made by mixing 10 ml of each stock solution from Table 1, (items 1-6) and 1 ml of items 7–10 with 1 L of distilled water in a volumetric flask.

**Table:- 1 Composition of Bold’s basal medium [41]**

|  |  |  |
| --- | --- | --- |
| **S. No** | **Stocks of chemicals** | **g/L** |
| **1** | **NaNO3** | **25.00** |
| **2** | **KH2PO4** | **17.50** |
| **3** | **MgSO4. 7H2O** | **7.50** |
| **4** | **K2HPO4** | **7.50** |
| **5** | **NaCl** | **2.50** |
| **6** | **CaCl2. 2H2O** | **2.50** |
| **7** | **Trace elements:** |  |
|  | * **ZnSO4 . 7H2O** | **4.42** |
|  | * **CuSO4 . 5H2O** | **1.57** |
|  | * **MnCl2 . 4H2O** | **1.44** |
|  | * **Co (NO3)2 . 6H2O** | **0.49** |
|  | * **MoO3** | **0.71** |
| **8** | **H3BO3** | **11.40** |
| **9** | **EDTA and KOH solution:** |  |
|  | **EDTA Na2** | **50.00** |
|  | **KOH** | **30.00** |
| **10** | **FeSO4 7 H2O with 1.0 mL concentrated H2SO4** | **4.98** |

**C. 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.[42].

**V. Cultivation of algae**

Algal cultivation requires water, carbon dioxide, and sunshine in addition to nutrients including phosphorus, nitrogen, potassium, zinc, and calcium in order to produce biomass through photosynthesis, which transforms solar energy into chemical energy stored in the microalgal cells. Based on the specific circumstances needed for development, four primary types of algal culture methods have been identified. They are photoautotrophic, heterotrophic, photoheterotrophic, and mixotrophic cultures [43]. Microalgae may drive photoautotrophy and heterotrophy in the mixotrophic mode of culture, and they can equally utilise organic and inorganic sources of carbon. In the photoautotrophic mode, processed microalgae use light as an energy source and inorganic carbon as a carbon source to carry out the process of photosynthesis, which produces chemical energy. The productivity of biomass could be slightly increased in a CO2-rich environment [44]. In addition to photoassimilation, photoheterotrophic culture also referred to as photometabolism, is a kind of cultivation that needs light in order to use organic molecules as a source of carbon [45]. In order to stimulate algae development, heterotrophic cultures use organic carbon sources as a source of energy [46].

Because it is suitable for producing huge amounts of algal biomass, photoautotrophic production is one of the aforementioned methods that is most frequently utilised. There are three basic cultivation methods for producing algae: open, closed, and hybrid systems. The hybrid systems are a combination of open and closed systems specifically designed for high productivity in terms of biomass generation, while the open systems are more cost-effective and the closed systems are more efficient in removing nutrients [47].

**A. Open pond systems**

The raceway pond, the closed pond, the shallow huge pond, and the circular pond tank are the most often used research and industrialised algal culture strategies [48]. By using a greenhouse, the cultures grown in open ponds may be protected from adverse ecological conditions (rainfall, temperature, and brightness). Microalgae that flourish in difficult conditions, such as a basic medium or one that is excessively salty, should be allowed in order to produce axenic cultures [49]. Open ponds constructed in a wastewater treatment facility can either be circular or gravity-driven [50]. The most fundamental standard for open systems is the position of a pond. The location should be chosen to provide the appropriate sunshine and to have all the conditions the algal strains demand. Although these culture systems often lack a stirring unit, which results in poor mixing, they nonetheless make it possible to manage and monitor the culture process in the easiest and most cost-effective ways possible.

The natural pond is often just about half a metre deep, which allows light to enter the water and be absorbed by many algae cells. In these kinds of open systems, a variety of algae strains—most notably *Dunaliella salina*—can be grown for profitable purposes [51]. An extended spinning arm that performs the function of a paddlewheel and acts like a clock dial is placed in the centre of the pond. This arm's construction is similar to that of a raceway pond. Algae cells and culture media mixed together are more effective than a pool that hasn't been stirred; however, when the algae are exposed to the environment, contamination is inevitable. The effeciencies in circular ponds range between 8.5 and 21 g/(m2 d) [52].

Since the 1950s, raceway ponds have been used to cultivate algae. They were initially employed for the Spirulina cultures. They might be made up of an oval channel or a racecourse channel. Typically, a concrete slab is used to build them [53]. Raceway ponds offer recirculation of algal culture coupled with a constant supply of nutrients and carbon dioxide. They are equipped with a paddle wheel to provide gentle mixing that prevents sedimentation. An aerator may be used to increase the air flow rate, which will increase carbon dioxide utilisation [54].

Open ponds' effectiveness is in doubt, despite the low cost of their creation and maintenance. As there is a greater demand for land, a greater danger of contamination, and limitations because of the weather and light intensity, an open system is challenging to monitor.

The main drawbacks of an open-air system are its sensitivity to the season, time of year, and weather. Open pond systems have some limitations that make it impossible to utilise them, such as the impossibility to produce monocultures, because a variety of native algae and algae grazing contribute to pollution. The temperature of the pond is often out of control, and the amount of light depends on the timing of the solar insolation. As a result, the efficiency of the open ponds depends on the natural variations in daytime temperature and sun insolation. While the cooling brought on by the evaporative process helps to some extent regulate the temperatures of open ponds, it also indicates significant water loss [55].

**B. Horizontal tubular photobioreactor**

Algal growth is frequently carried out in horizontal tubular photobioreactors (HTB) at the commercial level. This type of bioreactor consists of a long arrangement of tubes made of transparent silicone, glass, or plastic.To increase the area for light penetration, these tubes are positioned horizontally and kept at a modest diameter. The tubular photobioreactor is a suitable option for growing algae because of its enormous lighting area. A centrifugal pump or airlift technique called quantum fracturing can be used to circulate algae cells in tubular PBR. Modern strategies for designing tubular PBRs have been developed to provide a thin layer of culture suspension that is free from contamination as well as exceptional exposure to light and low energy requirements. However, a smaller surface-area-to-volume ratio and less light might be the outcome of a larger tube diameter. A second potential impact of the wider tube is that algae cells at different levels inside the tube may get inconsistent amounts of solar radiation. The capacity of an algal strain to synthesise photons may be hampered by longer tubes due to an accumulation of oxygen. These obstacles might make it more difficult for the tubular photobioreactor to scale up. Another problem is that controlling temperature in tubular photobioreactors is difficult. Cooling tubes and thermostats are both usable, but their installation costs are high. Scaling up HTB is possible by stacking the tubes on top of one another or by employing coil tubes.

**C. Vertical column photobioreactor**

This type of bioreactor is constructed of vertically positioned glass or acrylic 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 effective mixing, mass transfer of carbon dioxide, and oxygen removal. A vertical column photobioreactor typically does not have a physical agitation mechanism. Based on arrays of liquid flow, vertical PBRs may be divided into bubble columns and airlift reactors [56].

**D. Bubble column reactors**

The height of a bubble column reactor is greater than two times that of its vessel diameter. These are affordable and offer a large surface area for illumination. Since an effective sparger is employed for mass transfer and mixing, these types of reactors don't require any moving parts. The design of the sparger has a considerable impact on how the photobioreactor is implemented. Usually, aggregated bubbles are broken apart and distributed using perforated plates as spargers. There is light coming from outside, by moving from the lower to the upper light zones, this liquid circulation generates a variable gas flow rate, which is crucial for photosynthetic efficiency.However, it appears that bubble size is equally important for minimising shear damage to cells [57]. Some bubble column PBRs are equipped with a rubber membrane difuser or two spargers to increase the mass transfer of gases, including the availability of carbon dioxide and the removal of oxygen. This is done because of the high mass transfer, cheap energy costs, and extraordinarily low physical stress. The efficiency of CO2 transfer is increased four times when twin spargers are used as opposed to standard sparging. According to the performance of the membrane diffuser, the slits of the membrane behave more like holes with elastic caps that serve as valves to keep bubbles from entering the gas stream. The membrane diffuser performs as a one-way valve to stop liquid backflow [58].

In airlift reactor, the gas mixture flows within the riser and the down comer as it rises to the surface from the sparger in the first cylinder, which is known as the gas riser. The second cylinder, known as the down comer, is where the medium flows down towards the base. It is critical to take into account the fact that the length of time a gas stays in a certain zone impacts gas-liquid mass transfer, heat transfer, mixing, and turbulence. The main disadvantage of using these vertical bubble column PBRs is that some algae strains, like *S*. *costatum* and *C*. *muelleri*, have been proven to experience shear stress in algal tubes, and some algal cells are unable to survive due to the pressure provided by the pumps [59]. Recently, the efficiency of a novel zigzag-fow column photobioreactor (ZZ-fow PBR) used to cultivate A. platensis with high biomass was assessed. Four enhanced zigzag beam structures were installed over the outer (riser) part of the ZZ-fow PBR. In compared to traditional column PBR, the rate of intracellular photosynthesis and electron transport was accelerated, improving biomass production and CO2 fixation [60].

**E. Helical-type photobioreactor**

A degassing device that may be detached from and reattach to the transparent, flexible tube that is made up of helical PBRs. The culture is transported via a long tube to the degassing device by a centrifugal pump. The energy needed by the centrifugal pump recirculating the culture and the resulting shear stress limit the practical usage of this type of photobioreactor, which may be scaled up by simply adding a light-harvesting device. Another issue with the technique is inner reactor pollution [61].A conical helical reactor was created by giving the helical PBR a cone form with a cone angle of 600. The height and angle of the conical helical system are carefully chosen. The conical helical reactor was made by coiling polyvinyl chloride tubing into a conical structure. An air pump was used to circulate the liquid. In addition, this system has a heat exchanger for controlling temperature and a degassing system. When using 60°, the photoreceptor area and hence photosynthetic productivity increase by a factor of two. The photosynthetic efficiency for this reactor was 6.84 %, which was the highest of all cone angles examined. The higher light-gathering efficiency while preserving the same basal area is the major advantage of the cone shape. This kind of reactor also has the advantage of using less energy and exerting less mechanical strain on algae cells. The only way to scale larger due to its defined angle and size is by adding more light-collecting units; however, this causes additional energy loss in the complicated branches of flow networks [62].The downsides of this type of reactor are little gas exchange, high shear stress, the accumulation of biomass in the tubes, and the high energy input [63].

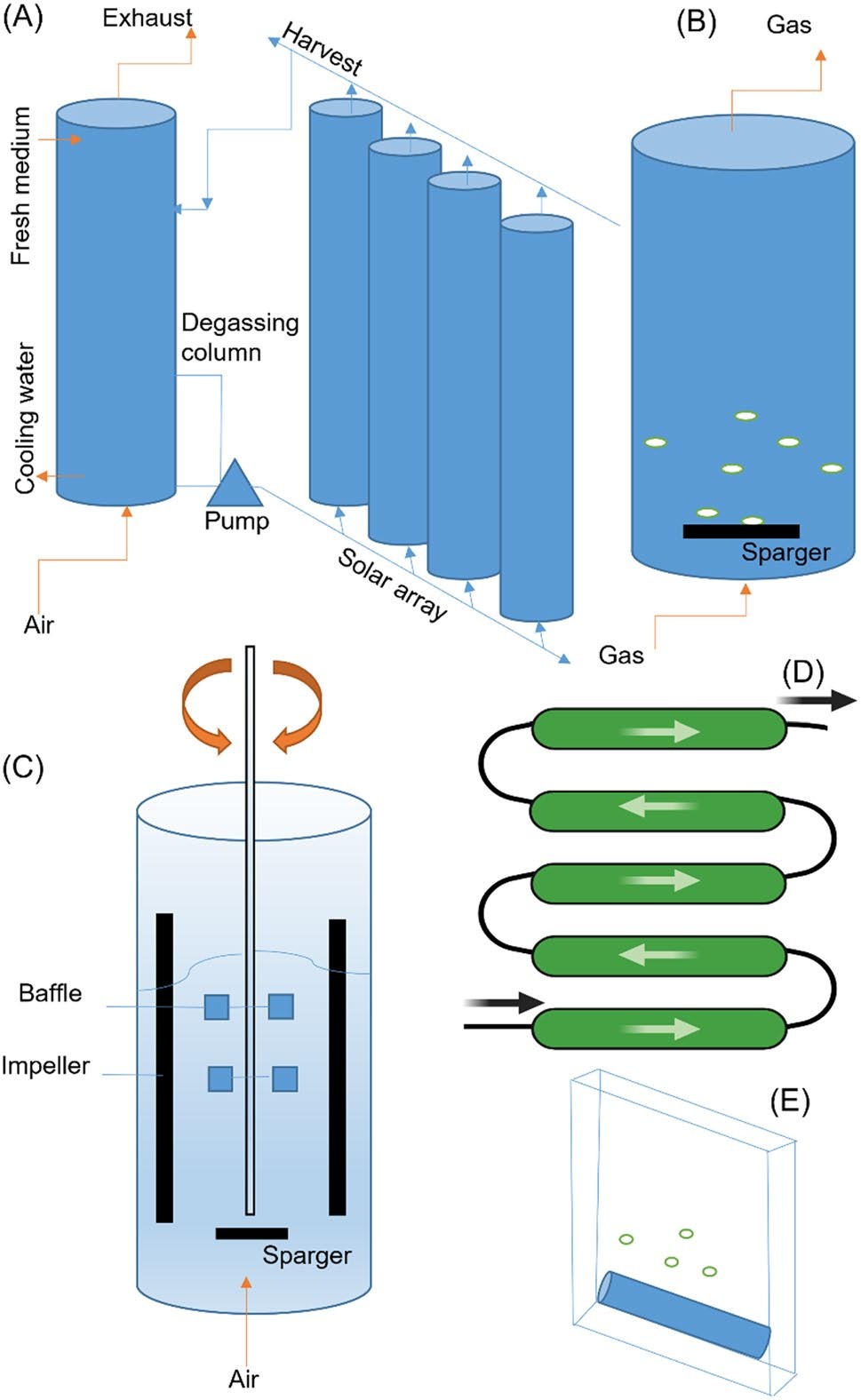
**F. Stirred tank photobioreactor (STR)**

The most useful type of reactor is the stirred tank reactor, which generates mechanical agitation with impellers of various sizes and shapes. With the use of baffles, the vortex is diminished. At the bottom, CO2-enhanced air is bubbled to give algae with a carbon source for growth. This type of bioreactor has to be externally illuminated using fluorescent or optical fibres in order to be transformed into a photobioreactor. A significant disengagement zone separates the unemployed sparged gas and oxygen produced during photosynthesis from the gassed liquid to the gas phase. Since stirred tank reactors are a standard, in industry and laboratories, they were initially proposed as a technique to generate microalgae photoautotrophically using artificial light or sunshine. A Hydraulically Integrated Serial Turbidostat Algal Reactor (HISTAR) system with a total capacity of 3.6 cubic metres was used to grow *Selenastrum* *capricornutum*. HISTAR (CFSTRs) was made up of two closed turbidostats and a series of open hydraulically connected continuous flow stirred tank reactors. The CFSTRs increased the biomass of the injected culture. Although there have been no reports of its deployment in the future, this technology has recently been used in the direction of developing a deterministic system to predict microalgal yield in order to assess its practical viability for broad application [64]. The system's tiny surface-to-volume ratio, which lowers its effectiveness at gathering light, is its main flaw.

It has also been tried to use optical fibres for lighting, however this has the drawback of obscuring the mixing pattern [65].

**G. 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].

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**Fig 1:- Different configurations for photobioreactor, A tubular photobioreactor, B bubble column photobioreactor, C flat panel photobioreactor, D helical tubular photobioreactor, E a simply stirred tank photobioreactor [47]**

**VI. Factors affecting algal cultivation and biofuel production**

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

**A. 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 feedstock 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].

**B. 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].

**C. Carbon dioxide**

Some of the major sources of carbon dioxide are the atmosphere, petrol emissions from industrial exhausts, and soluble carbonates [70].. Higher algal lipid content cannot be produced without more carbon dioxide being present [71]. Literature from various genres offers a variety of evidence regarding how carbon dioxide affects lipid synthesis. It has been noted that fatty acid synthesis is boosted by a larger quantity of carbon dioxide, whereas the development of algae is enhanced and the process of fatty acid synthesis is inhibited by a lower concentration of carbon dioxide. However, the carbon cycle will be significantly impacted if carbon dioxide concentrations rise. One study on Chlorella pyrenoidosa SJTU-2 and Scenedesmus obliquus SJTU-3 shown that the growth was improved at 10% carbon dioxide. However, when 30–50% of CO2 was used, the production of fatty acids and the accumulation of lipids both increased [72]..

**D. 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].

**E. 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 is the isolation of or separation from the algae's growing medium. The physiognomy of the chosen microalgae, the size and density of the microalgae cell, and the features of the final product, all play a significant role in the harvesting process. One of the crucial steps in the growth of microalgae is microalgae harvesting. It has a high energy need, which contributes for 20–30% of the entire production cost, according certain research [75]. Numerous mechanical, chemical, biological, and electrical methods, such as filtration, centrifugation, flocculation, and flotation, have been used to collect biomass [76]. To boost harvesting yield, it may occasionally be necessary to combine two or more techniques.

**A. 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 pore 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. Considering this obstacle, the filter membrane was constructed utilising inexpensive and easily accessible materials. Bejor et al. therefore succeeded in fabricating a stretch cotton filter membrane with a 66–93% harvesting efficiency.

**B. Centrifugation**

Based on the portion's density and particle size, 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 disc stack centrifuges, imperforated basket centrifuges, decanters, and hydro-cyclones, have been employed by the industry [76].

**C. 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 aluminium sulphate and iron chloride may eliminate around 95% of the microalgae biomass. But because of their high toxicity, the compounds are not environmentally friendly and need to be removed through additional processing steps that raise the cost of manufacture [79]. Bio-flocculants, as opposed to their chemical equivalents, are safer and more ecologically friendly. They are also economical to utilise, and often no pre-treatment is needed before reusing culture medium and processing microalgae further downstream [80]. The majority of bioflocculants utilised are biopolymers, such as acrylic acid and chitosan, which may 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].

**D. Flotation**

Flotation makes use of small bubbles that bond to microalgae cells to make it easier for cells to float on the surface of the culture fluid, enabling straightforward collection. The advantages of the flotation approach include comparably better harvesting performance, an easy maintenance regimen, and increased processing throughput at a low cost [83]. There are three main categories of flotation models, each of which employs a unique process to create air bubbles. By overpressurizing the culture with gas and then releasing it at room pressure, the dissolved air flotation technique produces air bubbles. This method has often been used to treat wastewater, but it is limited by the high costs caused by the use of resources and chemicals. Dispersed air flotation, in contrast, employs a sparger to create air bubbles, which thus requires less energy than dissolved air flotation. The third method is electro-flotation, which uses electrolysis to create microbubbles from its electrode to catch free-floating microalgae. This method allows for simultaneous cell disruption activity in addition to harvesting, when alternating current is used, but because of fouling, the comparable device consumes a lot of energy, and replacing the electrodes on a regular basis raises the cost of production.

**E. 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 rapid, automated, 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 bare (naked), coated, and surface-modified [86].

**a.) 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].

**b.) Coated and surface-modified nanoparticles**

A microorganism likes to bind to solid substrates in general to reduce free interfacial energy. Throughout algal attachment to magnetic particles in an aqueous environment, the whole amount of interactions, including non-covalent Lifshitz van der Waals forces, electrostatic forces, and acid-base interactions, must be determined. In order to prevent harvest suppression, magnetic nanoparticles must be changed. Due to the negative surface charges that cationic polymers have on the majority of microalgae, they are mostly used as coating chemicals [89].

Algae removal via magnetic separation was first documented approximately forty years ago. It can recover more than 90% of the cells during microalgae harvesting procedures in less than 5 minutes. By combining flocculation and magnetic separation into one process, magnetic harvesting offers advantages including speed, quickness, energy efficiency, and reduced cost. Additionally, the external magnetic field enables the evacuation of a greater volume of bulk liquid in a shorter amount of time as well as the concentration of magnetically converted cells into a compact slurry. Both naked and surface-modified magnetic nanoparticles are more effective in magnetic algae harvesting, but further study is needed to establish their cost-efficiency in commercial applications [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 2:- Advantages, drawbacks, and cost involvement of various methods of processing of micro-algae [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 toxic | High cost due to the cumulative expenses incurred by the use of solvent and 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 O2 is inhibitory. | Economically feasible. |
| Indirect bio photolysis | Blue-green algae can produce hydrogen from water and able to fix N2 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 specificity/selectivity for better efficiency. Cost intensive | High cost |
| Pulsed Electric FieldAssisted 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**

Currently, the globe is considering the development of bioenergy from algal biomass due to global warming and the depletion of biofuels. Other essential strategies to reduce poverty include expanding access and safeguarding energy. Creating biofuels from algal biomass is the only option now available to replace the usage and dependency on fossil fuels. Micro-algal biomass may be used to create a variety of biofuels, including biodiesel, bio-oil, bio-methane, bio-hydrogen, bio-gas, and bioethanol [55].

**A. 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].

**B. Bio-hydrogen**

Following are the three recommended processes for making hydrogen: Direct photolysis 1. 3. Direct photolysis 3. A path that is ATP-powered. Direct photolysis is only possible provided the hydrogen and oxygen created are continually eliminated. Here, the simultaneous production of hydrogen and oxygen raises safety concerns and raises the price of separating the two gases. This is because photosynthesis and water splitting are connected, which results in simultaneous hydrogen and oxygen formation. Additionally highly oxygen-sensitive, the hydrogenase enzyme utilised in the method. Indirect methods are furthermore preferred for these reasons. In anaerobic and sulphur-limited circumstances, the starch present in algal cell walls is largely transformed to 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].

**C. Bioetanol**

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].

**D.** **Biodiesel**

The cost of producing biodiesel is significantly influenced by the type of raw material utilised, which accounts for between 50 and 85% of the fuel's final cost. To create biodiesel that is cost-effective, it is crucial to evaluate the feedstock in terms of its effectiveness, quality, and potential for by-product utilisation. The process of transesterification turns lipids, chiefly triacylglycerols and free fatty acids, from raw materials into non-toxic, ecologically beneficial biodiesel. Fatty acid alkyl esters, which have a lower molecular weight, are produced from the crude algal oil with a high viscosity. During the transesterification procedure, crude oil and alcohol react in the presence of a catalyst; ideally, methanol and methyl esters of fatty acids (FAME) are formed as a byproduct along with glycerol. Although using acid catalysts has been found to be favourable, because of their speedy development, alkali catalysts are used in the commercial sector up to 400 times more often than acid catalysts.

A number of studies have focused on two significant species, *Chlorella* *vulgaris* and *Chlorella* *protothecoides*, for the production of biodiesel since they have a greater oil content [93]. The main component of microalgal biodiesel is unsaturated fatty acids. The algal biomass from wastewater contains a variety of different algae, and as a result, various fatty acid profiles can be produced. The economic viability of developing microalgal-based biofuels depends on the regulation of both biomass and lipid content. Creating effective processes for removing microalgal biomass from the culture broth is another significant problem in the production of biofuels. Researchers have focused on microalgae harvesting methods such as magnetic separation technology, pH-induced flocculation, and ultrafiltration in an effort to further lower the cost of microalgae harvesting and the manufacture of biodiesel [95].

Microalgae appear to be a promising renewable resource that might be used for a variety of industrial uses, such as the production of biofuels, human food, animal feed, cosmetics, medicines, and aquaculture [96]. Additionally, microalgae are an excellent source of natural compounds that are involved in a number of bioactivities.

**a.) Transesterification of algae oil for biodiesel production**

The process 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 fat or oil reacts reversibly with an alcohol to produce fatty acid alkyl ester and glycerol 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:-

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| CH2-COOR1  CH-COOR2 CH2-COOR3 | + | CH3-OH | Catalyst | CH2-OH  CH-COOR2 CH2-COOR3 |  | + | CH3-COOR1 |
| Triglyceride |  | Methanol |  | Diglyceride |  |  | Methyl ester |
| (Parental oil) |  | (Alcohol) |  |  |  |  | (Biodiesel) |
| **Step 2:-** |  |  |  |  |  |  |  |
| CH2-OH CH-COOR2 CH2-COOR3  Diglyceride | + | CH3-OH  Methanol | Catalyst | CH2-OH CH-COOR2 CH2-OH  Monoglyceride |  | + | CH3-COOR2  Methyl ester |
|  |  |  |  |  |  |  | (Biodiesel) |
| **Step 3:-** |  |  |  |  |  |  |  |
| CH2-OH CH-COOR2 CH2-OH  Monoglyceride | + | CH3-OH  Methanol | Catalyst | CH2-OH CH-OH CH2-OH  Glycerol | + |  | CH3-COOR3  Methyl ester |
|  |  |  |  |  |  |  | (Biodiesel) |

**Fig 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.

**b.) Steps followed in transesterification reaction**

Either wet biomass (10 g dry weight equivalent) or freeze-dried (10 g) algal biomass would be placed inside the glass vial. The glass was subsequently treated with n-hexane, an organic solvent, and methanol. After blending the mixture for 5 minutes, the mixture will be transferred into centrifuge tubes. Following that, distilled water and solvent will used to wash the glass twice. The tube also received these combinations. The mixture was then centrifuged at 4,000 rpm for 15 minutes. Organic material that contains algae oil will be taken out and placed in a glass vial that has previously been weighed. The algal oil will be heated for 5 minutes at 75 °C in order to evaporate the water content. The reaction mixture will be mixed for 35 minutes at a temperature of 75 °C with the algal oil, methanol, sulfuric acid, and solvent (n-hexane). After the reaction is complete, the samples will be cooled at room temperature, and the crude ester layer (the top phase) is separated from the glycerol layer using a separating funnel. The raw ester layer contained methyl ester, perhaps non-reactive oil, methanol, and glycerol. To separate the methanol, the organic layer will be washed twice with distilled water in a separating funnel until the washings become neutral. The FAMEs layer will be dried using a NaCl solution. The top phase containing the FAMEs will be poured into a glass test tube that has been previously weighed. The solvent will be evaporated using a Rotary Evaporator at 320 mbar and 36 °C. Then, n-hexane will be taken out, and the FAME content of the crude biodiesel fuel will be analysed using a gas chromatograph [101].

**X. Quality analysis of biodiesel**

**A. 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 190⁰c where in the injector and detector temperatures were 230⁰C and 240⁰C, respectively. FAME was identified by comparison of their retention times with those of the reference standards.

**B. 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:

1. Select the proper biodiesel sample size (using a prescribed methodology).

2. Weigh the material into a 250 ml beaker to the closest 0.1 mg.

3. Include 110 cc of the 90/10 blend.

4-6 drops of phenolphthalein indicator should be added.

5. Use a solution of 0.1 N alcohol, isopropanol, and KOH to titrate till the phenolphthalein end point (pink).

6. To check for errors, repeat the previous three times.

7. The acid number (AN) is calculated using the equation shown below:

AN= (VkoH-a) ∗ N∗56.1/W

Where:

VKOH = Volume of potassium hydroxide

a = Volume of potassium hydroxide for blank solution

N = Concentration of alcohol (isopropanol) KOH solution

W = mass of sample (g)

**C. Copper Strip Corrosion test**

Apparatus:- Hot plate, copper strips, beaker, and thermometer are the apparatus.

Chemicals: volatile sulfate-free hydrocarbon solvent and biodiesel.

Procedure:

1. Fill a beaker with biodiesel, then set it on a hot plate.

2. Heat to a constant temperature of 50 °C.

3. Insert a copper strip into the beaker.

4. After three hours, wash with acetone or another solvent.

5. Contrast the strip with the classification statistics for the Copper strip test that were received from NREL (2004).

6. Label the strip as shown in the table with a number and a letter.

7. To replicate the pH impact of fatty acids, add a little known amount of sulphuric acid to the biodiesel. Then, repeat the previous stages.

**D.** **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]

1. 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.

2. Add 2 ml of 1% isopropyl alcohol-based phenolphthalein indicator.

3. 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"

4. Include 1 ml of water with 0.4% bromophenol blue indicator.

At a pH of roughly 4.5, this indicator changes colour.

5. 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 developed a method for cultivating *Scenedesmus* *acutus*, a kind of green algae, and *Spirulina* *platensis*, a kind of blue-green algae, for Indian conditions. Spirulina is the most promising alga since it can be cultivated using low-tech techniques. The Indian Institute of Chemical Technology seeks to reduce the cost of producing oils derived from algae from INR 500 per litre to roughly INR 20. World Health Energy Holdings, Inc., an alternative energy company, has set aside $100 million to build a 250-acre commercial algae biodiesel plantation in Karnataka.  
Another research institute, the Dr. MGR Algae Biofuel Research Institute, has begun a biodiesel experiment utilising microalgae in Sivakas, one of Tamil Nadu's hottest and CO2-producing towns [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, they have attracted a lot of interest in 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.

Environmental issues are regularly discussed in public policy talks in India because of two factors: the energy-consuming sectors and the regions of the country where economic growth is occurring. The pace of urbanisation has been continuously increasing since 1971, despite the fact that a substantial majority of Indians—roughly 70%—live in 550,000 rural communities.

The megacities of Mumbai (12,57 million), Calcutta (10,92 million), Delhi (8,38 million), Chennai (5,36 million), and Bangalore (4,09 million) are among the 23 cities in India that today have at least a million citizens, up from 12 in 1981 [104]. As a result, Delhi saw an increase in the number of registered cars, from 841,000 in 1985 to around 3.5 million in 2001.

Due to India's rapid economic growth, there has been a major rise in air and water pollution, deforestation, water scarcity, and carbon emissions. Carbon emissions are rising as a result of the country's swift modernization, growing transportation sector, and widespread use of coal as fuel. India has experienced enormous increases in the use of nonrenewable energy sources as a result of its increasing urbanisation and industrialization, which might cause a serious scarcity of these fuels in the future. Therefore, the need for alternative energy sources is critical to preventing such situations. Recent research suggests that microalgae might be a source of renewable energy for India.

Making biodiesel in India has a number of extra advantages. Diesel fuel is marketed in India at government-subsidized rates to reduce transportation costs and increase GDP. A litre of petrol currently costs 2.5 times more than a litre of diesel fuel. In an effort to benefit from this price gap, Indian manufacturers have been heavily investing in the development of diesel vehicles. Because of this, a substantial number of today's automobiles run on diesel fuel and do not require the comparatively expensive retrofits required to run on compressed natural gas (CNG). The last factor that makes biodiesel production in India intriguing is the potential for cheap feedstocks to be grown. India has a natural advantage over other nations when it comes to the manufacture of algal biodiesel because of its tropical environment, which is suitable for growing a variety of microalgae species. The biodiesel generated by the microalgae was adequate to totally replace petroleum [105]. Algae can generate 19,000 to 57,000 litres of biodiesel per acre, whereas classic high-oil crops like palm may provide 2000 to 2500 litres per acre. Thus, the adoption of large-scale biodiesel production and consumption may help reduce India's reliance on foreign oil sources, improve the air quality in major cities like Delhi, Kolkatta, Bengaluru, and Chennai, reclaim unusable wastelands, employ Indians who are unemployed, and maintain the country's economy on course for its intended 8 to 10% annual GDP growth, as per India's 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.

Additionally, compared to open ponds, photobioreactors create an algal broth that is considerably more concentrated, which significantly lowers the dewatering expenses. It could be feasible to create dewatered algal biomass using tubular photobioreactors [108] for about €4 per kilogramme of dry weight [107]. Algal fuels are being attempted to be commercialised by dozens of startup businesses. Algae-based fuels seem quite promising. If the complete environmental effect of the later fuel types is taken into account, they may already be seen as being competitive with petroleum fuels. We may be forced to abandon petroleum long before it runs out by climate change-related issues. Significant advancements in our knowledge of algae growth have been accomplished during the past 40 years.

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.

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