ACTA TECHNICA CORVINIENSIS – BULLETIN OF ENGINEERING
TOME XVI [2023] | FASCICULE 3 [JULY – SEPTEMBER]

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VALORIZATION OF MICROALGAE IN WASTEWATER TREATMENT AND BIODIESEL PRODUCTION

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Abstract: Micro and macroalgae are renewable and carbon-neutral sources of energy that can grow naturally or can be cultivated in nutrient-rich wastewater, without compromising the production of food crops. Microalgae use nutrients, accumulate heavy metals and reduce coliform bacteria from wastewater, thus contributing to their phytoremediation. Furthermore, the conversion of algae biomass into biofuels contributes to reducing the dependence on fossil fuels and greenhouse gas emissions in the atmosphere. Depending on the species and cultivation method, microalgae have a very fast growth rate (12 days) and their potential to produce biodiesel is 15 to 300 times higher than that of agricultural and energy crops conventionally used for biofuels. Lipid content in microalgae can reach 75–80% by weight of dry biomass. To produce 39 billion liters of biodiesel, 15 million tons of nitrogen and 2 million tons of phosphorus are required during the growth process of microalgae. From algae cultivated in 500 billion m^3 of industrial wastewater, approximately 37 million tons of oil can be extracted that can be transformed into biodiesel. However, there are also challenges in large-scale utilization of microalgae, due to the high energy input, technical difficulties encountered in harvesting unicellular microalgae, and the need for subsequent pretreatment operations to improve lipid extraction. This paper reviews the potential of microalgae species to thrive in wastewater and the technological achievements in converting microalgae biomass for the sustainable biodiesel production.

Keywords: algae, wastewater, phytoremediation, biomass, biofuels

INTRODUCTION

Energy is essential for generating industrial, commercial and social well-being, as well as ensuring personal comfort and mobility. However, its predominant production from non-renewable sources (coal, natural gas, oil, tar sands, oil shale and nuclear sources) put considerable pressure on the environment: emissions of greenhouse gases and other polluting gases, land use, waste generation and oil spills. These pressures contribute to climate change, damage the natural ecosystems and the human environment, and have adverse effects on human health.

About 80% of the total amount of energy used globally each year comes from fossil fuels. It is estimated that by 2030, the total consumption of fossil fuels will decrease by 16% compared to current levels, and these fuels will represent 62% of the primary energy supply. Thus, the share of fossil fuels in the European Union's energy supply could be further reduced, to represent only 55% of the region's primary energy supply by 2030. The transition from imported fossil fuels to renewable energy is one of the key objectives of the European Union to achieve sustainability and climate neutrality. Over the last two decades, the European Union’s share of renewable energy has increased mainly due to climate and energy policies, and to the technological progress. In the European market, France, Italy, Germany, Spain, Denmark, and the Czech Republic are also permitting full tax exemption for a specific volume of biodiesel production. Recently, Brazil, Germany, and the U.S. have initiated tax incentives to increase the production of biofuels and reduce the price of biodiesel at pumps (*Grand View Research*).

Although biofuels (including biodiesel, bioethanol, biohydrogen, biogas, biohytane) produced from biomass waste and the biodegradable portion of industrial waste, are still more expensive than fossil fuels, their production and use are increasing worldwide. In 2017, the gross inland energy consumption of biofuels in European countries was estimated at approximately 16500 ktoe for biodiesel, 4000 ktoe for bioethanol and 1000 ktoe for other biofuels. Biofuels are the prime source of renewable energy (89%) used by the transport sector (*Calderon and Colla, 2019*). In addition to being used in vehicle transportation, algae oil can be used to blend aviation fuel as it has a positive impact on aircraft performance by lowering operating expenditures (*Grand View Research*).

Biomass has become one of the most frequently used sources of renewable energy in the last two decades and the second form of energy, after hydropower, in the generation of electricity. However, problems have arisen because conventional biomass crops for biofuel production require large areas of arable land and high water supply to grow, thus competing with food crops and endangering food security. For this reason, in recent years, attention has turned to biomass sources whose cultivation is not in conflict with food security.

Algae are a diverse group of highly productive organisms that include microalgae, macroalgae (seaweed), and cyanobacteria (formerly called "blue-green algae"). Major taxonometric orders are *Bacillariophyta* (diatoms), *Chlorophyta* (green algae), *Chrysophyta* (golden algae) and *Rhodophyta* (red algae) (*Udaiyappan et al., 2017*). Many of these groups of aquatic microorganisms use sunlight, CO_2 and nutrients to create biomass, which contains key components, including lipids, proteins, and carbohydrates, that can be turned into a variety of biofuels and products.

Caulerpa prolifera (green algae)

Sargassum (brown algae)

Laminaria (brown algae)

Figure 1 - Some algae species (*Ungureanu et al., 2019*)

Microalgae biomass results mainly from photosynthesis; they consume atmospheric CO_2 as carbon source during photosynthesis and they can capture more than 40% of global carbon; hence they are carbon-neutral biomass and reduce the greenhouse gas emissions. It was estimated that 100 tons of algal biomass can capture 183 tons of CO_2 from the atmosphere, along with soluble carbonates and heavy industry gases (*Qari et al., 2017*).

Biosynthesis of algal biomass ($C_{106}H_{263}O_{110}N_{16}$) is described by the following chemical reactions, where ammonium and nitrate serve as nitrogen sources (*Dalrymple et al., 2013*):

$$16NH_4^+ + 92CO_2 + 92H_2O + 14HCO_3^- + HPO_4^{2-} \rightarrow$$
$$C_{106}H_{263}O_{110}N_{16}P + 106O_2$$
$$16NO_3^- + 124CO_2 + 140H_2O + HPO_4^{2-} \rightarrow$$
$$C_{106}H_{263}O_{110}N_{16}P + 138O_2 + 18HCO_3^-$$

Microalgae have developed morphological, behavioral and chemical mechanisms to defend themselves from bacteria, fungus, protozoans, aquatic invertebrates, other algae and even viruses (*Hannon et al., 2010*). An undeniable advantage of algae is their tremendous ability to grow easily in areas that are not suitable for other crops, such as arid or desert areas using seawater, brackish water, wastewater, seashores, and lakes and even on systems placed on top of buildings.

Algae thrive in nutrient-rich wastewater (from domestic, agricultural, swine farms, cattle farms, agro-industrial and industrial sources etc.) consuming the nutrients (nitrogen, phosphorous and potassium) and converting them into useful biomass (*Komolafe et al., 2014*). Their small size offers a large surface area, which increases the rate of nutrient uptake in the wastewater (*Udaiyappan et al., 2017*).

Nutrient removal is an important step in wastewater treatment because the discharge of nutrient-rich effluents into natural water bodies leads to eutrophication. In wastewater treatment, algae could be integrated into the secondary stage or added as a tertiary (polishing) stage, because, apart from consuming nutrients, they are efficient in removing biochemical oxygen demand, chemical oxygen demand, and suspended solids (*Chamberlin J.F., 2016*). Algae improve the quality of final effluent through natural disinfection and incorporation of contaminants like heavy metals, pharmaceuticals, endocrine disrupters and coliform bacteria *Salmonella*, *Shigella*, viruses and protozoa (*Ungureanu et al., 2019*). A study conducted by *Zainal et al. (2022)* reported that *Spirulina platensis* removed heavy metals from palm oil mill effluent with good efficiencies: Fe by 45.1%, Cu by 52.8%, Zn by 55%, Ni by 61.9%, As by 71.4%, Cr by 83.8% and Mn by 84.9%. Growing of microalgae in wastewater reduces the need of chemical fertilizers and their related burden on life cycle (*Unpaprom et al., 2015*). Thus, microalgae-based wastewater treatment is a sustainable process and the treated effluents can be safely discharged into natural water courses, or they can be recovered for irrigation of agricultural and energy crops or for landscape purposes.

MATERIALS AND METHODS

Wastewater treatment by algae coupled with biofuels production emerged as a promising strategy to decrease the economic and environmental costs of energy (*Cheah et al., 2016*). Micro and macroalgae earned global attention as feedstock for the production of third generation biofuels. Microalgae-derived biofuels are renewable, highly biodegradable, nontoxic and eco-friendly in comparison to fossil fuels.

Initial testing of microalgae as potential sources for biofuel production began in 1970, but it was temporarily stopped due to economic and technical problems. Studies resumed from 1980 onwards showed that there is high potential in biofuel production from microalgae (*Medipally et al., 2015*).

Microalgae biomass can be converted into different biofuels by means of biochemical and thermochemical processes, chemical reactions and direct combustion (Figure 2).

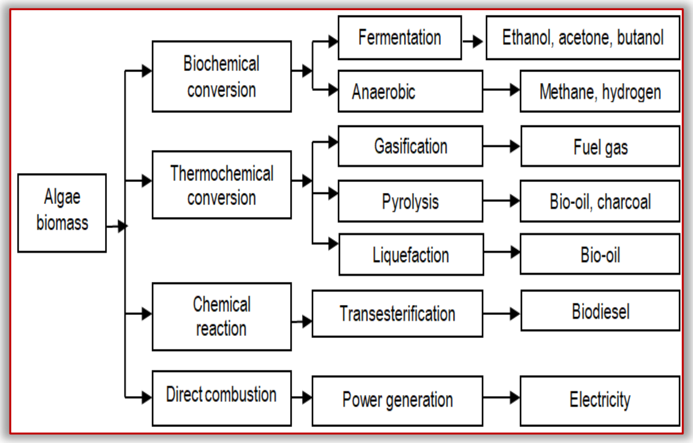

Figure 2 - Possibilities of converting the algae biomass into biofuels *(Medipally et al., 2015)*

The technology for producing biodiesel from conventional oily crops has been known for more than 60 years. The production of methyl esters or biodiesel from microalgae oil uses the same processes (Figure 3). Microalgae grow easier and faster and contain more oil than macroalgae, the latter being less used in biodiesel production.

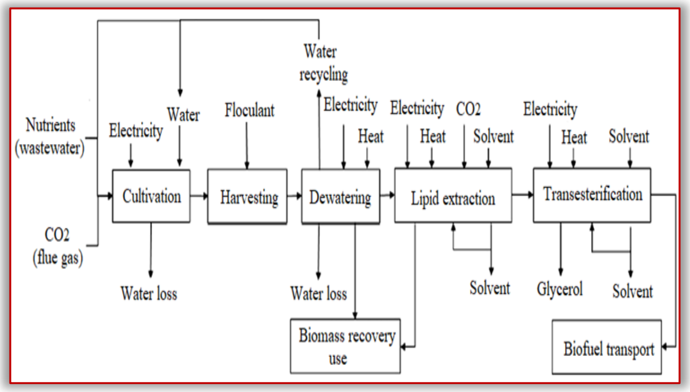

Figure 3 - Generalized processes for the production of biodiesel from microalgae *(Collotta et al., 2018)*

However, there are some drawbacks in large-scale commercial production of microalgae for biofuels industry. Industrial production of algae biomass can be more expensive than growing conventional crops; it is an energy intensive process that requires large amounts of energy and water, and the use of off-site generated CO_2. Harvesting of algal biomass can account for 20–30% of the total cost of production (*Guttierez et al., 2016*). To minimize expenses, biodiesel production must rely on freely available sunlight, regardless of the daily and seasonal variations in light levels (*Chisti Y., 2007*). Biodiesel production from microalgae would be more easily implemented at large scale if viable advances would be achieved in designing advanced photobioreactors, low cost technologies for biomass harvesting, drying and oil extraction.

Selection of adequate microalgae species has an important role in obtaining the desired product and the maximum microalgae productivity (Figure 4). Fast growing and high lipid producing microalgae strains should be carefully chosen for cultivation in order to increase the feasibility of biodiesel production.

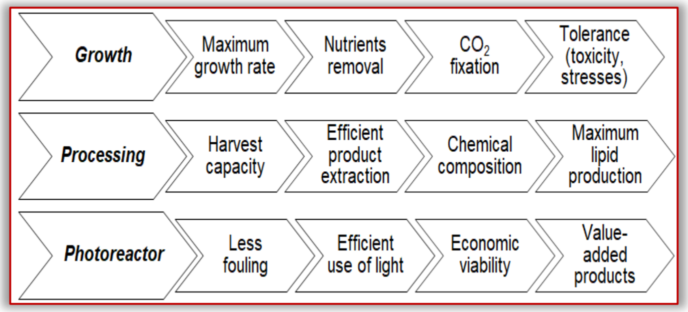

Figure 4 - Important criteria in the selection of microalgae for biodiesel production *(adapted from SundarRajan et al., 2019)*

Cultivation methods, from lab scale to industrial facilities, include man-made algae cultivation systems, like open pond systems such as high-rate algal ponds or raceway ponds, and closed systems such as different types of photobioreactors exposed to sunlight or artificial UV light and independent of seasons.

Open systems are preferred in most existing large scale microalgae cultivation plants because they provide easy operation, low costs of investments and maintenance (*Bilad et al., 2014*), are more durable than the photobioreactors (*Abinandan et al., 2015*) although they achieve low biomass productivities and mono-algae culture is not fully secured (*Bilad et al., 2014*).

Closed systems were developed to expand the yield of algae biomass, allow the monoculture under controlled conditions and prevent water evaporation and CO_2 loss (*Ugwu et al., 2008*). Photosynthetic growth requires light (sunlight or artificial UV light), CO_2, water and inorganic salts. Temperature must remain within 20–30°C. Microalgae have a very fast growing rate which can double just in one day (*Rittman B.E., 2008*) and biomass doubling times during exponential growth are commonly as short as 3.5 hours (*Chisti Y., 2007*).

Harvesting and dewatering of microalgae biomass can be considered both as a single operation and as combinations of multiple unit operations in a sequence.

Harvesting is one of the main challenges in biofuel production, due to the high energy input and recovery cost for microscopic microalgae and from diluted microalgal suspension (*Cheah et al., 2016*). Harvesting of microalgae is usually done by sedimentation (one of the simplest techniques), flocculation, flotation and thickening (by centrifugation or filtration) (*Abinandan et al., 2015*). Aiming to reduce the high costs of algae harvesting and streamline the process, other technologies have been tested: ozone-flotation which helps increase the lipid availability (*Nguyen et al., 2013*), coagulation by aluminum and ferric chloride, harvesting using Fe_3O_4 nanoparticles, harvesting using aluminum and magnesium based amino saline clays, harvesting using autoflocculation performed by microalgae due to CO_2 assimilation in cells, bioflocculation (*Abinandan et al., 2015*) and the use of microalgal cells immobilization in suspended media (*Cheah et al., 2016*). Harvesting of microalgae can be done each 12 days, these tiny organisms producing 15 to 300 times more oil for biodiesel production compared to the traditional oily crops on an area basis (*Chisti Y., 2007*).

Dewatering (thickening) processes must be tailored based on the species of microalgae and its growth conditions. Often, for the dewatering of algal biomass are used processes such as membrane filtration, vacuum and pressure filtration, centrifugation, and spiral plate technology, and sometimes dewatering can be followed by a drying step because the water content of harvested algal biomass should be reduced to about 5% (*Fasaei et al., 2018*). Secondary dewatering thickens the biomass to 15–25%, and if it is followed by drying process, it increases the total solid matter to 90–95%.

Extraction of lipids (oils) from microalgal biomass and their conversion to biodiesel are not affected by whether the biomass is produced in raceways or in photobioreactors (*Chisti Y., 2007*). Algae biomass consists of proteins, carbohydrates and natural oil (*Udaiyappan et al., 2017*). The latter is very high in unsaturated fatty acid that can be extracted and converted into biodiesel by esterification/ transesterification, in the presence of acid or alkali as a catalyst.

Algae has higher oil yield per unit area than other oilseed crops. For biofuel production, the lipid content in algae should be at least 20–40% (*Dalrymple et al., 2013*) and it can be adjusted by changing the composition of growth medium. It was reported that *Chlorella* has up to 50% lipids and *Botryococcus braunii* produces the highest oil content of about 80% (*Krishnamoorthy et al., 2022*). Composition of some microalgae strains suitable for biodiesel production is presented in Table 1.

Table 1. Biochemical composition of some algae strains expressed on dry matter basis (*Shalaby E.A., 2011*)

Algae strain	Proteins	Carbohydrates	Lipids	Nucleic acid
Anabaea cylindrica	43 – 56	25 – 30	4 – 7	–
Chlamydomonas rheinhardii	48	17	21	–
Chlorella vulgaris	51 – 58	12 – 17	14 – 22	4-5
Chlorella pyrenoidosa	57	26	2	–
Dundaliella bioculata	49	4	8	–
Dundaliella salina	57	32	6	–
Euglena gracilis	39 – 61	14 – 18	14 – 20	–
Prymnesium parvum	28 – 45	25 – 33	22 – 39	1-2
Porphyridium cruentum	28 – 39	40 – 57	9 – 14	–
Scenedesmus obliquus	50 – 56	10 – 17	12 – 14	3 – 6
Scenedesmus quadricauda	47	–	1.9	–
Scenedesmus dimorphus	8 – 18	21 – 52	16 – 40	–
Spirogyra sp.	6 – 20	22 – 64	11 – 21	–
Spirulina maxima	60 – 71	13 – 16	6 – 7	3 – 4.5
Spirulina platensis	46 – 63	8 – 14	4 – 9	2 – 5
Synechoccus sp.	63	15	11	5
Tetraselmis maculata	52	15	3	–

Other algal strains, including *Phaeodactylum tricornutum* (*Udaiyappan et al., 2017*), *Crypthecodinium cohnii, Dunaliella primolecta, Nannochloropsis* sp. (*Medipally et al., 2015*), *Oedogonium* (*Sharif Hossain et al., 2008*), *Arthrospira platensis, Nostoc* sp., *Oscillatoria* sp., and *Scenedesmus acuminatus* (*Unpaprom et al., 2015*) contain large quantities of hydrocarbons and lipids and have been employed in biofuels production.

The energy content of algal oils is 35800 $kJ\cdot kg^{-1}$, which is about 80% of the energy contained in petroleum (*Kligerman and Bouwer, 2015*). Nevertheless, not all oils produced by microalgae are considered legitimate biofuels, but suitable oils are common (*Udaiyappan et al., 2017*). Algae can produce 250 times the amount of oil per acre as soybeans and 7 to 31 times higher oil than palm oil (*Sharif Hossain et al., 2008*). Depending on the differences in nutrients concentrations in wastewater and the ability of algal strains to accumulate lipids, 500 billion m^3 of industrial wastewater could produce 37 million tons of algal oil (*Chinnasamy et al., 2010*). Hence, microalgae are a promising feedstock for biodiesel production, with an estimated yield of 58700–136900 $L\cdot ha^{-1}\cdot year^{-1}$ (*Unpaprom et al., 2015*). A realistic estimate of microalgae biomass production lies between 15–25 $t\cdot ha^{-1}\cdot year^{-1}$, while lipid production, with no optimized growth conditions is of 4.5–7.525 $t\cdot ha^{-1}\cdot year^{-1}$ at 30% lipid content in microalgae cells (*Lam and Lee, 2012*). Table 2 presents the oil content of some microalgae species, expressed as % dry wt.

Oil productivity, or the mass of produced oil/ volume of the microalgae broth/day, depends on the algal growth rate and the oil content in the biomass. Microalgae for biomass production have been cultivated under photoautotrophic, heterotrophic and mixotrophic conditions (*Abinandan et al.*, 2015).

Table 2. Oil content of some microalgae *(Chisti Y., 2007)*

Microalgae	Oil content (% dry wt)	Microalgae	Oil content (% dry wt)
Botryococcus braunii	25 – 75	*Nannochloris sp.*	20 – 35
Chlorella sp.	28 – 32	*Nannochloropsis sp.*	31 – 68
Crypthecodinium cohnii	20	*Neochloris oleoabundans*	35 – 54
Cylindrotheca sp.	16 – 37	*Nitzschia sp.*	45 – 47
Dunaliella primolecta	23	*Phaeodactylum tricornutum*	20 – 30
Isochrysis sp.	25 – 33	*Schizochytrium sp.*	50 – 77
Monallaanthus salina	>20	*Tetraselmis sueica*	15 – 23

The simplest method for lipid extraction is the mechanical crushing of microalgae cells using glass beads, screw presses, extruders and pulverization. Other methods of extraction, such as supercritical CO_2, sonication, autoclaving, microwaving, freezing and osmotic shock are less practical for commercial scale biofuel production (*Cheah et al.*, 2016).

Table 3. Biomass and lipid productivities of some microalgae in different cultivation methods *(Medipally et al., 2015)*

Cultivation method	Microalgae	Biomass productivity ($g{\cdot}L^{-1}{\cdot}d^{-1}$)	Lipid content (% dry wt)	Lipid productivity ($mg{\cdot}L^{-1}{\cdot}d^{-1}$)
Phototrophic	*Chlorella vulgaris*	0.02 – 0.2	50 – 58	11.2 – 40
	Chlorella protothecoides	2 – 7.7	14.6 – 57.8	1214
	Chlorella sorokiniana	0.23 – 1.47	19 – 22	44.7
Heterotrophic	*Chlorella vulgaris*	0.15	23	35
	Chlorella protothecoides	3.1 – 3.9	–	2400
	Chlorella sorokiniana	1.48	23.3	–
Mixotrophic	*Chlorella vulgaris*	0.25 – 0.26	20 – 22	52 – 56
	Chlorella protothecoides	23.9	58.4	11800
	Chlorella sorokiniana	0.58	–	29 – 56

Transesterification. Raw microalgal oil (triglycerides) has high viscosity which could ruin the vehicle's engine quickly due to the rapid accumulation of oil sludge, so it requires a chemical conversion (transesterification) into low molecular weight, non-toxic, biodegradable biofuel that offers smooth engine operation. Transesterification uses excess methanol or ethanol in the presence of a catalyst (sodium hydroxide in methanol and sodium methoxide for the former, hydrochloric and sulphuric acid in methanol for the latter) (*Zeng et al.*, 2011), to maintain the equilibrium shift towards fatty acid esters production and accelerate the reaction rate (*Cheah et al.*, 2016). The transesterification of triglycerides producing fatty acid esters is described by the reaction presented in Figure 5.

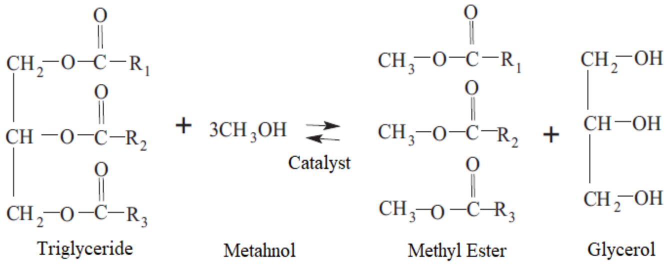

Figure 5 - Transesterification of triglycerides with production of fatty acid esters *(Cheah et al., 2016)*

If triglycerides with short chain alcohol are displaced by alcohol for fatty acid esters and glycerol formation, the process is referred to as alcoholysis. Despite being efficient, transesterification is also energy intensive, while glycerol is difficult to recover, and if the microalgal cell contains wastewater or moisture it could result in variations in pH, saponification, reduced catalytic efficiency and reduced biodiesel yield. Industrial scale transesterification can be improved by using solid catalysts (zeolites, metal oxides and ion-exchange resins), which are selective, active, stable at high temperatures and which can prevent water formation and saponification (*DuPont A.*, 2013).

RESULTS

Extensive researches have stated that biodiesel yield depends on the lipid content in algae biomass, which in turn depends on nutrient concentration in the wastewater. At the moment, significant advancements have been obtained mostly at laboratory scale. Improvements in lipid productivity are mandatory for the economic viability of microalgae-based biodiesel at pilot-scale. According to *Rosmahadi et al.* (2021), *Chlorella vulgaris* is the most utilized microalgae species for biodiesel production, due to its high lipid content (60–70%) and high productivity (7.4 $g{\cdot}L^{-1}$). *Unpaprom et al.* (2015) coupled wastewater treatment with biodiesel production, by growing *Scenedesmus acuminatus* in continuous stirred photobioreactor, in piggery wastewater effluent.

The batch feeding operation by replacing 10% of algae culture with piggery wastewater effluent every day could provide a stable net biomass productivity of 3.24 $g{\cdot}L^{-1}{\cdot}day^{-1}$. Total lipids from 100 mg microalgae were extracted using 2 mL chloroform/ methanol (v/v: 2/1), ultrasonic treatment for 10 min and centrifugation at 4000 rpm for 5 min. The effect of acid hydrolysis of lipids from *Scenedesmus acuminatus* on fatty acid methyl esters production was investigated. Direct transesterification (a one-stage process) of the harvested *Scenedesmus acuminatus* biomass resulted in a higher biodiesel yield content than that in a two-stage process, so it could be feasible to produce biodiesel from wet microalgae biomass directly without drying and lipid extraction.

Guldhe et al. (2017) tested the tungstated zirconia catalyst and obtained a high biodiesel conversion of lipids extracted from microalgae *Scenedesmus obliquus*, namely 94.85% at 100°C, with 15 wt% of catalyst (based on oil weight) and methanol to oil molar ratio of 12:1 in 3 hours. A study by *Hena et al. (2015)* evaluated the production of biodiesel from consortium of native microalgae (*Chlorella, Ankistrodesmus, Chlamydomonas* and *Scenedesmus*) grown in dairy farm treated and untreated wastewater. The microalgae were able to remove more than 98% nutrients and 98% chemical oxygen demand from treated wastewater. To obtain 100 g of algal oil from biomass of consortium grown in treated wastewater, 650 g dry biomass were treated by the dynamic hexane method.

In treated wastewater, the consortium produced 219.8 tons of biomass and algal oil yield of 51.37 thousand $L \cdot ha^{-1} \cdot year^{-1}$, while in untreated wastewater were obtained 137.68 tons of biomass and 33.38 thousand $L \cdot ha^{-1} \cdot year^{-1}$ of algal oil. It was found that 72.7% of algal lipid obtained from consortium could be converted into biodiesel, and loses were mainly due to oil impurities.

Zhu et al. (2013) tested the algae *Chlorella zofingiensis* grown in piggery industry wastewater, which at optimum COD concentration of 1900 $mg \cdot L^{-1}$, showed the highest biomass, lipid, and biodiesel productivities of 296.16 $mg \cdot L^{-1} \cdot day^{-1}$, 110.56 $mg \cdot L^{-1} \cdot day^{-1}$, respectively 30.14 $mg \cdot L^{-1} \cdot day^{-1}$.

Ahmad et al. (2013) showed that 95% of biodiesel yield could be obtained from microalgae *Chlorella vulgaris* by using sodium methoxide, CH_3ONa (base catalyst) at reaction time of 51 minutes and temperature of 160°C. This high yield is due to the alkaline metal oxides (sodium methoxide) which are highly active catalysts even when used in small concentrations. Microalgae *Botryococcus braunii* grown in carpet mill wastewater achieved 34 $mg \cdot L^{-1} \cdot d^{-1}$ of biomass productivity (dry weight) and 4.5 $mg \cdot L^{-1} \cdot d^{-1}$ of lipid productivity (13.2% lipid content), while the same microalgae grown with pig manure showed a biomass productivity of 700 $mg \cdot L^{-1} \cdot d^{-1}$ (dry weight) and lipid productivity of 69 $mg \cdot L^{-1} \cdot d^{-1}$ (9.8 % lipid content) (*Pittman et al., 2011*). In another study, *Chlamydomonas reinhardtii* removed 55.8 $mg \cdot L^{-1} \cdot d^{-1}$ nitrogen and 17.4 $mg \cdot L^{-1} \cdot d^{-1}$ phosphorus from municipal wastewater. The highest biomass productivity was 2 $g \cdot L^{-1} \cdot d^{-1}$ and lipid content of the strain was 25.25% (*Kong et al., 2010*).

Komolafe et al. (2014) tested microalgae *Desmodesmus sp.* and mixed culture of *Oscillatoria* and *Arthrospira*, grown in photobioreactors filled with wastewater, for biodiesel production. Decreases of total nitrogen by 55.4–83.9% and total coliforms by 99.8% were obtained. Ozone-flotation was used for algae harvesting and to reduce the unsaturation of fatty acid methyl esters. *Desmodesmus sp.* grew rapidly; the highest biomass concentration was 0.58 $g \cdot L^{-1}$, while the mixed culture reached 0.45 $g \cdot L^{-1}$. The mixed culture had the highest lipid and fatty acid methyl esters yield.

Shariff Hossain et al. (2008) tested common species *Oedogonium* and *Spirogyra* to compare the amount of biodiesel production. Algae samples were dried for 20 min at 80°C for water removal and then mixed with 20 mL hexane and ether solution to extract the oil. Using 0.25 g NaOH as catalyst and 24 mL methanol, algal oil and biodiesel (methyl ester) production was higher in *Oedogonium* than *Spirogyra* sp., but biomass yield (after oil extraction) was higher in *Spirogyra* than *Oedogonium* sp.

Ihsanulla et al. (2015) extracted oil from *Spirogyra* using a combination of n-hexane and Di-ethyl Ether. The yield of extracted oil was enhanced by smaller algal size, higher algal to solvent ratio and longer contact time. The maximum extracted oil was 0.09 fraction of biomass, by using algal biomass size of 0.4 mm, a blend of both solvents at solvent to biomass ratio of 3.5, and contact time of 24 hours. Transesterification was influenced by oil to methanol ratio, amount of catalyst, reaction time and temperature. The maximum yield > 95% was obtained at 60°C, oil to methanol ratio 8, reaction time 25 minutes and catalyst amount 0.5% of weight of oil.

Jaiswar et al. (2017) reported that the lipid content of freshwater *Neochloris aquatica* grown in artificial pond was 12%, calculated through Nile red fluorescence method and analysis of the fatty acid methyl ester of the tested strain showed saturated, monounsaturated and polyunsaturated fatty acids content of 29.15%, 37.95% and 32.90%.

The results of experimental studies demonstrate that microalgae have the potential to accumulate nutrients present in wastewater and their lipid-rich biomass can be used as a source of biodiesel production.

CONCLUSIONS

Microalgae are useful in wastewater phytoremediation, as they consume high amounts of nutrients, heavy metals and coliform bacteria, and reduce the chemical oxygen demand, biochemical oxygen demand and suspended solids. Depending on their species, wastewater-grown microalgae contain different concentrations of lipids, hydrocarbons and carbohydrates, making them useful products in the conversion into biofuels. To produce biodiesel, transesterification is mandatory after the extraction of lipids, because the extracted (raw) oil has high viscosity which would affect the engines. Although many studies have been developed so far on the production of biodiesel from microalgae biomass, the research is still on-going in order to make this type of biofuel competitive to other fossil fuel reserves. Reducing the production costs for wastewater-grown algae biofuels (especially the costs of microalgae harvesting and pretreatment) is an important goal for this industry.

Acknowledgement

This work was supported by a grant of the Romanian Ministry of Research and Innovation CCDI - UEFISCDI, Project INNOVATIVE TECHNOLOGIES FOR IRRIGATION OF AGRICULTURAL CROPS IN ARID, SEMIARID AND SUBHUMID-DRY CLIMATE, project number PN-III-P1-1.2-PCCDI-2017-0254, Contract no. 27 PCCDI / 2018, within PNCDI III.

Note: This paper was presented at ISB–INMA TEH' 2022 – International Symposium on Technologies and Technical Systems in Agriculture, Food Industry and Environment, organized by University "POLITEHNICA" of Bucuresti, Faculty of Biotechnical Systems Engineering, National Institute for Research–Development of Machines and Installations designed for Agriculture and Food Industry (INMA Bucuresti), National Research & Development Institute for Food Bioresources (IBA Bucuresti), University of Agronomic Sciences and Veterinary Medicine of Bucuresti (UASVMB), Research–Development Institute for Plant Protection – (ICDPP Bucuresti), Research and Development Institute for Processing and Marketing of the Horticultural Products (HORTING), Hydraulics and Pneumatics Research Institute (INOE 2000 IHP) and Romanian Agricultural Mechanical Engineers Society (SIMAR), in Bucuresti, ROMANIA, in 6–7 October, 2022.

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ISSN: 2067-3809

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