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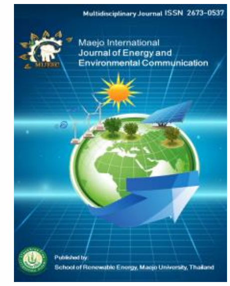

ARTICLE

Production of biofuel from microalgae grown in wastewater- A review

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ABSTRACT

There is an urgent demand for an evolution of renewable energy resources due to the rising global energy demand, resource depletion, growing emphasis on reducing climate change, and resource scarcity. Among many resources, Microalgae are thought to be the cleanest alternative feedstock for biofuels and biorefineries because of their advantages of quick growth, effective carbon dioxide fixation, lack of competition for agricultural production and potable water, and the potential to accumulate massive volumes of lipids and carbohydrates. Therefore, this review has discussed the different wastewater which can support and enhance the growth of microalgae, biosynthesis of lipids, production of biofuels from microalgae, and biochemical properties of biofuel. In order to minimize the accumulation of wastewater to protect the environment from pollution, apply wastewater to grow microalgae to achieve the goal of bio circular economy. This review provides clear details and a summary of the production of biofuel from microalgae grown in wastewater.

1. Introduction

There is a negative impact on our environment because of the quick social development and ongoing rise in the human population (Gotore et al., 2022a). Water coming from different industries and manufacturing units has affected our water reserves and it does not applicable for agricultural due to the presence of organic compounds, heavy metals, total nitrogen, total phosphorus, and other chemicals (Gotore et al., 2022b). There is an urgent need to treat these large quantities of wastewater and prevent environmental pollution and protect public health by providing safe water for consumption (Whangchai et al., 2022). There are different types of wastewaters and can be categorized into textile wastewater, anaerobic digestion wastewater, agro-industrial wastewater, metal-containing wastewater, and wastewater coming from pharmaceutical companies (Krishnamoorthy et al., 2021). Conventional treatment techniques have many problems associated with them, such as large energy requirements, large land

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requirements with high operational costs and regular maintenance requirements (Manmai et al., 2022).

Algae provide an alternative method for the treatment of wastewater for the removal of unwanted material and chemicals from the wastewater, algae use these unwanted chemicals as a nutrient for their growth and convert them into biomass (Manmai et al., 2022). The simulation of wastewater treatment with algae biomass cultivation for liquid biofuel generation is shown in Figure 1. There are some species of algae that could take up other pollutants, such as heavy metals, compounds of nitrogen, and other harmful chemicals (Pimpimol et al., 2020). It has been confirmed that some species of microalgae can eliminate chemicals like nitrogen and phosphorus from wastewater and achieve a significantly low concentration of 2.2 and 0.15 mgL^{-1} and use the chemical as nutrients for biomass production (Boelee et al., 2011). Previous research suggests that wastewater is a rich source of different nutrients, having a good quantity of organic carbon (Unpaprom et al., 2015; Wang et al., 2015). *Chlamydomonaas reinharditi* has been stated to have properties of Pb removal (380.7 mg g^{-1}) (Bayramoğlu et al., 2006). Algae offer many other advantages as rapid growth, high lipid content, more environmental tolerance, not depend on land usage, and no limitation of seasons for cultivation (Unpaprom et al., 2015).

Many researchers have demonstrated cultivation of algae using wastewater helps to reduce the cost of cultivation and much more effective when compared to the traditional method of algae cultivation (Wilkie et al., 2002; Wang et al., 2011). Normally, in wastewater algae grows with bacteria. Bacteria can aid in efficiently oxidizing the chemical oxidation demands (COD), in combination with CO_2 production. Algae utilising of CO_2 and into biomass by the process of photosynthesis, and O_2 is produced to aid in bacterial growth (Tsai et al., 2017). Therefore, wastewater is treated well by an algae-bacterial consortium system with no need for additional oxygen supply. Also, nutrients can be converted into biomass, and CO_2 emission in the atmosphere will be reduced (Ramaraj et al., 2015). Algae are a cutting-edge form of water treatment since they are economical, environmentally friendly, and produce goods with a market value. Algae are more effective at removing nutrients from the environment than other types of microbes because Ammonia, nitrate, phosphate, and trace minerals found in different wastewaters are among the nutrients that algae can use (Tsai et al., 2017). Open and closed microalgae farming method increase the productivity of biomass.

Cultivating algae is an efficient method for removing toxins from wastewater while simultaneously providing a source of energy that is necessary for development. This review focuses primarily on analysing the different types of biofuels that can be generated from wastewater algae cultivation, the different types of biofuels that can be generated from algae cultivation, and the use of wastewater to promote algae-based biofuel production and wastewater treatment to achieve the goal of commercialization.

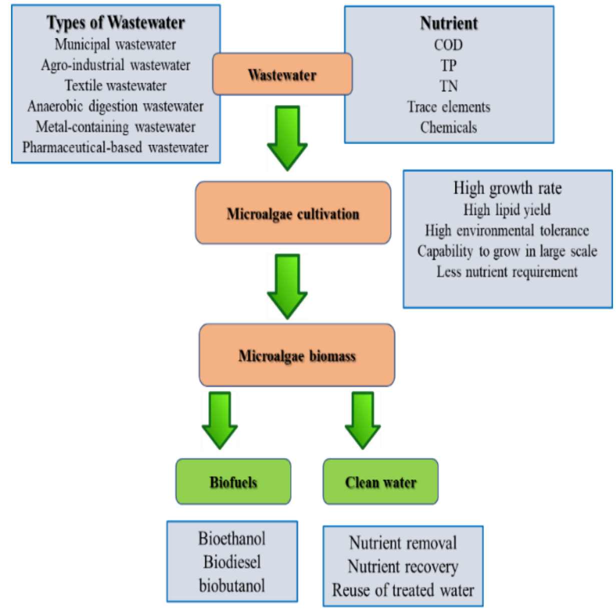

Figure 1 Schematical representation of simulation of wastewater treatment with algae biomass cultivation for liquid biofuel generation.

2. Different Wastewater Treatment by Algae

2.1 Municipal wastewater

Municipal wastewater production has grown because of growing urbanization and urban population growth. Nitrogen and phosphorus are present in municipal wastewater, but there are higher levels of HMs like Pb, Zn, and Cu. There are three phases taking part in the conventional municipal wastewater treatment. Removing out dissolved inorganic elements, in the advanced treatment process such as post aeration, carbon adsorption, filtration, and membrane filtration which helps to eliminate the pollutant compounds (Cai et al., 2013). Particularly energy-intensive procedures like aeration make up 45% to 75% of a wastewater treatment plant's net energy expense. According to research, aerobic processes fueled by algal-bacterial interactions present a workable substitute for treating wastewater. Algae supply the necessary oxygen for bacteria to eliminate contaminants, as well as the CO_2 that bacteria require (Arcila et al., 2017).

According to studies, chlorophyte algae, such *Chlorella*, can grow well in municipal wastewater (Arcila et at., 2017). *Chlorella sp.* has grown in every sample of wastewater examined. *S. obliquus* and *C. stigmatophora* were cultivated in urban wastewater at different ratio of nitrogen and phosphorus from 1:1 to 35:1, and Arbib et al. examined the microalgal growth rate and nutrient removal (together with CO_2 bio fixation). The optimal nitrogen-to-phosphorus ratios for batch biomass productivity ranged from 9 to 13 (Arbib et al., 2013). For the treatment of municipal wastewater, algae-bacteria aggregates were investigated. Low irradiance levels encouraged the development of algal-bacterial flocs and granules, achieving the highest TN, total COD, and P elimination values of 60 5%, 89 3%, and 28 7% (Arcila et al., 2017).

2.2 Agro-Industrial wastewater

Swine-and-dairy manure wastewater is a common example of Agro-industrial wastewater contain high content of organic matter, TN, and TP nutrients (Hilhorst et al., 2001). *C. vulgaris* JSC-6, a carbohydrate-rich microalga, effectively removes 40%–90% NH_3–N and 60%–70% COD from swine effluent (Wang et al., 2015). *C. vulgaris* was also noted as a successful bioremediation agent for POME, resulting in decreases of 61%, 84.0%, 50.5%, and 61.6% in ammonia-nitrogen, phosphorus, COD, and BOD, respectively (Kamarudin et al., 2013). The farming system may lose N and P from fertilizers through runoff or leaching channels, which have the capacity to sustain algal growth (Hilhorst et al., 2001). Additionally, agro-industrial wastewater frequently exhibits high ammonium concentrations, which are closely associated with eutrophication (Wang et al., 2015). Fortunately, many species of algae can thrive in "nutrient-rich" conditions and quickly produce biomass from the nutrients found in agro-industrial wastewaters (Hernández et al., 2016). Additionally, cyanobacteria like *Oscilatoria*, *Anabaena*, and *Spirulina* can use elemental nitrogen as their only supply of nitrogen by reducing N_2 to ammonium. In general speaking, cyanobacteria prefer to use ammonium over nitrate since nitrate uptake depends on light (Palanisamy et al., 2021a). Algae and cyanobacteria working together could be a potential solution for ammonium-rich agro-industrial wastewater. High ammonium concentrations would prevent nitrate uptake because nitrate reductase production is suppressed by ammonium, whereas ammonia uptake is impeded by high nitrate concentrations (Ohmori et al., 1977).

2.3 Textiles wastewater

The textile industry, which produces more than 7×10^5 tons of dyestuffs year worldwide, is Z one of the primary traditional industries in the world that uses a variety of commercially accessible dyes (Robinson et al., 2001). Textile wastewaters contain dyes, binder, waxes and reducing agents. This composition of these dye effluents varies greatly. It has quite intense color with high COD, pH, salinity, and temperature. These effluents can have a negative impact on water quality and gas solubility, which makes aquatic plants and animals more harm and causes serious environmental issues on a global scale. Algae are intriguing bacteria because they can accumulate lipids that can be converted to biodiesel by transesterification and can cleanse textile effluent by absorbing fertilizers and dyes (Fazal et al., 2018). There are algal bioremediation of textile effluent takes place in two methods such as bioconversion and processes of bioaccumulation and biosorption. In addition to adsorbing colours to their surface, algae also use dyes as carbon sources, which they then bio convert into metabolites (Fazal et al., 2018). The degradation of azo dyes into simple aromatic amines by algae species like *Chlorella vulagris*, *C. pyrenoidosa*, and *Oscillatoria tenuisin* results in the decolorization of dye effluent (Fazal et al., 2018). *C. vulgaris* to breakdown azo bond has also been reported (Lim et al., 2010).

2.4 Anaerobic digestion wastewater

One of the biological processes that are most frequently used to effectively turn waste activated sludge into bioenergy is anaerobic digestion (e.g., H_2 and CH_4) (Zahedi et al., 2016). However, because AD effluents include high levels of COD, TN, and TP, AD by-products (both residues and effluents) are seen as a threat to the environment (Cheng et al., 2015). Additionally, the produced biogas often contains 3000-5000 ppm H_2S and 20%–60% CO_2, making it unsuitable for use as fuel gas without previous purification (Kao et al., 2012). Therefore, it is vitally necessary to create a low-cost method to treat AD wastewater and improve the quality of biogas in order to fulfil the actual demand.

Algae are thought to be a practical way to turn effluent wastes into biofuels due to their rapid growth rate, excellent environmental adaptation, and remarkable nutrient-removal ability (e.g., biodiesel) (Ho et al., 2018). Photosynthesis during microalgal culture can turn CO_2 into biomass (Campbell et al., 2006). CO_2 can be converted into sugars and glyceraldehyde 3-phosphate (GAP) through Calvin cycle, the main precursor for the synthesis of triacylglycerol, which can build up under stressful environmental conditions (Mohan et al., 2015). It is preferred to use algae for mutageneis which can tolerate high CO_2 and CH_4 concentrations. (Boelee et al., 2011).

2.5 Metal-containing wastewater

Metal-containing wastewater occurs from metal plants, battery production and petroleum refineries (Lim et al., 2010). That use industrial procedures that incorporate HMs (or substances containing HMs). Wastewaters containing HMs are produced because of these activities and released into surface water. HMs are among the most dangerous pollutants because of their poisonous and nonbiodegradable qualities, which pose a serious risk to both human health and natural systems. According to reports, several harmful and carcinogenic heavy metals such as Ni, Cu, Cr, Pb, Cd, and Zn, may seriously harm human health and should be eliminated from the environment (Ho et al., 2017). There are many studies that have reported heavy metals (HM) being removed from wastewater by algal biomass (Kalin et al., 2005). In general, the two categories of the algal remediation phenomenon are bioaccumulation by living cells and biosorption by non-living, nongrowing biomass or biomass products (Kumar et al., 2015). The biosorption of HMs by living algae cells is a difficult process. However, according to Monteiro et al., the two-stage process by which HMs are normally accumulated by algae is I the first quick removal of HMs at the cell surface, and (ii) a considerably slower process that takes place inside the cell (Monteiro et al., 2012). The first phase, known as passive elimination, happens in both living and non-living cells and is nonmetabolic, quick, and virtually reversible. Here, HMs are tightly bound to the organic functional groups of algae, resulting in the adsorption of HMs onto both cell surfaces and extracellular polysaccharides. Other adsorption mechanisms include physical adsorption, ion exchange, electrostatic adsorption, chemisorption, coordination, microprecipitation, complexation, chelation, entrapment in the network of structural polysaccharides, and diffusion through membrane (Monteiro et al., 2012).

2.6 Pharmaceutical-based wastewater

Pharmaceutical substances (PCs) are pervasive in aquatic ecosystems and present a serious concern to both wildlife and people (Kolpin et al., 2002). Unfortunately, a lot of PCs have been consistently discovered in wastewater over the past few decades since they were ejected from industrial wastes, home wastes of the pharmaceutical business, industrial wastes, and hospital effluents (Ternes et al., 1998). Even though the majority of PC concentrations in the environment fall between ng L1 and g L1 levels, data suggests that PCs have the potential to have disastrous ecological consequences on both target and nontarget organisms. For instance, they can alter microbial communities, restrain microbial communities, restrict microbial communities, scale back microbial communities, they can alter microbial communities, restrain microbial communities, restrain microbial communities (Xiong et al., 2018)

Additionally, PCs often have a long environmental half-life and are challenging to biodegrade. High-trophic level species (including humans) have harmful PC concentrations due to biomagnification in food chains (Kelly et al., 2007). Energy is used to power bioaccumulation, an active metabolic process for absorbing substrates. Previous studies shown that algae can bioaccumulate nutrients for growth as well as organic contaminants. For instance, the green alga *Nannochloris* sp. may remove the drugs triclosan, trimethoprim, and sulfamethoxazole through bioaccumulation, and *Desmodesmus subspicatus* can accumulate about 23% of radiolabeled 17-ethinylestradiol in under 24 hours (Maes et al., 2014).

3. Biosynthesis of lipid in microalgae

Lipid content microalgae have become the practical feedstock for the large-scale production of biofuels due advantages of doesn't need fresh water. Their variety of species can accumulate triacylglycerol (TAG) up to 70% of its dry cell weight. However, there are some challenges that need to be gone through to come up with microalgal biofuel as a sustainable and cost-effective alternative biofuel. Studies reported that Inefficient light harvesting capacity in native development conditions and the absence of two characteristics, high lipid content and rapid growth rate, in extant microalgal species, are the main constraints. Therefore, Sharma et al. (2018) reported that understanding the genetic and metabolic controls for triggered metabolic flux, target subcellular storage location, and potential release of lipids, particularly TAG biosynthesis, could help in strain improvement and maximize the cost-effectiveness of microalgal biofuel production (Behera et al., 2021). This helps to overcome the major strides which can be made in strain improvement by biotechnological treatments to produce biofuel on a commercial basis.

Biosynthesis of lipid involves numerous metabolic pathways to produce lipid molecules. The process begins at the chloroplast of microalgal cells. This organelle utilise the atmospheric carbon to produce starch, which is then catabolized by glycolysis to create the constituents of fatty acids (FA) and TAGs. The Kennedy process, which requires the integration of FAs into a glycerol backbone to generate TAG, is the pathway that is specifically used to synthesize store lipids. Stress situations cause lipid accumulation, although the underlying process is not well understood. TAG accumulation in growing seeds is regulated by developmental signalling, whereas lipid accumulation is triggered by stress conditions (Palanisamy et al., 2021b). Cornell (1977) reported that, lipid synthesis is regulated by specific cycle in the microalgae cell. Most often, stressors resist the cell cycle progression, which led to the theory that they really cause lipid build-up. Stressors frequently prevent cell cycle progression, which led to the theory that they really cause lipid accumulation (Palanisamy et al., 2022a). Thus, validation is necessary for this kind of induction procedure. In aquatic situations, changes in buoyancy brought on by lipid enhancement may aid in motility and provide protection for microalgae.

There are some modifications can be made to enhance the lipid synthesis by certain factors such as pH, temperature, salinity, light intensity, and nutrient depletion. Nitrogen starvation highly influence the lipid accumulation in cells according to the study of Latsos et al. (2020). Besides physical parameters, enhancing cellular lipid accumulation under typical growth circumstances is possible by genetic modification of microalgae as shown in Figure 2, which provides greater opportunities for the precise regulation of target systems (Sharma et al., 2018). Modification of enzymes is primer route to convert the carbon and reductive equivalents flux for lipid enhancement. Moreover, the modification of specific genes is responsible for different metabolic process steps. Nevertheless, due to the complex regulation of lipid production in microalgae, this approach has had varying degrees of success.

There are several earliest efforts have been undertaken to boost the expression of key enzymes involved in FA production because the FA supply governs the lipid biosynthesis process. Ohlrogge et al. (1997), mentioned that fatty acid supply might regulate lipid biosynthesis by overexpressing the acetyl coA carboxylase gene. However, it does not highly support lipid synthesis. Besides that, blocking the metabolic pathways that are competitive to lipogenesis is an effective way to improve lipid accumulation in microalgae. Conventional genetic engineering techniques depend on a single gene to change the elements of a metabolic pathway. Transcriptional factor engineering is an alternate approach that can simultaneously edit several elements of a metabolic pathway; such methods have made it possible to significantly re-engineer the cellular lipid content of microalgae (Sun et al., 2019)

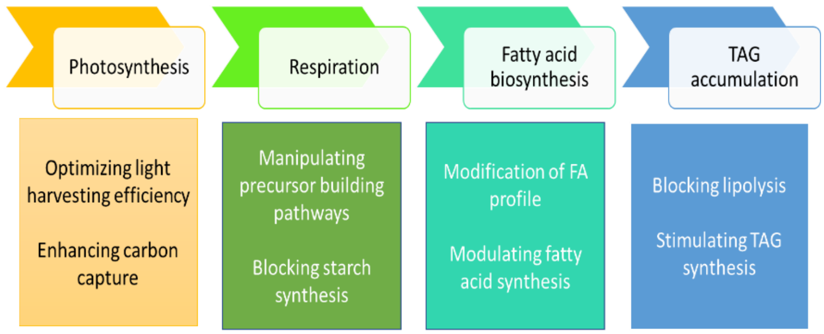

Figure 2 Genetic engineering techniques applied in microalgae biodiesel production

4. Cultivation method of microalgae

A range of growth modes and production strategies explicitly impacts the productivity and biochemical makeup of microalgae. Photoautotrophic, Heterotrophy, and mixotrophy are a few of the growth modes that can be used, along with combinations of other culture systems in closed or open systems (Palanisamy et al., 2021a). The different methods that may be utilized to develop microalgae are compared in Table 1, which can be seen below. Even though most microalgae are phototrophic, which means that they get their energy from exposure to light, there are those that can get their energy from organic substrates (Palanisamy et al., 2022a). This system offers an excellent method for managing algal culture because heterotrophic microalgae are typically grown in fermenters using well-established traditional technologies that have high productivity, production repeatability, provide appropriate light intensity, and cost-effective harvesting (Chisti, 2007; Palanisamy et al., 2022b).

In addition, streams of heterotrophically cultivated microalgae generate a greater amount of lipid when compared to the same microalgae produced using sunlight as the primary source of photosynthesis. Taking this into account, *Chlorella* sp. microalgal cells may build up 55.2% of their lipids by heterotrophic means, compared to just 14.6% through phototrophic means. Despite the fact that such a culture is suited for the production of biodiesel, the most major negative of such a culture is the expensive and labor-intensive nature of the fermentation process. There are many different growth methods, each of which has its own set of benefits. One example is open systems, which have low operating and capital expenses, are capable of producing algae on a big scale, but are especially prone to contamination.

The thoughtful selection of microalgal growth modes is the key to accomplishing successful production and algae cultivation goals in aquaculture (Mahari et al., 2022). Saratale (2022) reported that, despite being very inexpensive and simple to cultivate, photoheterotrophic microalgal production requires occasional cleaning. While mixotrophic culture offers the possibility of separating CO_2 and increased lipid and carbohydrate build-up in addition to an easier scale-up process, the heterotrophic mode has been used to avoid the high expense, connected with feedstocks (Saratale et al., 2022).

One type of organic stream that can support a sustainable economy is one that contains open ponds or raceways for the development of microalgae. Open ponds typically range in depth from 1 to 100 cm in this oldest and most widely used farming technique. It comprises a straightforward pond made of concrete or dug into the ground with the aid of a paddle wheel system and a polymer raceway of various diameters (Singh et al., 2014). It produces between 15 to 25 tons of dry biomass per hectare annually. This method of farming is recognized as a low-cost technique for generating more biomass and lipids, hence it is becoming increasingly popular (Saratale et al., 2022).

Traditional closed photobioreactors were developed as a solution to the primary challenges that were present in open or raceway pond culture setups. With the help of photobioreactors, large quantities of microalgal biomass may be grown, and these devices can also be used to develop a single species of microalgae in a way that is environmentally friendly over long periods of time (Nageshwari et al., 2021). The photobioreactor was applied in various sizes and shapes such as tubular, column, vertical, bubble column, airlift, flat inclined, column aeration and multistage continuous flow specification. Photobioreactors have the potential to benefit from the utilization of wastewater as well as the flue gases that are released by power plants; however, this utilization is prohibitively expensive.

Photobioreactors that can be positioned either vertically or horizontally and have tubular forms are the ones that see the most use. They are constructed up of several transparent, translucent tubes with a diameter of 10 ems that are manufactured out of glass and plastic and allow for optimal sunlight dispersion. In this method of plant growth, an algal culture is pumped back into a reservoir after being cycled through tubes that are capable of producing effective photosynthesis (Chisti et al., 2007).

Table 1 Cultivation method of microalgae for biomass production (Rashid et al., 2019)

Autotrophic	Heterotrophic	Mixotrophic
Use CO_2 as a source of carbon	Use organic carbon	Use organic and inorganic carbon sources
Low efficiency of bioconversion	High efficiency of bioconversion	High efficiency of bioconversion
Limited biomass production	High productivity of biomass	High productivity of biomass
Low growth in wastewater	High growth in wastewater	High growth in wastewater
High purity of biomass	Low purity of biomass	Variable biomass composition
Limited ability to tolerate environmental factors	High tolerance of extreme environmental conditions	Acceptable tolerance to environmental conditions

5. Type of biofuel

Many of the most urgent problems facing the globe, such as energy security and climate change, are said to be solved by biofuels (Arabi et al., 2019). In the most likely scenario, the demand for biofuels will increase by 41 billion litters, or 28%, between 2021 and 2026. One-fifth of this demand surge is due to a return to pre-Covid-19 consumption levels (IAE, 2021). Microalgae have the tendency of higher photosynthetic levels which can convert into desirable biofuels. Thylakoid membranes contain the lipids which is nontoxic and biodegradable.

5.1 Biodiesel

A sustainable, biodegradable fuel made domestically from vegetable oils, animal fats, or used restaurant grease is called biodiesel. The Renewable Fuel Standard's biomass-based diesel and total advanced biofuel requirements are both satisfied by biodiesel. Biodiesel is not the same as renewable diesel, sometimes known as "green diesel." Biodiesel known as B100 which is a liquid fuel. It’s like petroleum diesel which apply to fuel compression-ignition engines. It can be produced through chemical process known as transesterification (Palanisamy et al., 2021a). Triglycerides are reacted with an alcohol catalyst in this reaction, which results in the production of glycerol and fatty acid. Triglyceride feedstocks, which are typically utilized in the production of biodiesel, are subjected to a catalytic reaction in the presence of hydro to produce renewable diesel fuel, also referred to as "green diesel." (Bhuyar et al., 2021). The results of this procedure are hydrocarbons rather than fatty acid alkyl esters, alcohol is not necessary, and no glycerol by-product is produced. It is possible that the present diesel crisis can be remedied by using biodiesel that is created from microalgae; however, in order to use this technology, strains of microalgae that have a high growth rate and oil content will need to be selected (Tiwari and Kiran, 2018).

5.2 Bioethanol

Microalgae highly rich in cellulose. It can be breakdown into glucose by pre-treatment of hydrolysis. Beside cellulose, microalgae cells present with lignin and hemicellulose. These are the material which has limit conversion and combined with polysaccharides. To convert the complex molecules into simple form delignification process are needed to perform before hydrolysis. In this process, there are three steps such as reduction of lignin content and enhance the polysaccharides exposure through pre-treatment. Then, polysaccharides convert into glucose and xylose monomers. At last, sugar fermentation to ethanol. Microalgal biomass that has been defatted can be hydrolysed to yield a sizable amount of reducing sugars, which can then be fermented to ethanol. The biomass that is left behind after extracting oil and other useful chemicals can be used in the production of ethanol after suitable processing (mechanically, enzymatically, and chemically) to release fermentable sugars in order to solve the economic issues associated with microalga production (Fetyan et al., 2022).

5.3 Biobutanol

Biobutanol is seen as an appropriate replacement for traditional fuels. Due to a variety of benefits, it is more efficient as a biofuel than biomethanol or bioethanol due to its higher energy density and chemical resemblance to gasoline. This shows that it can be used for in-place fuel engines more easily, either alone or in combination with diesel (excellent inter-solubility). The limited product yields of the biochemical process used to manufacture this biofuel, acetone-butanol-ethanol (ABE), severely limit the amount of biobutanol that can be produced (Figueroa-Torres et al., 2020). Acidogenesis and solventogenesis are the two steps in the two-stage ABE fermentation process carried out by Clostridia species. The solventogenic phase, where accumulated acids are transformed into the final ABE products, begins when carbohydrate-rich biomass is first metabolized into organic acids (such as acetic acid and butyric acid). It has been suggested that methods for increasing butanol yields include altering the fermentation media (such as the type of carbon substrate) and externally supplementing acetic acid and butyric acid (the two main precursors for solventogenesis), provided that an ideal composition has already been determined (Figueroa-Torres et al., 2020).

6. Properties of Biofuels.

6.1 Biobutanol

Nowadays, butanol is now thought to be the most advanced biofuel that may be used as a substitute for gasoline, diesel, and kerosene. Butanol has a greater tolerance for water contamination, mixes at higher percentages without requiring vehicle modifications, has a higher energy density, makes vegetable oils less viscous, and can dissolve in vegetable oils at any ratio (Niemistö et al., 2013). Butanol can be converted into jet fuel, it mixes with gasoline, and it also extends the richness of fuel. Butanol can be added to gasoline as a fuel additive and utilized in internal combustion, which produces just carbon dioxide, making it a more ecologically friendly biofuel. Groundwater pollution is less likely since butanol is less soluble in water (Ranjan et al., 2011). Additionally, through a biochemical fermentation process, biobutanol may also be made from biomass feedstock (Niemistö et al., 2013). The biofuel properties were compared among biodiesel, bioethanol, and biobutanol in Table 2 below.

6.2 Biodiesel

The term "biodiesel" refers to a fuel made from mono-alkyl esters of long-chain fatty acids generated from renewable energy sources, such as vegetable oil, animal fat, etc. The American Society of Testing and Materials (ASTM) D 6751 and the European Union's (EU) EN 14214 criteria must both be met for biodiesel to be classified as B100 (Siraj et al., 2017; Bryan et al., 2009). Greater cetane number, higher biodegradability, higher combustion efficiency, and lower carbon monoxide emissions are some of the benefits of biodiesel over diesel fuel. Biodiesel fuels are becoming popular all over the world as an alternative to diesel fuel in automobile engines or as blending components (Siraj et al., 2017). The transesterification method is mostly employed in the industrial manufacturing of biodiesel due to its low cost and widespread usage.

6.3 Bioethanol

The fermentation of sugars derived from biomass results in the production of bioethanol. Sugarcane, sugar beet, corn, wheat, and lignocellulosic materials, such as sugarcane bagasse, wood, and straw, can all be used as bioethanol feedstocks (Dias et al., 2009). Bioethanol has about 35% of oxygen content, which helps the fuel burn completely and reduces the emission of nitrogen oxide and particulates (Elangovan et al., 2017). it has a higher-octane number, faster flames, larger flammability ranges, and higher heat of vaporization than gasoline, and it has more benefits over gasoline in internal combustion engines in terms of compression ratio and short burn period (Choudhary et al., 2016). Below are some properties of biodiesel, bioethanol, and biobutanol.

Table 2 Properties of Biodiesel, Bioethanol, and Butanol

Parameters	Biodiesel	Bioethanol	Biobutanol	Unit	References
Lower heating value	37.12	26.9	-	MJ/kg	Elangovan et al., 2017
Density at 15°C	1.9-6.0	790	0.81	kg/m3	Siraj et al., 2017
Kinematic Viscosity at 40	1.9-6.0	-	3.7	cSt	Chiaramonti et al., 2007
Iodine Value	-	2.5	29.2	psi	Husam et al., 2017
Pour point	-15 - 16	-	96	kJ/kg	Istvan et al., 2011
Cetane number	48 - 60	below 8	17	-	Kamiski et al., 2011
Flash point	100-170	13	36	°C	Istvan et al., 2011
Boiling point	182-338	78	78	°C	Siraj et al., 2017
Cloud point	-3 - 12	-	-89	°C	Istvan et al., 2011
Auto ignition temperature	-	366	343	°C	Siraj et al., 2017
Flammability limits	-	13-42	-		Istvan et al., 2011
Vaporization heat	-	842	-		Istvan et al., 2011

4. **Conclusion**

Microalgae has the potential to be successfully applied to treat wastewater from different sources. Microalgae create biomass and biofuels from some of the nitrogen/phosphate, organic carbons, VFAs, medicinal compounds, dye compounds, and HMs, creating an effective and reliable mechanism for the simultaneous reduction of waste and production of biofuels. Biofuel feedstock, fertilizers, and animal feed can all be made from the harvested microalgae biomass. Wastewater treatment by using the procedure of microalgae cultivation is the most cost-effective way of microalgae cultivation. Based on the successful results obtained in recent times in wastewater treatment by microalgae, further research should target the economic aspects of harvesting biomass to make microalga-based wastewater treatment a certainty of the future.

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