# BIOREMEDIATION OF WASTE WATER USING MICROALGAE

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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 $CO_2$, temperature, and contamination. Centrifugation, filtration, flotation, coagulation, and flocculation are some of the commonly used biomass separation technologies. Microalgae require only light, sugar, $CO_2$, 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.

KEY WORDS: Microalgae, Cultivation methods, Waste water treatment, Lipid production.

## 1. 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.

## 2. 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. Different types of culturing conditions were set up in microalgal cultivation, including photoautotrophic, heterotrophic, mixotrophic, and photo-heterotrophic cultivation. In photoautotrophic conditions, the microalgal cells civilized in an open system with solar energy and microalgae obtain 5% to 68% of lipid. In heterotrophic development,

the microalgal cells nurture under solar energy and dark conditions like bacteria, and 40% of lipid content is obtained from this fostering (*Chlorella protothecoides*). In mixotrophic cultivation, microalgal cells raise in phototrophic or heterotrophic conditions. In photo-heterotrophic development, the microalgal cells have need of both sugars and light, simultaneously [11]. Accordingly, microorganisms that can grow hastily and renovate solar energy into chemical energy through the purpose of $CO_2$ are at the moment considered a promising source for biodiesel production [12]. The handling of membrane technologies in microalgal cultivation and giving out involves microfiltration (MF), ultrafiltration (UF), dialysis and forward membrane diffusion (FO) during cultivation harvesting [13].Microbial cultivation can be incorporated with wastewater treatment plants (municipal wastewater and agricultural waste) for the reason that they have the potential to utilize nutrients (e.g., nitrates and phosphates). The microalgae are constantly grown in photoperiods under nutrient conditions adequate for high bioavailability, and then, partially transferred to nutrient scarce raceway pools for greater fat amassing [14].

## 2.1 OPEN SYSTEMS – PONDS

Shallow raceway ponds and circular ponds with a rotating arm to mix the cultures are typically used for commercial algae cultivation. With paddle wheel mixers that use low shearing forces, the raceway pond is arranged in a meandering pattern [15]. High-rate algal ponds (HRAP) and facultative ponds are frequently used for wastewater treatment. A facultative pond is typically deeper than one meter, has anoxic water near the bottom and algae growing in the surface water layers. In contrast, an HRAP is typically less than a meter deep, gently stirred continuously, and aerobic throughout its volume. In HRAPs, nutrients in the wastewater are converted into algal and bacterial biomass, and microalgae provide heterotrophic bacteria with oxygen [9]. Similar to facultative ponds, the elevated pH causes ammonia stripping and phosphate precipitation. According to the majority of studies on the role of algae in HRAPs, indirect nutrient removal is frequently more significant than direct uptake. However, because of the aerobic environment present in an HRAP, the amount of denitrification that takes place in facultative ponds should be viewed as negligible [16] asserts that properly constructed and run HRAPs are capable of removing up to 80% of nitrogen and phosphorus and more than 90% of the biochemical oxygen demand (BOD). The open system premeditated depends upon the natural sunlight and climatic changes. The open system has the same distinctiveness as natural ponds. Generally, outdoor

cultivation systems are constructed for commercial production with the minority exceptions from the closed cultivation system. For commercial production, the open system is effortless and uncomplicated to manufacture large amounts of yield, and also, the construction of open ponds is uncomplicated and has a low cost-effect. The construction of an open system depends upon the restricted climatic environment due to high temperatures, which causes high evaporation [17]. The outdoor ponds are endlessly and semi-continuously operated during daylight time with paddle wheels' help. The paddle wheels thoroughly mix culture and nutrients with an average speed of 15 cm/sec. The cells' density in outdoor ponds ranges from 200 to 700 mg/L [18]. The infectivity is a noteworthy trouble in outdoor ponds. In up-to-date reports on the large scale production of microalgae in outdoor bioreactors, a high concentration of bicarbonate prevents the contaminations in *Spirulina* and *Chlorella* cultivation. Four types of open cultivation systems are present generally in the microalgal culture system, including big shallow ponds, tanks, circular ponds, and raceway ponds. The open type tubular bioreactor is one of the most significant vessels to culture the microalgae in various shapes such as horizontal/serpentine, vertical, near horizontal, conical, and inclined photo-bioreactors [19]. In numerous open cultivation systems, an organic carbon source was incessantly added to the culture medium in spare time only due to the heterotrophic growth of bacteria [11]. *Tetraselmis suecica*, *Dunaliella tertiolecta* and *Chlorella* sp., (Chlorophyta) were effectively cultivated and obtained high lipid content in an outdoor bag bioreactor with the help of solar irradiation. The highest solar irradiation was experiential in the spring and summer season (30–34.8 MJ /$m^2$), and the lowest irradiation of solar energy was experiential in the autumn and winter seasons (3–6 M.J. /$m^2$) [20].*Dunaliella viridis* was mass civilized in outdoor ponds to produce β-carotene at too high salinities and light intensities.

### 2.1.1 UNSTIRRED POND

Unstirred ponds are very uncomplicated for working, and cost effective. These types of ponds are used commercially to cultivate microalgal species such as *Dunaliella salina.* In large scale production, unstirred ponds are constructed, and natural ponds have less than 50 cm of depth. The unstirred ponds are constructed with plastic covers [21]. More than 30 species of algal culture (in dry cells) are harvested from the natural unstirred ponds in Southeast asia. In unstirred ponds, the infectivity is superficial than in other open cultivation systems This kind of pond is being used in many countries with high yields, such as Western Biotechnology Ltd and Western Australia. Many

companies carry out large scale production of algae by unstirred ponds including Whyalla, South Australia (7 to 10 tons per year of ß-carotene in 460 ha from *Dunaliella salina*), Hutt Lagoon, Western Australia (6 tons per year of ß-carotene in 250 ha from *Dunaliella salina*) and natural lakes in Southeast Asia (30 tons per year of microalgal biomass). Although the use of immobile ponds is limited to microalgal species that may be in poor conditions. These species must overcome protozoa-like contaminants that affect the cultural medium. The use of herbicides / pesticides can control biological smog in the cultivation of untreated ponds [22].

### 2.1.2 RACEWAY POND

The open raceway pond is acknowledged in the 1950 s as a primary choice for large scale microalgal biomass production due to its low-cost effect. The airlift driven open raceway reactor provided energy efficient biomass productivity and improved efficient consumption of $CO_2$ for the most favorable biomass production of *Scenedesmus* sp. The raceway pond is prepared of a 0.3 m deep closed circle channel for the circulation of algal broth [23]. The raceway pond's central divider is ended up of a concrete wall with 0.1 m of thickness, and the whole channel has 3.95 m of thickness. The paddle wheels are fixed in the raceway pond's center to mix the cells, nutrients, and air. The paddle wheels play an vital role in the raceway pond system, and 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. The major drawback of raceway pond is the extensive light path and is easily impure by external factors such as birds, dust particles from a contaminated environment, and free-living microorganisms from air [11]. The standard raceway pond has 0.10–0.24 m/sec of flow velocity, with the paddle wheel's rotation speed, which is 35–55 rpm. The raceway pond cultures are second-hand as feed for animals and biodiesel production. Several algal species are successfully cultivated by this system (for instance, *Coccolithophorid* and *Pleurochrysis carterae*) [24]. The raceway pond was constructed for pilot-scale production in Bharathidasan University, Tiruchirappalli, Tamil Nadu, with a 5 K L (7.5 m × 2.5 m) and 35 K L (30 m × 5 m). This raceway pond is seen at 10.6817◦N, 78.7412◦E, in a satellite view [18]. In natural ponds, the algae are residential with different microorganisms inhibiting the potential energy of algae. Hence, the man-made open raceway ponds are constructed to build up individual species of algae with a tiny number of other organisms. The tiny number of other microorganisms doesn't influence the mass

cells of individual algae. The raceway pond is cleaned with some chemicals such as chlorine powder, acetic acid, and HCL. The circular ponds are widely used in many countries such as Taiwan, Japan, and Indonesia. The rotating scraper is fixed in the middle part of the circular ponds to mix the nutrients and air with algal cells (less than 5 cm). This type of pond is not pertinent for large scale production due to their iniquitous mixing quality, contamination tribulations [25]. Perceptive raceway pond contains ~ 50% of algal concentration that produces an algal culture with 20.5% (w/w) oil content. In tropics, algal biomass's once a year production has been 15000 kg/ha/year, which means ~ 16% of biomass has been harvested [26]. The significant disadvantages of open pond systems are too much light utilization, which causes cell damage, significant evaporative losses, and large area necessity [27]. The infectivity is another major crisis of open pond cultivation. Microorganisms affect the algal growth from the environment, such as fungi, bacteria, etc. [17]. The porous plastic covers provide $CO_2$ from the atmosphere and maintain temperature during the night. The major shortcoming of raceway pond is that the low attention of culture can be made (0.25–1 g/L) with a small amount of gas exchange [28].[29] reported photosynthetic efficiency of marine *Chlorophyta* sp., and freshwater *Chlorella* sp., in open raceway pond with 0.986 m2 of area and 8 fins paddle wheels. In this trial, *Chlorophyta* sp., and *Chlorella* sp., have 4.15% (PAR) and 6.56% (PAR) photosynthetic efficiency.

### 2.1.3 LIMITATIONS OF OPEN SYSTEM

In an open system, the upgrade process is very challengeable due to the variations present between small scale to large scale production such as light Modulation, temperature, mixing of culture into the medium, nutrient provision, infectivity, infestation, predation, biomass film formation (fouling), loss of reactor wall transparency and oxygen upsurge. Man-made open cultivation system of algae depended upon several factors such as the size of the rotating scraper, depth of the pond, and cultivating strain. The most gigantic open circular pond was reported as 50 m. Single species culture of algae was profitably cultured with high salinity, high alkalinity, and high nutritional statuses such as *Dunaliella* (high salinity), *Spirulina* (high alkalinity), and *Chlorella* (high nutrition) [11]. In an open cultivation system, the biomass of algal cells was reliant on solar energy and essential of the surface of culturing vessels, nutrient energy available concepts of algal cells (light regime and light per cell). Raceway, like open cultivation ponds, was planned to improve algal biomass production with paddlewheel circulations, usually of 15–35 cm depth and

0.2 and 0.5 ha in size. The most gigantic raceway pond was reported with 440,000 m2 [30]. The once-a-year yield of 20 tons of oil per hectare was successfully achieved from *Nannochloropsis* culture in Tuscany through the open cultivation system. This is a large scale and gigantic cultivation of algal biomass in open cultivation system. Research publication as available on the hypothetical projections as 80–90 tons per hectare per year [31]. In an open pond system, only a tiny number of algal species can be profitably grown on a large scale; wild microbes are a crisis. There are major evaporation losses and water conservation becomes an issue; $CO_2$ is not used proficiently. A large area is required, so only infertile or waste land can be used; bio-productivity is subordinate than the closed cultivation methods and the cost of harvesting algae organisms is tranquil high. Although efforts have been made to improve open ponds with temperature control systems, appropriate nutrients, improving pond depth, and CO2 infusion systems, productivity is still very stumpy compared to closed systems. Due to these boundaries, the focus is on improving the little cost closed cultivation methods [11].

## 2.2 CLOSED SYSTEM – PONDS

Covered raceways and tubular reactors are the two main classes of closed photo-bioreactors. It is typically possible to sustain high biomass and productivity with less retention time in closed photo-bioreactors than in open ponds because they typically have better light penetrating characteristics than open ponds. However, their operating costs are higher than open systems because they are more technically complex, frequently need specialized personnel, and frequently use more energy. The internal shadowing effect between the algae is reduced and the cells can receive illumination from multiple angles by using transparent pipes for cultivation. However, because of the light refraction, there will be shadowed areas in the tubes, so there must be enough turbulence to illuminate every cell [32]. Tubular reactors come in a variety of rigid and flexible materials, and they can be positioned either vertically or horizontally. Aeration and agitation in a vertical column reactor can be achieved by injecting $CO_2$ enriched air at the bottom of the column. The fact that these reactors are basically parallel to the sun's rays has a drawback because a significant amount of solar energy is reflected in the summer. The closed system is specifically made for the non-natural growth of photosynthetic microorganisms in a photo-bioreactor. It is one of the finest methods to cultivate microalgae than the open system. The aseptic condition thoroughly prohibits the impurity, water evaporation, and the growth of unnecessary algal species.

In a closed system, the algal cells are maintained in a particular vessel for their mass cultivation. Different types of photo-bioreactors were introduced earlier to cultivate algal cell biomass without any infectivity [26]. The photo-bioreactor is specifically premeditated to culture the photosynthetic microorganisms without infectivity, and sunlight is not openly provided. As an alternative, the light is passed through the crystal-clear glass fixed in bioreactors [17]. The closed system's crucial challenge is oxygen removal from algal culture after respiration and pH and $CO_2$ gradients, overheating, bio-fouling, high material, and maintenance of costs [28]. The advantage of a closed reactor is production of a high yield than an open system. Although light and other growth parameters are manually introduced in the indoor bioreactor for algal cultivation. Closed bioreactors are usually made up of glass or crystal clear material [33]. The first generation of closed PPRs rapidly faced harsh limitations of simple closed container based systems (tanks, hanging plastic bags) for the reason that they could not effectively initiate light at 50–100 L for successful biological growth. Many technological approaches to setting up underwater lamps, submerged lamps or light emitting optical fibers on one hand, or pillar shaped illumination on the other, have been tried, but have not been successful in application [34].

### 2.2.1 TUBULAR PHOTO-BIOREACTOR

Davis and his teammates reported the first algal cultured tubular bioreactor made of plastic or glass with 40 feet (12.2 m) length and ~ 8 mm diameter. Crystal clear glass materials make sunlight obtainable for tubular photo-bioreactors placed in an outdoor environment condition. Tubular photo-bioreactor are made in variety of shapes for large scale production including vertical, horizontal, and helical structures. Tubular reactors are linked with an aeration system to provide air to the growth media and cultures. The tubular photo-bioreactor provides a sympathetic pH and environment than the open culture system. The productivity of the culture is also high compared to the other methods (20 to 40 mg/L/day) [18]. The diameter of the tube is designed based on major exterior factors such as light absorption, biomass concentration, daily volumetric productivity, concentration of oxygen in the culture, $CO_2$ storage capacity of the bioreactor, temperature course of the culture and flow pattern and head loss for culture recycling in the bioreactor. The tubular bioreactor is operated with a high density of culture without any exterior factor's turbulence due to the high productivity of algal biomass [23]. Aeration in the tubular reactor is provided using a aeration motor. The crystal clear tubular arrays are capturing the sunlight for the development of

microalgae. Tubular arrays are generally 0.1 m or less in diameter. The tubular arrays' diameter is planned to penetrate sunlight into the growth medium. Two air-lift type tubular photo-bioreactor (TPB) is one of the designs of tubular photo-bioreactors that contains two identical reactors (diameter of the first reactor is 0.06 m and second reactor is 0.03 m), an external loop and a solar receiver. TPB is specifically made for the mass cultivation of *Spirulina* sp., cultures and has two types of air lifts for the motion of the culture [35]. The tubular photo-bioreactor is classified into four types based on their structural characters (serpentine, manifold, helical photo-bioreactors, and fence arrangement with manifolds) [17]. Essential n − 3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are mainly extracted from the fish. On the other hand, this source is not enough for global demand (1.1 million tons of EPA + DHA are needed once a year). So, microalgae are an alternative source for EPA and DHA. *Nannochloropsis* sp., is one of the sources of EPA and DHA and is cultivated in tubular photobioreactor [36]. Tubular PBR is highly producible compared with open raceway pond production. *Chlorella* sp., was cultivated in a helical tubular PBR with an unadulterated anaerobic digestion of piggery effluent resulting in 2.1-fold increasing biomass productivity than the open raceway pond [37]. *C. vulgaris* and *Tetradesmus obliquus* were cultivated in a novel PBR designed to establish a high light intensity with the treatment of landfill leachate (with an increased nitrogen removal of 21%) [38].

### 2.2.2 PLASTIC BAG PHOTO-BIOREACTOR

The plastic bag photo-bioreactors are the 1st generation containers of a closed system made up of polyethylene sheets related to the tubular bioreactor. Large scale production of algal biomass is primarily started with plastic bag photo-bioreactors in a closed system. From this bioreactor, 50–100 L of algal cultures are civilized with a low-cost effect [34]. Generally, the polyethylene covers permit the sunlight to pass from one side to another side easily. In this bioreactor, the cultures are mixed with air at the bottom of polyethylene bags that are positioned in sunlight. In this type of cultivation, 50–100 L polythene bags are used to yield 20–30 g/m3 d for Tetraselmis [39]. The plastic bag bioreactor also mechanism as a tubular reactor in the vertical form with a radius at 0.2 m and a height of 4 m [40]. *Nannochloropsis oceanica* CY2, a deep sea microalga was civilized in a 5 L plastic bag type photo-bioreactor with a growing amount of eicosapentaenoic acid production, EPA content (4.12%) and biomass productivity (7.49 mg/L/day) [41]. Euhalothece sp.,

ZM001 was civilized in small scale, horizontal plastic bag photo-bioreactors producing a yield of 17.06 g/m2 /day of algal biomass in 10 cm depth [42].

### 2.2.3 AIRLIFT BIOREACTOR

The airlift bioreactor is specifically made for bio-processing fermentation technology that helps to stir up cultures with nutrients gently. This device can help to accomplish large scale production with an inevitable loss of productivity. The nonstop culture was routinely produced with *Botriococcus braunii* in lab scale [43]. Airlift bioreactor's noteworthy improvement is the prevention of cell damage during agitation and it is less classy [26]. They have been classified into two main groups (internal loop and external loop air-lift bioreactors). The internal loops have three different structures of airlift bioreactors (split cylinder internal loop, concentric draught tube internal loop, and concentric draught tube (vertically split) internal loop). The airlift bioreactor can be used to incubate and harvest algal biomass by maintaining a less glutinous broth culture in low-cost oxygen system. In this airlift bioreactor, the broth cultures are mixed with nutrients and cells using air force [44]. The consequence of NaCl on the carotenoid production by *Haematococcus pluvialis* was premeditated using airlift bioreactor. The results of the learning showed that the cell density was maintained at a particular concentration (25 × 104 cells m/L) . It is helpful tool to measure the rate of nitrogen removal and addition of autotrophic bacteria in the bioreactor [45]. The large scale production of split cylinder internal loop air-lift bioreactor was effectively achieved under untreated flue gas condition from coal fired power station with 178.9 ± 30 mg/L/day of *Tetraselmis suecica* biomass productivity [46].

### 2.2.4 LIMITATIONS OF A CLOSED SYSTEM

The closed system has been residential for the sample culture growth and continuation to large scale production. In this cultivation system, the cultures were maintained without any infectivity under aseptic conditions. A small number of cells can enlarge under this system with adequate nutrients in the growth medium [45]. In recent times, various types of large scale photo-bioreactors were introduced with some of the boundaries. The production scale photo-bioreactors were premeditated based on intensity and wavelength of light, light conversion efficiency of strain, and constant level of algal cells in the growth medium [47]. The leading closed bioreactor (made by glass tubes) was profitably reported with 500,000 m of length and 700 $m^3$ of total volume. In

Central European conditions, glass made closed bioreactors were effectively run occupying 10,000 $m^2$ of area and produced 130–150 tones dry biomass of algae [34]. At some stage in the scaling up process of microalgal cells, pumping plays an crucial role in circulating the medium and algal cells, and 75% of biomass yield was increased due to the substitution airlift for pumping medium into the reactor.

## 3. WASTEWATER OF THE TEXTILE INDUSTRY

The textile industry wastewater contains many carcinogenic chemical compounds that cause poor effects to humans, animals, and plants through the groundwater. Carcinogenic chemical compounds are released from the industries, and they are openly mixed with river water. These carcinogenic compounds contact the marine water and cause bioaccumulation in marine organisms [48]. A few years ago, several studies reported the treatment of microalgae and macrophytes (floating or rooted plants) in wastewater treatment. In wastewater, extensive matters, plastic papers, and woods can be found. These large particles should be aloof before the development of microalgae in wastewater. Most of the *Chlorella* sp. have helped in the removal of heavy metals, nitrogen, phosphorous, and carcinogenic compounds. The textile wastewater has a lot of essential compounds for microalgal growth including carbon, (C), nitrogen (N), phosphorous (P), magnesium (Mg), potassium (K), zinc (Zn), iron (Fe), molybdenum (Mo), chromium (Cr) and copper (Cu) [49]. *Chlorella* sp. produced the high amount of FAME (20 ± 4%) in textile industry wastewater treatment with the more addition of nitrogen sources (that promotes the removal rate of Chemical Oxygen Demand (COD) and $NH_4^+$-N up to 75%) . *C. vulgaris* UMACC 001 effectively treated the textile effluents with 41.8% to 50.0% of colour deletion, 44.4 to 45.1% of $NH_4$ -N deletion, 33.1 to 33.3% of $PO_4$ -P removal and 38.3 – 62.3% of COD deletion . *Micractinium* sp., CCAP 211/92 (NM 1), *Chlorella sorokiniana* UTEX 1665 (NM 2), Chlorella sorokiniana UTEX 246 (AB 1), Chlorella sp., CB4 (OJ 2), *Chlorella* sp. KU211a (IL 1) and *Chlorella* sp. KU211b (IL 3) were civilized in textile wastewater and they have been isolated from various sources including fish ponds in Nigeria, textile wastewater discharge and stream. Those chlorellaceae family microalgal strains aloof the colour and heavy metals including aluminium (Al), copper (Cu), vanadium (V), lead (Pb) and selenium (Se) in textile wastewater [50]. *Chlorella* and *Scenedesmus* sp. were civilized as consortium in textile wastewater via fed batch operation. About 68–72% of color was reduced and 100% of nitrogen and phosphorous compounds were also eliminated by the consortium cultivation

of *Chlorella* and *Scenedesmus* sp., in textile wastewater [51]. *Chlorella variabilis* was successfully cultivated in textile wastewater with 74.96 ± 2.62 mg/L of biomass productivity and 20.1 ± 2.2% of lipid productivity. *Chlorella variabilis* also remediated 100% of Al, 82.72% boron, 45.66% Ca, 100% Co, 14.5% K, 0.1% Mg, 42.18% Na, 100%, Ni 100%, Fe 78.17%, of total phosphate ($PO_3^4$) and 25.22% of total inorganic phosphate with the value added medium [52]. [53] reported about dye deletion from textile wastewater using marine microalgae (including *Chlorella marina*, *Isochrysis galbana*, *Tetraselmis* sp., *Dunaliella salina* and *Nannochloropsis* sp.) and freshwater microalgae (*Chlorella* sp.) *Acutodesmus obliquus* strain PSV 2 was a successful strain in the deletion of orange G dye from the textile effluent [54]. Duckweed (Lemna minor) and algae (natural colonisation) treated textile industry wastewater led to heavy metal elimination including 36% and 33% for Pb, 33% and 21% for Cd and 27% and 29% for Cu, respectively [55].

## 4. ALGAL CULTIVATION IN WASTEWATER

The microalgal culture inoculated onto the large particles removes textile wastewater. Microalgae is one of the best ever upward microorganisms that imprison $CO_2$ and natural sunlight to produce its food and metabolic products. The microalgal culture is maintained at 20– 30◦C. This algal cultivation system setup is gently agitated for 10 days to avoid cell damage [56]. (Fig.1) Different types of algal strains were reported in scientific literature to cultivate microalgae in wastewater, such as *C. vulgaris* UTEX 259 and *Chlorella zofingiensis* [57]. Culture is maintained at an average light intensity (12 h light and 12 h dark per day) at 110 μE $m^{-2}$ $s^{-1}$ with 12 × 20 W ‘warm white’ fluorescent tubes. Inoculated algal culture would grow in 10 days. This incubation period depends upon the algal species. After 10 days, the algal biomass can be harvested by different methods [58].

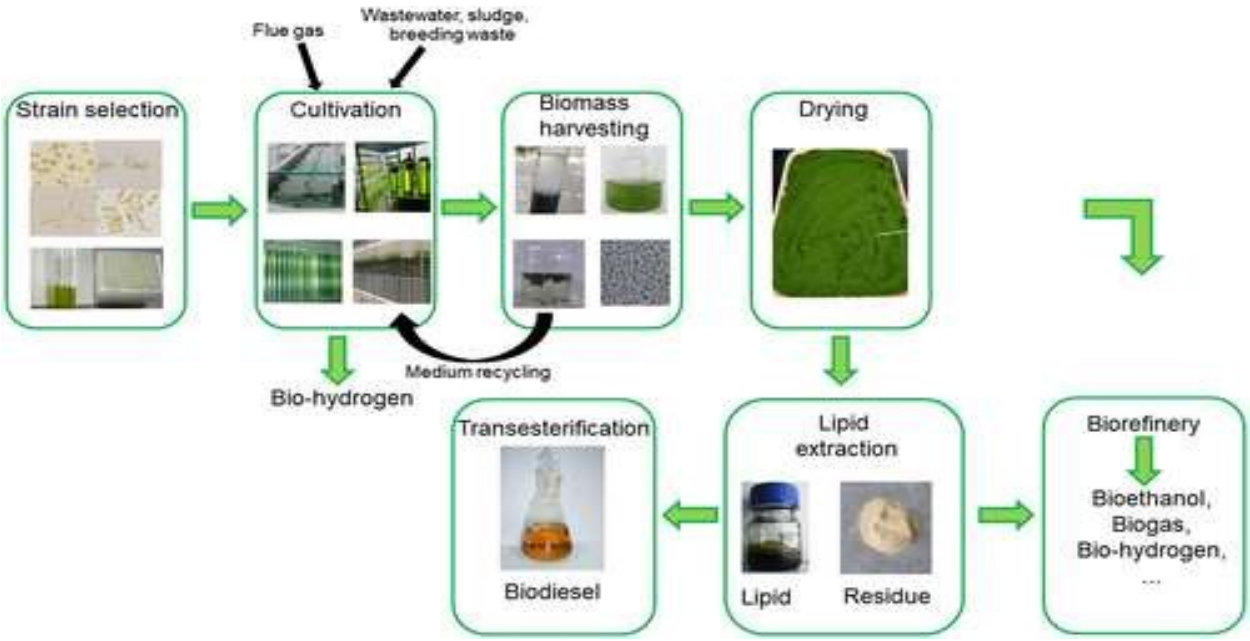


Figure 1: Process flow diagram for microalgal bio-fuel production [58].

## 5. ALGAL HARVESTING

In general, the harvesting methods are classified into three types based on the methods (chemical based, mechanical based and biological based methods) [28].The harvesting techniques are based on algal biomass properties, including size, density, and value of desired products. Harvesting techniques are divided into two major groups based on the civilization (bulk harvesting and thickening). In bulk harvesting, a high quantity of algal biomass is divided from the suspension culture. From this method, 2–7% of biomass can be divided by flocculation, flotation, or gravity sedimentation methods. The thickening method is a cost effective and high energy consumption method compared to bulk harvesting. Most of the suspension cultures are harvested by centrifugation method at 500–1000 rpm for 2–5 min [12]. The centrifugation method is generally applicable for large scale production with some exceptions. The centrifugation method's major disadvantage is that it can be applied only for small scale cultivated biomass [59]. several techniques are followed for harvesting algal biomass in large scale productions, including filtration, centrifugation, sedimentation, flocculation, and flotation [60]. The flotation method is used in pond cultivation with a culture concentration of less than 0.5 g/L [61].

## 6. CELL DISRUPTION

The cell disruption process extracts the lipid content of microalgal cells. In this process, the algal cell wall is disrupted to get the intercellular metabolites (lipid content). There are many types of cell disruption methods, such as microwaves (94.9%), water bath (87.7%), ultrasonics (67.7%), blender (93.0%), and laser (96.5%) [62]. The laser method is the most costeffective process, and is effective in cell disruptions but it is used only in lab scale production due to the low amount of sample (1.06 μm) used in this method. The microwave is one of the best cell disruption methods that have achieved the most effective algal cells and higher disruption values. It is the fastest method compared to the other methods and is applicable for large scale productions. The algal cell disruption in the ultrasonics method is less significant and has a low disruption rate. In this method, algal cell debris and lipid content can be separated [63].

## 7. LIPID EXTRACTION

Pretreatment helps to improve the recovery of lipid content from algal biomass. In this treatment, the cell wall of microalgae is disrupted, pressed, and weakened. Mainly, four different pretreatment methods are followed (chemical, physical, mechanical, and biological) [64]. In general, oil extraction is a necessary biofuel production process that has been done by two different methods (chemical and mechanical methods). The mechanical methods include the expeller press and the ultrasound effect, whereas the chemical method includes hexane solvent, soxhlet, and supercritical fluid extractions [65].The total lipid content is separated from the disrupted cells by Bligh and Dyer's method. In general, the disrupted cells contain proteins, carbohydrates, and lipid molecules. In this method, the solvent is prepared by mixing chloroform–methanol (1:1 v/v). Then, the solvent-sample is mixed (1:1 v/v), and the lipid content is separated using a separating funnel after evaporating the solvent. The separated lipid molecules undergo the trans-esterification process to produce biodiesel [62]. High Lipid content and biomass are usually difficult to accomplish simultaneously. Instead of giving pressure on the microorganisms at the cultivation stage, a stressful environment such as nutrient malnourishment, salinity and light effect was introduced to *C. vulgaris* after harvest. Within a day of cultivation in saline pressure of 6.0 g/L under dark room had a high lipid content of 38.8% (based on dry weight) due to nutritional malnourishment. The lipid content was recorded at 40.28% (based on dry weight) when the working volume was increased. In addition, the fatty acids identified in the extracted microalgal

lipid were mainly linoleic, linolenic and palmitic acids. Microbial fat production is 15 to 300 times higher compared to oil bearing crops such as maize, soybean, sunflower and palm oil. In addition to nutrient malnourishment, continuous lighting with white glowing light or dark room condition was used to study the effect of light on the fat content produced by the microalgae. The dark stage cultivation was performed in a dark container placed in a dark room. Then, when the number of days of malnourishment was reached, the microbial lipid was extracted. In the same way, the extracted lipid was measured and recorded by gravimetric method [66]. Repeated use of PU foam support material expended on the same liquefied bed biodegradation system introduced with new N concentrate real wastewater at each rated cycle was found to be stable for at least 4 cycles of attached microorganisms to satisfy sewage discharge quality. Later, the neutral lipid content extracted from the attached microorganism was recorded to be four times higher than the content obtained from the suspended culture method. The neutral lipid extracted from the attached microalgal biomass was finally converted to 97% − 98% yield in FAMEs mixture (by wt of lipid) [67]. Finally, early *C. vulgaris* cultivation in SWE affected the fat accumulation. The concentration corresponding to the COD concentration of SWE at 365.67 ± 3.45 mg/L was 25–35 mg/L [68]. In day 20, cultivation in PBR and NPBR showed maximum fat yield of 14.43% and 4.83%, respectively. The most important fat accumulation was achieved on day 20 in NPBR, which resulted in a lipid content three times higher than that of CY-1 [58].

## 8. CONCLUSION

The only way to be successful is to use renewable energy sources including sunlight, wind, thermal energy, biomass, and flowing water. Biomass is the only renewable energy source that produces solid, gaseous, and liquid fuels, hence, a lot of investigate goes into extracting energy from biomass. Depending on the source, biomass derived biofuel feedstock can be classified into four groups. They come from agricultural, wood, and crop leftovers, aquatic biomass (algae, water weed, and water hyacinth), energy crops (corn, wheat, and barley), sugar crops, and oil producing crops like jatropha, castor, palm, soyabean, and sunflower, and forest products (wood, logging residues, trees and shrubs). The obtainable biomass is off the record into generations with reverence to its edibility where edible crops are listed to 1st and non–edible crops for 2nd generations. The Department of Energy of United States initiated the research on algal biofuels during the late 19th century to assess the forthcoming aspects of algal sp. The next generation

biofuel from microalgae has been investigated by [69]. Biodiesel generation via lipid transesterification, bioethanol production via algal biomass fermentation, biogas production via anaerobic digestion, and biocrude production via thermochemical amendment are only a few of the diverse procedures for revolving microalgae into biofuel. Few concerns, such as managing a high energy and expensive dewatering process, coping with the high number of residues left after lipid extraction in the case of lipid-based biofuel production, are necessary for a viable production of biofuel from microalgae. The creation of biofuel from microalgae, on the other hand, has yet to be realized on a big scale. Major research gaps, such as energy input lessening, yield maximization, and material and energy efficiency, are all waiting to be filled. The nutrient supply has a substantial crash on cost, sustainability, and production sittings in microalgae rising, whereas the key nutrients (nitrogen and phosphorous) necessitate primary attention. Integration of microalgal biofuel production with industrial or power services has been optional as a way to improve the process profitability.

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