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Phycoremediation of Domestic Waste Water and Biodiesel Extraction from Fresh-Water Microalgae

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Microalgae have recently drawn attention as a potential source for the sustainable production of biotechnologically valuable resources and improving the environment in various ways. They are widely distributed and can thrive even under extreme circumstances like high temperatures or high salinity. However, producing microalgae takes a lot of nutrients, which may have an adverse impact on the environment and the economy. The use of wastewater, particularly those from agro-industrial facilities, domestic waste waters, and industrial discharges, which often contain high nutrient concentrations, can serve as an alternative to synthetic culture media. Because the composition of wastewater and usual culture media is relatively similar, wastewater can be utilized to both clean itself and culture microalgae at the same time. Utilizing microalgae as wastewater bioremediation agents can efficiently remove N and P from domestic wastewater, maintain dissolved oxygen concentration, and reduce the various disease-causing pathogens and fecal bacteria that are present in domestic wastewater. The potential of microalgae to be used as a feedstock is increased by their ability to change the composition of their biomass under stress and accumulate lipids or carbohydrates that might be used to produce biodiesel. Methyl or ethyl esters of fatty acids produced from triglycerides by transesterification process by using renewable feedstocks are known as biodiesel. The microalgal biomass is considered as the next generation of feedstock for biofuel production. The Dual function of microalgae in domestic wastewater treatment and biomass growth for biodiesel production is outlined and discussed in detail in this review paper.

Keywords: Biodiesel; Domestic Waste water; Lipid extraction; Microalgae; Phycoremediation.

Microalgae have recently drawn an attention as a potential source for the sustainable production of biotechnologically valuable resources and improving the environment in various ways. They are unicellular photosynthetic microorganisms that belong to a diverse group of eukaryotic single-cell algae and prokaryotic cyanobacteria. It ranges from 0.2 to 2 µm (picoplankton) up to filamentous forms with sizes of 100 ìm or higher[1]. Microalgae are found in various aquatic environments like oceans, lakes, ponds, rivers even damp soil and they are capable of growing in different habitats. They are widely distributed and can thrive even under extreme circumstances like high temperatures or high salinity[2].

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Researchers' interest in the cultivation of microalgae due to their capacity to produce biomass quickly. However, producing microalgae takes a lot of nutrients, which may have an adverse impact on the environment, such as increased nutrient runoff and eutrophication in surrounding bodies of water. Implementing sustainable practices, such as recycling nutrients can help mitigate these environmental impacts. Additionally, incorporating algae cultivation into wastewater treatment facilities can provide a dual benefit of nutrient removal and biofuel production[3]. Use of wastewaters, particularly those from agro-industrial facilities, Domestic waste waters and Industrial discharges which often contain high nutrient concentrations, can serve as an alternative to synthetic culture media. Because the composition of wastewater and usual culture media is relatively similar, wastewater can be utilized to both clean itself and culture microalgae at the same time[4].

Wastewater is a mixture of organic and inorganic substances and contaminants, and its components, including ammonium, phosphate, carbohydrates, and magnesium, can support microalgal growth. Utilizing microalgae as wastewater bioremediation agents can efficiently remove N and P from Domestic wastewater, dissolved oxygen concentration was maintained, and reduce the various disease-causing pathogens and faecal bacteria which are present in domestic wastewater[5].

The aim of some recent research was to identify novel microalgae strains that are highly effective at treating wastewater and produce high-growth, high-value products. The potential of microalgae to be used as a feedstock is increased by their ability to change the composition of their biomass under stress and accumulate lipids or carbohydrates that might be used to produce biofuel. Methyl or ethyl esters of fatty acids produced from triglycerides by transesterification process by using renewable feedstocks are known as biodiesel[6]. Transesterification is a continuous process which takes place in stirred tanks at the optimum temperature 60 ° to 70 °C and the after the esterification process completed the byproduct glycerol is removed by continuous centrifugation. One of the highly efficient and high values reaching process is Transesterification. Compared to other terrestrial crops which are used for the biofuel production, microalgae require less water which can lower cultivation costs. Lipids, carbohydrates, and proteins are the primary microalgal components that can be of particular interest. Lipids can be used as a feedstock for the production of biodiesel, however only neutral lipids, such as triacyl glycerides (TAGs), are suitable. The lipids are transformed into biodiesel (FAME) in the presence of a catalyst and an alcohol.

The composition of the fatty acids determines the quality of biodiesel produced, which have a significant impact on the combustion and power of engines. Production of microalgae biomass and biofuels can be classified as two significant phases that combine upstream and downstream operations. While the downstream stage concentrates on harvesting methods and the sustainable production of biofuel, the upstream stage uses various cultivation technologies to maximize biomass quality and quantity. Dual function of microalgae in domestic wastewater treatment and biomass growth for biodiesel production is outlined and discussed in detail in this review paper.

Waste Water Treatment

Domestic waste water's chemical composition varies depending on where it was produced. The lipids, carbohydrates, proteins, and volatile acids make up more than 70% of the organic carbons in wastewater. Kitchen waste is rich in lipids, proteins, carbohydrates, and cooking oils. It also contains phosphate and nitrogen as well as minor amounts of numerous synthetic organic compounds[7]. Untreated domestic water contains phosphorus from household detergent and soap use, nitrogen from nitrate from diapers washed in bathrooms, and phosphorus[8]. Depending on the sorts and lifestyle of human's trash are being created by various kinds of industry at the moment. The final composition of wastewater is heavily determined by the type of sewer system used and the technology.

Microalgae can be cultivated in various types of wastewaters including municipal wastewater which are all drained from our daily usages and wastewater from agricultural process and industrial discharges wastewater with toxic substances. The availability of toxic substances, microorganisms and other competitors in wastewater may cause a negative influence on algae even though it includes vital substances for microalgal cultivation. The characteristics of a particular wastewater also vary depending on the source. Because of this, each microalgae strain and each waste stream have a different capacity for utilizing waste streams and accumulating lipid. Variations in nitrogen and phosphorus concentrations in wastewater can directly impact algal biomass productivity. Low levels of these nutrients can limit growth, while excessive concentrations can lead to nutrient imbalance and hinder lipid accumulation. Microalgae also require trace elements such as iron, manganese, and zinc for optimal growth. Variability in the availability of these trace elements in wastewater can affect microalgal cultivation and overall lipid yield. To mitigate the effects of variability in wastewater composition, strategies such as strain selection, process optimization, and wastewater pretreatment can be employed. Selecting robust algal strains capable of tolerating fluctuations in nutrient levels and contaminants can enhance cultivation success. Therefore, strain selection is a crucial factor in ensuring the efficiency and stability of algal cultivation systems. Agricultural wastewater with a high turbidity would allow less light to pass through, which is essential algae growth. Therefore, wastewater needs to be diluted both before and during storage. It has been demonstrated that dilution affects the removal of nutrients and biomass accumulation from agricultural wastewater. Three different types of wastewaters are present inside the wastewater treatment plant. Primarily treated wastewater after the solids and fats are removed, secondary treated waste water, in this most of the organic material has been removed and after the anaerobic digestion, which has a high contaminant concentration. The primary contaminants in wastewater include iron, manganese, phosphorus, nitrogen, and organic matter (COD), all of which are necessary for the growth of microalgae. The wastewater composition which has to be treated must be known in order to design an effective process, particularly to know whether additional nutritional source must be added and corresponding with the system's biological and engineering capacity the maximum flow will be determined which can be treated. Primary settled wastewater is typically used without modification (dilution, additional treatments) hence this practice is common. Likewise, wastewater from the secondary treatment can be treated and any remaining N and P from secondary treatment can be removed using microalgae treatment, which serves as the tertiary treatment. Other pollutants in wastewater include heavy metals and developing compounds (cosmetics, medicines, surfactants, etc.) any of which can be hazardous to microalgae. Heavy metal and emerging chemical concentrations in wastewater are typically so low that they have not much change on the system's function. But even at high concentrations, heavy metals like cadmium, lead, and mercury can disrupt the photosynthetic apparatus of microalgae. They may interfere with the electron transport chain, disrupt chlorophyll synthesis, or damage photosystem II (PSII) proteins, leading to reduced photosynthetic efficiency. As a result, microalgae exposed to high concentrations of heavy metals may exhibit decreased growth rates and biomass productivity[9]. Raw wastewater should be treated with pre physicochemical process for cultivating the microalgae to increase its ability for biodegradation. Before microalgae culture, the nutrient load and color of the wastewater should be reduced this can be done by some oxidation techniques like wet air oxidation, ozonation[10].

Domestic wastewater may be effectively cleaned up by microalgae by removing N and P. Currently, municipal wastewater treatment is the main focus of investigations on the combining of domestic wastewater treatment with production of biofuel. Due to the amount of nutrients, it contains, agricultural wastewater can be used as another source of essential nutrients for the microalgae cultivation. However, a more concentration of fertilizers and many substances in wastewater from agriculture may prevent the cultivation of microalgae[11]. Microalgae culturing in wastewater released from industries emphasizes on the process of removing of metal pollutants and some chemical toxic substances, other than N and P. whereas the concentration of high metal and presence of toxic organic chemicals, and less amount of N and P concentration in wastewater from industrials cause an adverse impact on microalgae culture.

Selection Of Microalgal Strains

Selecting the best microalgal strain for wastewater treatment and biodiesel production involves considering several key factors related to the strain's growth characteristics, nutrient utilization efficiency, lipid productivity, and environmental tolerance. Determine the specific goals of wastewater treatment, such as nutrient removal, organic matter degradation, or biomass production. Select a microalgal strain that is well-suited to achieving these objectives efficiently and effectively. Conduct screening experiments to evaluate the growth performance and biomass productivity of different microalgal strains under conditions similar to those of the target wastewater. Consider parameters such as growth rate, biomass yield, lipid content, and nutrient uptake kinetics. Evaluate the lipid productivity potential of candidate microalgal strains, as biodiesel production efficiency depends on lipid content and lipid productivity. Select strains with high lipid content and robust lipid accumulation capabilities, ideally under nutrient-replete conditions, to maximize productivity. *Scenedesmus* sp., *Chlorella* sp. are the microalgae species that have been investigated for waste water treatment and biomass production under varied growing circumstances. Chlorella and Scenedesmus are the most employed microalgae species in this genera Chlorella vulgaris and Scenedesmus obliquus species are cultivated in waste water due to their high tolerance, and more biomass production and lipid accumulation potential. Enhancing lipid productivity in microalgae cultivated in wastewater is essential for efficient biodiesel production. Choose or engineer microalgal strains with inherently high lipid content and efficient lipid biosynthesis pathways. Genetic modification techniques can be used to enhance lipid accumulation by overexpressing key lipid biosynthesis genes or modifying metabolic pathways to redirect carbon flux towards lipid production. Optimize light availability and distribution in the cultivation system to enhance photosynthetic efficiency and lipid productivity. Implement light supplementation strategies, adjust photoperiods, and optimize reactor geometry to maximize light penetration and utilization by microalgal cells. High nutrient removal rates and strain robustness are frequently positively correlated with high growth rates, which show a strain's adaptability. Even if the lipid content is relatively low, the rapid growth rate will make up for this drawback and produce well results in the biomass and lipid production and domestic wastewater treatment[13].

Most widely used microalgal species are listed in Table 1. Some other species, such as *Anabaena* sp. *Arthrospira* sp. *Hindakia* sp. *Phormidium* sp. *Auxenochlorella* sp. *Botryococcus* sp. *Neochloris* sp., *Chlamydomonas* sp., *Desmodesmus* sp., *Dunaliella* sp., *Haematococcus* sp. *Isochrysis* sp. *Nannochloropsis* sp., *Microcystic* sp. *Oscillatoria* sp., *Phaeodactylum* sp., *Scenedesmus* sp., *Spirulina* sp., *Synechococcus*sp., *Trentepohlia*sp.

Phycoremediation

Phycoremediation, which has been in use since 1957, it is the process of treatment of wastewater by using microalgae. Oswald et al., (1957) published one of the first reports of this application. Biotransformation of organic nutrients and xenobiotics present inside the microalgal cell, this technology is a safer, more effective, and more sustainable method for removing pollutants by using microalgae or macroalgae[30]. Outstanding research efforts have been made over the past 20 years in the fields of phycoremediation and wastewater-based microalgal culture. These studies showed that the nutritional substances in wastewater significantly impact the development of microalgae and their chemical composition such as their production of protein and lipids. Using microalgae in the treatment of wastewater and the biomass generation of several microalgal strains, including Chlorella spp. and Scenedesmus spp., result in the process of phycoremediation and nutrient removal[31].

According to Martnez et al.'s research, wastewater functions as a complete medium that is a replacement of chemical media in terms of kinetics. Using wastewater for microalgal cultivation has the potential to consume 90% less freshwater and up to 94% less nitrogen. The use of wastewater in place of freshwater can completely eliminate the requirement for the addition of K, Mg, and S [32]. With significant removal efficiency, *Botyrococcusbraunii* to successfully remove N and *Scenedesmus obliquus* for the removal of P.

Phycoremediation is the process of converting pollutants present in water and other impurities dissolved in wastewater into biomass through photosynthesis and other metabolic processes, it is an efficient way to treat wastewater using a microalgae-based system. The symbiotic association between bacteria and microalgae that produced great effectiveness in removing pollutants from wastewater, microalgae will be the potential candidate for domestic wastewater treatment in tertiary phase. Because of production of Oxygen by microalgae through photosynthesis process, which aerobic bacteria can use as an electron acceptor to break down organic contaminants. Microalgae can use the CO2 that is generated during bacterial mineralization as a source for carbon and the cells can grow through the process photosynthesis in the presence of light[33].

Nutrient Removal—Nitrogen and Phosphorus

High amounts of nutrients in untreated or just partially treated wastewater cause eutrophication when they discharge into water bodies. The primary sources of the anthropogenic nutrients that accumulate up in these environments are urban and agricultural wastewater. Because they need a lot of nitrogen and phosphorus to make proteins, nucleic acids, and phospholipids, microalgae are effective ways to remove a lot of nutrients. Microalgae cells consume NO3- and PO_4^{3-} for growth can significantly decrease the N and P content in domestic wastewater and improve the quality of the discharging wastewater[34].

After carbon, nitrogen is the second requirement for the growth of microalgae. Although there are inorganic and organic forms of it in the wastewater, microalgae prefer the organic form even in little amounts such as ammonium. The removal in the direct process is carried on by the microalgal cells assimilation. While during indirect method, the removal process is brought about by differing the wastewater's concentration brought on by microalgae activity. Since ammonia is lost to the atmosphere at high pH levels and the most common nitrogen source in domestic wastewater, it will be removed by the phycoremediation process from the wastewater. Denitrification, in which nitrates are converted to nitrogen gas, is the most common technique for removing nitrogen. Microalgae cells' dry weight may contain 7-10 % of Nitrogen.

In wastewater the inorganic phosphorus, plays a vital role for the cultivation and metabolism of microalgae which can be found in lipid content of microalgae, nucleic acids, and other proteins. organic nitrogen can enter in to wastewater through sewage discharge from Domestic usages. Chemical precipitation with ferric chloride is a common method for removing phosphorus. Eukaryotic microalgae have the ability of converting inorganic nitrogen into organic forms through absorption. The conversion of nitrate to nitrite and then to ammonium, which is eventually incorporated into amino acids, is the transformation mechanism which takes place on plasma membrane of the microalgal cells[35].

The Total Nitrogen and Total Phosphorus removal during the phycoremediation process of domestic wastewater is based on the factors like nutrients, temperature, light, pH all are important for the growth of microalgae. The absorption process that occurs during the microalgae cell's photosynthesis or chemosynthesis is the mechanism by which nutrients are removed from wastewater using microalgae. Additionally, the pH values that these processes cause to vary in the wastewater might play a role in the volatilization and precipitation processes that remove these contaminants[36].

Heavy Metals Removal

The potential of the microalgae cells to act as a biosorbent for heavy metals is very high. The potential of algal biomass to remove metal from wastewater has been demonstrated in various researches. Carbohydrates and polysaccharides having negatively charged groups, such as hydroxyl, amino, carboxyl, or sulfhydryl groups, make up the cell walls of algae and cyanobacteria. The majority of positively charged metals may form strong bonds with ligand groups which are all have negative charges, this is the basic process of metal removal from wastewater that contains metals[37]. Other effective methods of metal removal other than adsorption on cell surfaces include bioaccumulation, chelate synthesis (such as the production of aragonite complexes), and internal or surface precipitation. During bioaccumulation process the metal ions are accumulate within the cell cytoplasm by the transportation system which is active and found in live cells. Therefore, the microalgal cell has the ability to withstand the toxic effects of heavy metals determines the rate at which heavy metals bioaccumulate[38]. Low concentrations of some heavy metals, such Copper, nickel, and zinc play a major role as cofactors for the enzymes in the cells of microalgae. However, they become hazardous for cell functions at high concentrations.

Hg, Pb and Cd are some other metals which are toxic even if they present in low concentrations[39].

The concentrations of heavy metals in waste water may vary based upon the water source like industrial wastewater, Agriculture waste water, and Domestic waste water. Due to their tolerance to the adverse effects of heavy metal stress. The various self-defense techniques used by microalgae species include heavy metal immobilization, gene regulation, exclusion, and chelation, as well as antioxidants or reducing enzymes that reduce heavy metals through redox reactions[40]. In addition to heavy metals, microalgae have active binding sites in their surface with reactive groups that can combine with contaminants in domestic wastewater to cause flocculation, which lowers the concentration of total dissolved solids (TDS) and total suspended solids (TSS).

Pathogens Removal

Pathogens in waste water pose serious risks to the public's health since they may cause the outbreak of many diseases. Through many mechanisms, including as nutrient competition, pH elevation by changing the pH base to acid and oxygenation process attachment and sedimentation, and microalgal toxins, microalgae show a promising method for the elimination and inactivation of harmful bacteria. Bacterial cells will starve as a result of the fight for nutrients between microalgae and bacteria, which ultimately causes the bacterial cells to die off [35, 41]. Because CO2 is assimilated during photosynthesis in microalgae culture, pH value often increases. OH- ion was produced when every nitrate ion which converts to ammonia and nitrogen absorption by microalgal strains also raises the pH of the culture medium and the oxygen content of the waste water. Pathogens will be eliminated as a result of various variations in waste water characteristics, which may inhibit pathogen growth in waste water. *Escherichia coli, Enterococci,* and *Clostridium perfringens* are just a few examples of the faecal coliforms that are known to be significantly eliminated from waterbodies as a result of pH fluctuations and these changes negatively impact the survival of *E. coli*[35]. Microalgae's photosynthetic activity is essential to raise waterbodies' oxygen contents to levels that are harmful to bacteria. It has been determined that oxygen concentrations above 0.5 mg/L indicate the elimination of faecal microorganisms. By producing more chlorophyll-a and secreting it, green algae can remove faecal coliforms. The amount of chlorophyll-a increased along with the amount of fecal coliform decay under light conditions[42].

Dyes Removal

The dye in the wastewater discharged to the environment is primarily responsible for the water pollution caused by the textile industry. In addition, the quality of fresh water, which is essential for maintaining the ecosystem's biodiversity, has been severely decreased by the anthropogenic discharge of organic dyes. There are several water treatment technologies that have been developed and researched by scientists worldwide to treat wastewater that contains dyes [43].One of the potential methods for treating the wastewater which contains dye released by the textile industry is dye adsorption using microalgae. A number of factors are considering such as initial dye concentration, Dye contact time, acidic nature, temperature and dosage value of the adsorbent, are critical for dye removal. Therefore, these factors are taken into account when choosing the effectiveness of microalgal dye removal. For a successful decontamination process, a Consortium of microalgae, bacteria, and fungi is more effective than pure cultures[44].*Spirogyra*, a species of microalga, has demonstrated as an effective biosorbent for the removal of reactive dyes.

Pesticides Removal

Pesticides are one of the organic pollutants that microalgae are capable of absorbing and used as energy in wastewater. The removal of pesticides by using microalgae has a variety of methods; including bio adsorption in this the pollutants are removed by adsorption process, bioaccumulation, and biological degradation[45]. Pesticides and organic contaminants can be absorbed by microalgae such as aromatic compounds. Microalgae are susceptible to organic contaminants such as pesticides, which may induce cell organelles like chloroplasts, mitochondria, and peroxidases to produce reactive oxygen species (ROS). It results in functional problems and even cell death in algal cells by oxidation of membrane lipids and DNA. Superoxide dismutase, catalase, and ascorbate peroxidase are the antioxidant enzymes, which are in charge of removing ROS to reduce algal cell damage. Inducible genes can be activated by these pollutants, which cause the algae cells to release antioxidant enzymes. Microalgae's detoxification and protection systems are susceptible to contamination, which indicates the potential for bioaccumulation in microalgae in an indirect way. Microalgae have been shown to be capable of both bioaccumulating and degrading pesticides[46, 47]. Organic material in water can be broken down by microalgae into smaller molecules, which serves as sources of nutrients for microalgae growth. The metabolic reactions of various enzymes are important for the degradation of pesticides. The most important enzymes in the processes of pesticide biotransformation are esterase, transferase and for detoxification cytochrome P450[48].

Cultivation

It is simple to cultivate microalgae using different types of wastewaters. Low-density growth and algal biomass accumulation are the primary problems. Although various systems have been created to produce microalgae in wastewater, achieving high productivity is still challenging. A culture system should have a low cost of construction, an essential light resource, should easy to handle, allow best transfer via the barrier of liquid gas, and must have very less risk of contamination[70]. In addition to wastewater treatment, cultivating microalgae are effective in the fight against the greenhouse effect and global warming by lowering atmospheric carbon dioxide levels through photosynthesis. In recent years, numerous techniques and instruments have been developed for enhancing microalgae productivity. Cultivating microalgae in large scale-controlled system is difficult. During cultivation, soluble algal compounds are mixed into culture media which are all presented inside the microalgal cells. Those compounds may form in the cultivation medium, restrict growth of the microalgal cells, higher the fair for microalgal biomass recovery, and reduce the efficiency of the subsequent downstream process waste water treatment process after the cultivation microalgae[71]. Depending on the purpose for which it is used and application of the algal biomass, there are a variety of methods for growing microalgae on a large scale and laboratory conditions. The cost for the cultivation of microalgae, productivity of biomass, and control condition for the cultivation system are just a few of the differences across cultivation technologies.

Cultivation Conditions

The four main types of cultivation conditions are photoheterotrophic, photoautotrophic, mixotrophic, heterotrophic[53]. The most frequently used cultivation method for producing microalgae is photoautotrophic cultivation. When microalgae are in autotrophic mode, they fix CO2 and generate biomass using an energy source like sunlight. The Carbon dioxide may be converted from the atmospheric air, industrial operations, power plant exhaust, and soluble carbonate in aqueous phase. Due to slow cell development, phototrophic cultivation often produces substantially less oil than heterotrophic production. The open ponds or raceway ponds are the outdoor microalgae cultivation system they are usually set up under phototrophic cultivation conditions[8]. In Heterotrophic culture energy source and a carbon source for microalgae is organic carbon. Organic carbon sources are broken down in the heterotrophic mode without a need for light. Wastewater contains a variety of organic carbons, including ethanol, glycerol, and various sugars. Comparatively cultivation in heterotrophic showed many advantages over autotrophic cultivation. Nearly 20 times as much lipid could be produced with heterotrophic cultivation than under phototrophic cultivation, at its highest point. However, especially in open cultivation of microalgae in heterotrophic culture, the microalgal cultures are easily contaminated that fail to produce lipids, which can lead to failures in large-scale lipid synthesis. The major drawback is also the cost of adding an organic carbon source. Application still faces difficulties with regard to organic utilization and contamination prevention. On the other side, employing autotrophic growth reduces the impact of the pollution problems. Both heterotrophic cultivation and autotrophic cultivation contribute to mixotrophic cultivation, and both organic carbon and inorganic carbon are taken up with the sunlight. Selected microalgae can exist in phototrophic, heterotrophic or both conditions in the mixotrophic system. Microalgae grown in a photoheterotrophic environment requires light when using organic molecules as their carbon source. Mixotrophic mode of algal cultivation gives microalgae more efficiency in their growth, which leads to more effective organic and inorganic nutrient removal process and microalgal biomass productivity. This is because of the light limitation and light inhibitions are less effective when both Carbon dioxide and carbohydrates can be used for catabolic reaction[72, 73].

Cultivation Techniques

Open and closed systems are the two main types of microalgae cultivation. The most popular of these systems are turf scrubbers and open ponds with lanes (Raceway Ponds). Closed systems, also known as photobioreactors, are more diverse in terms of their shapes and configurations, with tubular, Bubble Column, Air-lift, and Flat Panel being the most popular**.** The main difference between the open and close system of cultivation is requirement of light as a source of energy, whereas the former can do it using organic chemicals. However, because of the possibility of contamination and the need for light, neither of the cultivation system is frequently used in the manufacture of microalgae oil. Scaling up microalgae cultivation in wastewater for practical applications poses several challenges, despite promising results at the laboratory scale. Transitioning to large-scale production involves addressing complexities related to reactor design, light distribution, nutrient supply, and operational considerations. Selecting the appropriate reactor type and configuration is crucial. Light availability and distribution are critical factors influencing microalgal growth and productivity in large-scale cultivation systems. Large-scale microalgae cultivation in wastewater involves complex operational considerations, including temperature control, pH regulation, CO2 supply, and biomass harvesting. Addressing these challenges requires interdisciplinary collaboration among researchers, engineers, biologists, and industry stakeholders to develop innovative technologies, optimise cultivation processes, and overcome technical and economic barriers to large-scale microalgae production in wastewater.

Microalgae Harvesting Methods

The production of enzymes and biomass can both be done using wastewater, which is a rich media for microalgal cultivation. The microalgal cultivation and biomass produced from it was harvested to use in various fields, including biodiesel, animal feed, various form of biofuel and soil fertilizer. The appropriate harvesting technique was selected based on how the microalgae biomass yield is used. Mechanical, chemical, and biological harvesting techniques are the most common techniques. Researchers and industry practitioners are exploring alternative harvesting technologies that are more cost-effective, energy-efficient, and environmentally sustainable. Some emerging harvesting technologies, like membrane-based separation techniques such as microfiltration, ultrafiltration, and membrane distillation, offer potential advantages for microalgal harvesting from wastewater. These methods utilize porous membranes to selectively separate microalgae cells from the surrounding water based on size or other properties. Electrocoagulation involves the use of electric current to destabilize suspended particles and facilitate their aggregation and precipitation. This method has shown promise for microalgal harvesting from wastewater due to its relatively low energy consumption and ability to remove a wide range of impurities. Bioflocculation harnesses the natural flocculation properties of certain microorganisms or polymers to induce the aggregation of microalgae cells into larger, settleable flocs. This approach can be more sustainable and cost-effective than chemical flocculants. Integrating microalgal cultivation with wastewater treatment processes can streamline harvesting by exploiting synergies between biomass production and nutrient removal. This approach can reduce the overall energy and resource requirements of microalgal harvesting from wastewater. The biomass concentration in the culture may change based on the type of photobioreactor and the culture conditions utilized throughout the wastewater treatment. To be in compliance with regulations concerning the quality of released water, all of the biomass must be recovered. For reducing the water used in domestics for growing microalgae, the technique of harvesting that is chosen has significant effects on how much of the recycled water may be used again[49,50]. Microalgae from the production media are harvested throughout the process of two phases. The biofilm of microalgae about 2-7% on the surface of the growth media is separated in the first phase using flotation, precipitation, and coagulant additions. In second phase the suspended microalgae cells are separated using filtration or centrifugation. it could not be harvested using the method previously mentioned. The total process must be economically feasible and energy-efficient for these procedures to be effective. Choosing microalgae strains that are easy to harvest is important [51].

Physical Harvesting Method

The most reliable and efficient methods for harvesting microalgae are physical ones, commonly referred to as mechanical biomass harvesting methods. These techniques make use of centrifugation, filtration, flotation, and gravity sedimentation processes. With the help of gravity and the density of the microalgal biomass, concentrated slurry is removed using the energy-efficient solid-liquid separation technique known as gravity sedimentation. The supernatant then condenses into a clear aqueous layer[52]. One of the methods for separating solid material from the liquid phase is centrifugation. Bacterial biomass was effectively separated from the culture media by centrifugation. For microalgal biomass recovery process, this method similarly gives results of 80–90% efficiency. In big centrifuges, ice bath water is provided to the centrifuge in order to prevent the temperature from rising. The biomass of cultured microalgae used for the extraction of nutrition's is more appropriate for the centrifugation harvesting technique. Because there are no chemical additives employed in this technique like there are in the chemical harvesting method, the microalgae biomass harvested may be safely stored for a very long time[53]. Filtration is less expensive, uses less energy, doesn't require any chemicals, and works effectively for cells which are not harvested shear process due to the sensitivity. 5-27% of the solid content can also be recovered by the procedure, and 70-90% of the biomass can be recovered. A porous membrane of a specific size is used in the filtration process to pass wastewater medium containing microalgae through. The filtrate is made up of the leftover wastewater after the microalgae slurry has been retained by the porous membrane. The removal of suspended particles and bacteria from water and wastewater is commonly accomplished using the membrane filtering method[10]. Due to the absence of chemicals, in filtration process the supernatant can be reused as a production medium for microalgae biomass it was the main advantage. The main consequence of membrane filtration is the development of microalgae on the filter surface, which can increase the resistance and decrease filtration flux in response to a steady pressure drop[52]. The microalgae cell and bubble size formed are important considerations in the flotation-based method for collecting microalgae biomass. In the flotation process gas bubbles was produced by maintaining aeration that act as lifters to move and separate particles. Microalgal biomass has low density due to that the flotation is widely used for the processes of wastewater treatment and is more efficient than sedimentation. The microalgal cells are very tiny and making the flotation process more appropriate for harvesting them[54,55].

Chemical Harvesting Method

In chemical harvesting flocculants, coagulants, and chelators such as salts and alum, as well as organic polymers, inorganic polymers, and also the naturally available biological flocculants are used. The auto flocculation of microalgal biomass occurs naturally in the environment as a result of an increase in pH to an alkalinity level, which is connected to the photosynthetic process of algae. This process is considered to be an effective method to collect microalgae from wastewater[56]. Different types of flocculants or coagulants are used in the flocculation process, which causes microalgae cells to flocks of in the culture medium as a result of sedimentation. Microalgae cells typically have a diameter of about <15 ìm, on their cell surface they have negative charge, and a higher density than that of water. While adding the chemical agents' coagulants or flocculants, which have a positive charge in the medium, causes them to bind on membrane of the microalgae cells because they have negative charge[10]. The flocculation process depends on positive charges interactions of the inorganic flocculant or coagulant and the microalgae cell surface has negative charges caused by the presence of different functional groups. The organic polymers in organic flocculants serve as a pathway between the chelating metal ions and microalgal cells, increasing the floc size and facilitating better sedimentation of the microalgae biomass[57, 58]. The harvesting of microalgae biomass was successfully accomplished with the help of inorganic flocculants such ferric chloride and alum. The adverse effects associated with the obtained biomass, it may have a higher concentration of metals,

Table 1. Most widely used microalgal species for waste water treatment

Microalgae	Wastewater type	References
Chlorella vulgaris	Municipal wastewater	Gao *et al.,* 2014.
	Piggery wastewater	Abou-Shanab *et al.,* 2013.
Chlorella pyrenoidosa	Degradation of azo dyes wastewater	Wang *et al.,* 2012.Jinqi *et al.,* 1992.
	Diluted primary piggery wastewater	Lin *et al.,* 2007.
	Landfill leachate	
Scenedesmus sp.	Wet market wastewater	Hena *et al.,* 2015.bte Jais *et al.,* 2015.
	Dairy farm wastewaterpaper	Narro *et al.,* 1987.
Botryococcusbraunii	Secondarily treated sewage in batch and	Sawayama *et al.,* 1992.
	industry wastewater	Sawayama *et al.,* 1994.
	continuous culture	
Chlamydomonas sp.	Meta cleavage in wastewater	Jacobson *et al.,* 1981.
	Dairy industry wastewater	Kothari *et al.,* 2013.
spirulina platensis	Domestic wastewater treatment	Lalibert *et al.,* 1997.
Protothecazopf	Degraded petroleum hydrocarbons	Walker *et al.,* 1975.
Gonium sp.	Textile effluent	Boduroðlu *et al.,* 2014.
Tetraselmis	Fish farm wastewater	Michels *et al.,* 2014.

is where these chemical elements implications are found[59]. Organic polymers like chitosan and starch which are biodegradable as well as the natural flocculants *Strychnospotatorum, Moringa oleifera* demonstrated great effective process of flocculation of wastewater and microalgal biomass was harvested without toxic effect on the microalgae cells. The presence of several metal ions may make flocculation less successful when collecting the biomass of microalgae produced in the phycoremediation of wastewater[36]. Some metals will reduce the ability of the coagulant and flocculant to interact with the cell surface of microalgae. Chemical flocculation is considered as a reliable, high-yield method because it may be used on large quantities of culture without affecting cellular structure. Furthermore, the recovered microalgae biomass is not significantly at risk of contamination when using these, inexpensive, simple to use chemicals. As a result, chemical harvesting techniques are useful in the effective harvesting of microalgae biomass for a variety of uses[60].

Lipid Extraction

The dried biomass of microalgae has a lipid content that ranges from 20 to 50%. However, by using a specially designed culturing technique, the microalgae lipid content can be increased. Because of they include fats, oils and triglyceride molecules that can be converted into alcohol esters by esterification process, various oils obtained from microalgae have a lot of applications. The resulting “ester fuels” have demonstrated that they may be blended up to 30% with diesel stocks without affecting engine performance[61]. For the production of biofuels with relatively low energy and cost requirements, lipid extraction from microalgae is an important challenge. The cell structures and compositions of various microalgal strains vary. Some microalgae can be extracted relatively easily, but in other cases the lipid may be present intracellular and protected by rigid cell walls, making extraction more difficult. Even though they have a low production cost, microalgal lipid extractions have drawbacks such as low mass transfer, poor yield, and prolonged extraction times[62]. Chemical, physical, or combinations of the two methods are used to extract lipids from biological material. In order to retrieve sugar and lipids present intracellular in microalgae for the production of biofuels, cell disruption is often necessary. Mechanically disrupt the cell to release the desired component followed by extraction process by using chemical solvent is the typical method for large-scale lipid extraction from microalgae. Many methods are used to extract lipids from microalgal biomass including mixed Bligh-Dryer method methanol-chloroform, ultrasound-assisted extraction, hexane Soxhlet Extraction and microwave assisted extraction.

Each microalgae biomass extract was filtered from each of those processes to remove any remaining biomass. After that, the filtered solution will begin to evaporate, and the remaining crude lipid will be gravimetrically measured. The Soxhlet Extraction method is usually considered as the best method of extraction because it can fully extract the entire lipid present in microalgae, yielding a 100% recovery[63]. Two ranges for lipid contents in various microalgae species have been categorized, related to the lipid content. The information provided above show that several microalgal species accumulate large amounts of lipids[64, 65].

Biodiesel Production

After the lipids extracted from microalgae it has a high viscosity and can't be used directly as fuel, a conversion process is required in order to make biodiesel[66]. Due to its characteristics of renewable energy, undergoes biodegradable process, environmental friendliness, and reduced greenhouse gas (GHG) emission and the most valuable third generation biodiesel is produced from microalgae is thought to be the most valuable, possible substitute for fossil fuels[67]. It is possible to grow microalgae on non-agricultural land, suggesting that a small area is enough to produce algal biodiesel. According to recent reports, wastewater can be used to cultivate microalgae that can breaks down organic pollutants and produce biomass. In this regard, there are various techniques for converting microalgal biomass into biofuel like Chemical, biochemical, and thermochemical conversion are the processes that can be used[68]. Therefore, in order to produce a biofuel that provides good engine performance, the microalgae oil viscosity must be lowered before it's used. A common technique is transesterification for reducing the viscosity of microalgae oil.

Transesterification

A common method for reducing the density and viscosity of the microalgae oil extracted from biomass is transesterification, which converts microalgae oils into the equivalent FAME which is also referred to as biodiesel by chemically. Alkalis, acids, and enzymes are the primary types of catalysts that can be used to the production of biodiesel[6].

In catalytic transesterification process the base catalysts which are not high cost and use of optimum temperatures and pressures for reaction are used. Sulfuric acid was commonly used because it was highly catalytic it is the most widely used acid catalyst. However, base catalysts are more frequently utilized in transesterification than acid catalysts. The reaction rate of acid catalyst is four thousand times slower than that of base catalysts[69].

In non-catalytic transesterification method methanol is used at a critical temperature, in a single reaction the algal lipids can extract from cell and transesterify. Supercritical methanol (SCM) is a desirable material for the synthesis of industrial biodiesel because coupling the two processes saves much time and lots money. Similar to the SCM, in-situ transesterification also known as direct transesterification involves simultaneously performing the extraction and transesterification processes. Compared to the traditional process of biodiesel production, this simplified method has an advantage. In-situ transesterification has the benefits of use of minimal solvent consumption, simple product separation, and fast reaction times. Although homogeneous catalysts are producing a lot of toxic substances at the end of reactions since the catalyst is neutralized and the products are separated. Even though heterogeneous catalysts don't produce large waste, their costs prevent them from being used on a large-scale operation. In-situ transesterification, which combines extraction and conversion into a single phase, was created to reduce production expenses as a result of these economic challenges[62].

CONCLUSION

In the production of biodiesel and wastewater treatment using microalgae, sustainability is a fundamental concept. Due to the significant cost savings, alternate for nutrients the wastewater was used as a source of essential for the success of the production of fuels. It is commonly known that microalgae show great potential for use as a source of food, energy, and high-value bio compounds. The use of microalgae in wastewater treatment is useful because they can regulate waste and produce bioenergy at the same time. Domestic sewage, Industrial waste waters, Agricultural waste water, agro-industrial, and wastewaters from livestock are all capable of being treated by specific microalgal species. Additionally, some techniques using algae to remove high toxic minerals such as Lead, Scandium, Arsenic, Bromide, Cadmium, Mercury and Tin ions have been used separately or in combination[7]. The cost of crude oil is rising, therefore it appears like a reasonable approach to blending biodiesel with petrodiesel. In the short term, the current use of first-generation biofuels may have negative consequences on the environment, economy, and society. Making biodiesel from microalgae lipids appears to be a sustainable way to solve these issues because of its high productivity and low land usage needs. Researchers, entrepreneurs, and the general public have recently renewed their interest in using microalgae as a substitute source for biodiesel.

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