

BIOREMEDIATION OF WASTE WATER USING MICROALGAE

Abstract

Water is an essential part of life. Water shortage, which leads major issue throughout the worldwide, would also result from a diminish in water quality. By reason of rising water demand, contaminated water bodies, and a lack of technologies to recover used water. Microalgae and cyanobacteria-based process are primarily used to remove nutrients and heavy metals from waste water. As primary producer, microalgae can do good to the environment and contribute to the development of a circular economy. Microalgae systems are classified as open or closed, with each having advantages and disadvantages. Open systems are more susceptible to microbiological contamination and require more process control, whereas closed systems, despite their greater initial commercial grade, are easier to control for critical cultivation parameters such as availability of nutrients, pH, dissolved CO₂, temperature, and contamination. Centrifugation, filtration, flotation, coagulation, and flocculation are some of the commonly used biomass separation technologies. Microalgae require only light, sugar, CO₂, nitrogen, phosphorus, and potassium to grow, and they can synthesis large amounts of lipids, proteins, and carbohydrates that can be processed and converted into bio-fuels and high-value-added chemical products such as Docosahexaenoic acid (DHA) and Carotenoids.

KeyWords: Microalgae, Cultivation methods, Waste water treatment, Lipid production.

Authors

Kohila Durai
School of Life Sciences
Bharathidhasan University
Tiruchirapalli, Tamil Nadu, India.

Sri Sneha Jeyakumar
School of Life Sciences
Bharathidhasan University
Tiruchirapalli, Tamil Nadu, India.

I. INTRODUCTION

Water pollution is influenced by people such as industrial effluents, agricultural runoff, sewage discharge, and unplanned urbanization [1]. Microalgae are the photosynthetic microorganisms that can develop fast and survive under harsh settings due to their basic form. Cyanobacteria (Cyanophyceae) are prokaryotic microalgae, while green algae (Chlorophyta) and diatoms (Bacillariophyta) are eukaryotic [2]. Green algae are a diverse category of autotrophic organisms with photosynthetic complexes composed of chlorophyll-like molecules such as chlorophyll a, b, c, d, and e, bacteriochlorophylls, pheophytin a and b, and additional pigment molecules such as carotenoid a and b, xanthophylls, and others [3]. However, after rehabilitation with *Chlorella vulgaris*, it was discovered that microalgae not only proficiently cleared nutrients and COD, but also significant reductions in solids such as Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS) and Electrical Conductivity (EC) concentrations were observed. A magnetic stirrer was used to mix wastewater and microalgae, amplifying sensible gas transfer [4], nutrient dissolution, and light penetration [5], reducing temperature variation within the system and preventing microalgae from settling. However, because microalgae cells have limited capability, are found in low concentrations, and are highly stable in suspension, standard separation processes have constraints in terms of power consumption, cost, and efficiency. They can eliminate urea from water, which boosts their bioconversion activity. A growth process in which light is used as a viable source of energy that can be transformed into chemical energy via photosynthesis reactions. Microalgae, which has outstanding biological traits like high photosynthetic activity and a simple structure, has the capacity to flourish well under adverse conditions such as heavy metal presence, high salinity, nutrient stress, and extreme temperature. As a consequence of higher binding affinity, abundance of binding sites, and large surface area, microalgae are increasingly being used in phycoremediation of toxic heavy metals [6]. Furthermore, microalgae biomass, both living and non-living, can be used as biosorbents. Aside from superior removal capacity and environmental friendliness, biological treatment of heavy metals using microalgae has the advantages of a potent and simple process, a lack of toxicity constraint, a faster growth rate than higher plants, and the formation of value-added products such as bio-fuels and fertilizers [7,8]. Heavy metals such as boron (B), cobalt (Co), copper (Cu), iron (Fe), molybdenum (Mo), manganese (Mn), and zinc (Zn) are consumed by microalgae as trace elements for enzymatic processes and cell metabolism, whereas other heavy metals such as arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), and Mercury (Hg) are toxic to microalgae. Microalgae cultivation has advantages for waste water treatment because it provides tertiary bio-treatment as well as the production of biomass, which can be used for a variety of purposes (Rendón et al. 2015). In the process of bioremediation of water, algae use their photosynthetic capacity to convert energy from the sun into biomass, and then incorporate nutrients such as nitrogen and phosphorus that cause eutrophication [9]. Since the late 1950s, bioremediation processes based on microalgae cultivation have been used. Microalgae have since gained popularity in the treatment of urban industrial and agricultural wastewater using *Chlorella* sp., *Scenedesmus* sp., and *Muriellopsis* sp., .Microalgae cultivation systems are classified into two types: open and closed. The most common of these systems are open ponds in lane format (Race-way Ponds) and turf scrubbers. Closed systems, also known as photobioreactors, come in a wider range of shapes and configurations, the most common of which are tubular, Bubble Column, Air-lift, and Flat Panel. Raceway ponds and similar systems differ from open ponds in that they have artificial agitation mechanisms, which are typically performed by paddle impellers. Turf Scrubber, a new wastewater treatment

technology that employs clusters of several filamentous algae species, appears to be effective in improving the quality of agricultural wastewater as well as domestic and industrial sewage. This system is made up of a community of algae that grows attached to a screen or a support and is connected to a chute through which polluted water flows, providing treatment via the uptake of organic and/or inorganic compounds during photosynthesis. Agricultural runoff and manure effluents while also producing biomass suitable for harvesting and use as feedstock for bio-fuel production.

II. CULTIVATION METHODS

The two primary categories of microalgae cultivation systems are closed and open systems. While open systems are more dependent on outside elements and have interaction with the open air, closed systems offer greater control over the growth conditions [10]. Open systems, however, are frequently easier to build and maintain, and may thus be selected for financial reasons. Immobilisation, in which the cells are imprisoned in a solid media, is a third, completely different approach to phytoplankton culture.

Microalgal cultivation utilized a variety of culturing conditions;

1. Photoautotrophic,
 2. Heterotrophic,
 3. Mixotrophic,
 4. Photo-heterotrophic cultivation.
- **Photoautotrophic:** Under photoautotrophic circumstances, 5% to 68% of lipid is obtained by microalgal cells that have been solar-powered in an open system.
 - **Heterotrophic:** In heterotrophic development, microalgal cells grow in the dark and with sun energy like bacteria, and this encouragement provides 40% of the lipid content .
 - **Mixotrophic:** Microalgal cells grow under phototrophic or heterotrophic conditions in mixotrophic culturing.
 - **Photo-heterotrophic Cultivation:** The microalgal cells require both carbohydrates and light simultaneously for photo-heterotrophic growth [11].

Consequently, it is considered to be a promising source of biodiesel production at present for microorganism that can rapidly grow and transform solar energy into chemical energy by the use of CO₂ emissions [12]. Therefore, the promising sources of biodiesel production are currently microorganisms capable of growing quickly and converting solar energy to chemicals as a result of CO₂ use [13]. Because of their potential to use nutrients like nitrates and phosphates, microbial cultivation can be integrated into municipal or agricultural waste treatment plants.[14]

III. OPEN POND CULTIVATION METHOD:

1. **Open Pond Structure:** In the case of commercial algae cultivation, shallow racing pools or circle ponds with a rotation mechanism are typically employed for mixing cultures. The racetrack pond is set up in a meandering pattern with the paddle wheel mixer, which uses

little shearing force [15]. Facultative ponds and High-rate algal ponds (HRAP) are frequently used for wastewater treatment.

2. **Facultative Ponds:** The facultative pond is usually around one meter deep, has anoxic waters in proximity of the bottom and algae grows within the surface water layers.
3. **High-rate Algal Ponds (HRAP):** On the other hand, the HRAP is usually less than a meter deep, gently stirred continuously, and aerobic throughout its volume. In HRPs, nutrients in the sludge are converted into algal and bacterial biomass using microalgae to supply heterotrophic bacteria with oxygen. [9]. Asserts that HRAPs with proper construction and operation can remove up to 80% of nitrogen and phosphorus, as well as more than 90% of biochemical oxygen demand [16]. Because of high temperatures, which cause an increase in evaporation, the establishment of an open system depends upon a limited climatic environment [17]. In outdoor ponds, the density of cells may range from 200 mg to 700 mg per litre [18].

In the microalgae culture system, four types of organic growing systems are commonly used, i.e. large shallow reservoirs, tanks, cages and raceways ponds [19]. *Dunaliella viridis* was mass cultivated in outdoor ponds to produce β -carotene at too high salinities and light intensities [20].

4. Unstirred Pond System

- Untended ponds are produced in large volumes and natural ponds have depths of less than 50 cm. Unstirred ponds are built with plastic covers [21].
- The species must be able to overcome protozoa contaminants that can affect cultural media. In the cultivation of untreated ponds, the use of herbicides and pesticides may be used to control biological haze [22].
- The unstirred ponds are very simple to operate and cost effective. The cultivation of microalgae such as *Dunaliella salina* is carried out in these type of ponds on a commercial basis.

5. Raceway Pond System

- In order to circulate algal liquid, a raceway pond has been created in deep closed circle channels [23].
- The centre barrier of the racetrack pond is replaced by a concrete wall.
- In the middle of a race course pond, paddle wheels are set to mix cells, nutrients and air. In the racing pond system, paddle wheels have a vital role.
- only one wheel (eight-bladed paddle wheel) is fixed in a single raceway pond to stay away from interference between the paddle wheels.
- These ponds are low and constructed based on the flow of the culture medium.

6. **Advantages:** The system successfully cultivates a range of algae, such as *Coccolithophorid* and *Pleurochrysis carterae* [24]. In Equatorial, the algal biomass production is high and the yield of algal biomass is also more to be harvested [26].

7. **Disadvantages:** This sort of pond is not suitable for large scale production, owing to the iniquitous quality of mixing and contamination issues [25]. The major drawbacks of open pond systems are their excessive use of light which results in cell damage, an increased loss of air and a large amount of space required for the purpose of environmental protection [27]. One of the main shortcomings of Raceway Pond is that there may be a limited number of gas exchanges, leading to less attention being paid to culture [28]. [29] reported the marine microalgae and freshwater Microalgae cultivation in open pond system produce more photosynthetic efficiency.
8. **Constraints:** The man-made open algal culture system depended on conditional Factors such as the magnitude of the rotary scraper, pond depth and culture supply. . The raceway, like open culture ponds, was designed to improve algal biomass production through impulse rotation. The largest racing pool was 440,000 m² [30]. In Tuscany, a plantation of *Nannochloropsis* successfully harvested 20 tons of oil per hectare per year by means of an open cultivation system. In an open farming system, it is a huge scale and massive cultivation of algal biomass [31].

IV. CLOSED POND CULTIVATION METHOD

Covered raceways and tubular reactors are two major classes of closed photobioreactors. Closed photobioreactors typically have better light transmission characteristics than open ponds, allowing shorter residence times to sustain higher biomass and productivity than open ponds. However, open systems are technically more complex, often require specialized staff, consume more energy, and have higher operating costs than open systems [32].

1. **Closed Pond Structure:** Tubular reactors come in a variety of rigid and flexible materials and can be arranged vertically or horizontally. Aeration and agitation in a vertical column reactor can be achieved by blowing CO₂-rich air into the bottom of the column. However, light and other growth parameters are manually introduced into the indoor algae culture bioreactor. Closed bioreactors are typically made of glass or transparent materials [33]. Many technological approaches to constructing underwater lamps, submersible lamps or luminescent glass fibers on the one hand and columnar lighting on the other hand have been tried, but without success in their application [34].
2. **Tubular Photo-Bioreactor:** Tubular photobioreactors are manufactured in a variety of geometries, including vertical, horizontal, and helical configurations, for large-scale production. The tubular reactor is connected to an aeration system to add air to the growth medium and culture. Tubular photobioreactors provide a more comfortable pH and environment than open culture systems. The diameter of the tubing is critical for external factors such as light absorption, biomass concentration, daily volumetric productivity, oxygen concentration in the culture, CO₂ storage capacity of the bioreactor, temperature history and flow patterns in the culture, and pressure drop in the culture. Designed based on factors. Culture recycling in bioreactors. Tubular photo bioreactor is specially manufactured for mass cultivation of *Spirulina* seed cultures and is equipped with his two types of air lifters for culture transfer [35]. *Nannochloropsis* sp. is one of the sources of eicosapentaenoic acid and docosahexaenoic acid, cultivated in tubular photobioreactors [36]. Tubular photo-bioreactor is more productive compared to open pond production. Pure anaerobic digestion of *Chlorella* sp. piggery wastewater cultured

in spiral tubular photo-bioreactor's resulted in higher biomass productivity than open ponds [37]. *C. vulgaris* and *Tetradismus obliquus* were cultured in a new photo-bioreactor designed to achieve high light intensity (21% increase in nitrogen removal rate) when treating landfill leachate [38].

- 3. Plastic bag photo-bioreactor:** The first generation of closed system tanks consisting of polyethylene sheets attached to a tube bioreactor are lightweight plastic bag bioreactors. In a closed system with photobioreactors, the largescale production of algae biomass primarily takes place in plastic bags. Polyethylene shields generally allow sunlight to travel easily from one part of the enclosure to another. The culture is mixed with air at the bottom of polyethylene bags set under sunlight inside that bioreactor. This type of cultivation uses upto 100 liter polyethylene bags, results upto 25 g/m^3 per day for *Tetraselmis* [39]. The plastic bag bioreactor also works in a vertical tube reactor with appropriate radius and height [40]. The deep-sea microalgae *Nannochloropsis oceanica* CY2 was grown in a 5-L plastic bag photobioreactor, which increased eicosapentaenoic acid production, and high biomass productivity [41]. *Euhalothece* sp., ZM001 was cultivated in small-scale horizontal photobioreactors with plastic bags, which produced an adequate algal biomass based on their depth [42].
- 4. Airlift Bioreactor:** The Airlift bioreactor is specially designed for biotreatment for fermentation technology, which helps to gently mix the cultures with nutrients. This device may be able to assist in the achievement of major scale production, although there will inevitably be a loss of productivity. Continuous culture with *Botriococcus braunii* at a laboratory scale was usually carried out [43]. Broth cultures are mixed with nutrients and cells by air force in this air bioreactor [44]. Measuring the rate at which nitrogen is taken out and added to autotrophic bacteria in bioreactors is an appropriate instrument for this purpose [45]. Large-scale production of a split-cylinder in-loop air bioreactor was effectively achieved under raw smoke conditions of a coal-plane power plant with a biomass productivity of $178.9 \pm 30 \text{ mg/l/day}$ by *Tetraselmis Suecica* [46].
- 5. Constraints:** For the development of the sample culture and the continuance of large-scale manufacturing, the closed system has been built. In this cultivation system, aseptic conditions were used to maintain the cultures' infectivity-free status. Under this approach, a small number of cells can enlarge if the growth media has enough nutrients [45]. Recent years have seen the introduction of several different kinds of large-scale photo-bioreactors with some of the boundaries. Based on light intensity and wavelength, strain light conversion efficiency, and a constant concentration of algal cells in the growth medium, production scale photo-bioreactors were planned [47]

V. INDUSTRIAL WASTE WATER

Many carcinogenic chemical substances found in industrial effluent have negative impacts on people, animals, and plants through groundwater. Chemicals that cause cancer are discharged by factories and openly diluted with river water. These cancer-causing substances interact with marine water and lead to bioaccumulation in marine life [48]. Numerous nutrients necessary for microalgal growth can be found in wastewater, including carbon (C), nitrogen (N), phosphorous (P), potassium (K), zinc (Zn), iron (Fe), molybdenum (Mo), chromium (Cr), and copper (Cu) [49]. The heavy metals aluminum (Al), copper (Cu), vanadium (V), lead (Pb), and selenium (Se) in textile effluent are removed by the microalgal

strains of the chlorellaceae family [50]. Through fed batch operations, the Consortium of *Chlorella vulgaris* and *Scenedesmus* sp. were domesticated in the effluent, it reduces the colour of the effluent and Completely reduce the phosphorus and nitrogen level presents in the effluent [51]. *Chlorella variabilis* was effectively grown in the effluent with a biomass productivity of above 70 mg/L and adequate amount of lipid production . Using the value-added medium, *Chlorella variabilis* also remedied the nutrients present in the effluent and it's gives best results [52]. [53] discussed the removal of color from the effluent utilizing freshwater (*Chlorella* sp.) and marine microalgal species. Orange G dye was successfully deleted from the effluent by *Acutodesmus obliquus* strain PSV 2 [54]. The duckweed (*Lemna minor*) and algae (natural colonization) also removed the heavy metals present in the effluent [55].

VI. ALGAL CULTIVATION IN WASTEWATER

- One of the finest performing microorganisms ever discovered, microalgae use CO₂ and sunlight from the sun to make their own Nutrients.
- The algal samples is kept between 20 to 30 degrees Celsius.
- Stirring of algal samples prevent the cell damage in the algal culturing [56].
- (Fig.1) *Chlorella zofingiensis* and *C. vulgaris* UTEX 259 are two examples of the several algal strains that have been documented in scientific literature as being able to grow microalgae in wastewater [57].
- The type of algae species determines this incubation period. Finally the biomass is collected [58].

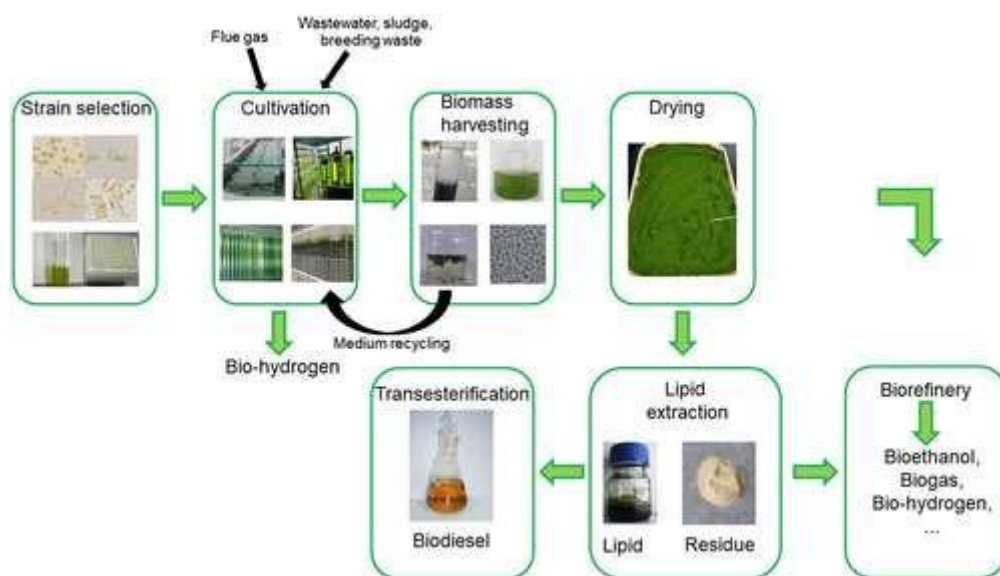


Figure 1: Process flow diagram for Microalgal Bio-Fuel Production [58].

- 1. Algal Harvesting:** The centrifugation method is only tested in lab-scale development [59]. Several methods, including filtration, centrifugation, settling, flotation, and flotation [60], are used to collect algal biomass. The flotation method is used in pilot scale with less amount of algal concentration [61].

2. Cell Disruption: There are several types of cell killing methods such as microwave (94.9%), water bath (87.7%), ultrasound (67.7%), stirrer (93.0%) and laser (96.5%) [62]. Disintegration of algal cells by ultrasound is less important and has a low interference ratio. This technique allows for the separation of lipid content and algal cell detritus [63]. One of the greatest cell lysis techniques that has produced the most effective algal cells and highest lysis values is microwaves. In comparison to other processes, it is the fastest and most appropriate for large-scale manufacturing. The lipid content of the microalgae cells is separated during the cell lysis procedure.

3. Lipid Extraction

- Lipid recovery from algal biomass is enhanced by pretreatment.
- The microalgae's cell wall is damaged, squeezed, and weakened during this procedure.
- The most common pretreatment techniques are chemical, physical, mechanical, and biological [64].
- Oil extraction, which may be done in two ways (chemically and mechanically), is an essential step in the manufacturing of biofuels.
- Pressure press and sonication are examples of mechanical procedures, whereas hexane solvent, soxhlet, and supercritical fluid extraction are examples of chemical approaches [65].
- By using the Bligh and Dyer technique, total lipid content is extracted from lysing cells.
- Degraded cells often include molecules of lipids, proteins, and carbohydrates.
- In this procedure, the solvent is made by combining methanol and chloroform (1:1 v/v).
- Next, the solvent sample is blended (1:1 v/v), and the lipid content is determined.
- To create biodiesel, the isolated lipid molecules are transesterified [62].
- After that, the microbial lipid was removed once the specified number of days of starvation had passed. Similar to that, a gravimetric approach was used to quantify and record the isolated lipid [66].
- Finally, a FAME mixture was created using the neutral lipid that was recovered from the fixed microalgae biomass [67].

VII. CONCLUSION

As the only renewable energy source that can also create solid, gaseous, and liquid fuels, biomass is the subject of extensive study to find ways to harness its energy. Biomass-derived biofuel feedstocks may be divided into four classes based on their source. They originate from energy crops (maize, wheat, and barley), sugar crops, wood and crop residues, web species (algae, water weeds, and water hyacinth), oil-producing plants like jatropha, castor, palm, soybean, and sunflower, as well as forest goods (timber, logging residues, trees, and shrubs). With edible grains indicated for generation 1 and inedible grains for generation 2, the biomass provided is of record generation un terms of its edibility. In the late 1800s, the US Department of Energy started investigating algae-based biofuels. [69] examined the development of the next-generation microalgal biofuel. There are several ways to turn microalgae into biofuel, including the creation of biodiesel by transesterification of lipids, bioethanol by fermentation of algal biomass, biogas by anaerobic fermentation, and biocrude

oil via thermochemical modification. It was a possibility to increase the process' profitability by integrating the production of microalgae biofuels with industrial or energy services.

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