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Enhanced biomass production from *Chlorella* micro-algae species: a review of new technologies towards sustainable energy development

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Chlorella microalgae species are among the most diverse, resilient, economical and sustainable biomass strains that are presently employed in renewable biofuel production. In this systematic literature review, the necessity for micro-algae biofuel development, the pros and cons of different *Chlorella* micro-algae cultivation modes and methods and their effects on biomass and total lipids productivities were critically examined. A comprehensive comparison of *Chlorella* biofuel properties with different conventional plant biomass resources was also carried out. Moreover, the use of mixotrophy and hybrid cultivation systems as high-yield and sustainable biomass production alternatives were analyzed. The effects of surface area to volume ratio (V/S) during *in situ* micro-algae cultivation in volumetric flasks were also examined because of its critical influence in large photobioreactors design most importantly in areas of light penetration, CO_2 diffusion and nutrient availability for enhanced micro-algae growth. *Chlorella* micro-algae species have higher total lipid productivity in the range of 15–70 mg L^{-1} depending on the route of nutrient metabolism with mixotrophic growth mode having the highest lipid accumulation of 45–70 mg L^{-1}. While photoautotrophic and heterotrophic modes have lipid contents of 15–30 mg L^{-1} and 35–68 mg L^{-1} respectively. This promotes sustainable energy production from the micro-algae species. Interestingly, the micro-algae biodiesel has a higher cetane index and calorific value between 50–56 and 38–43 MJ kg^{-1} respectively. This results in better ignition quality, enhanced fuel combustion and performance characteristics, lower engine fuel consumption and reduced carbon oxide (Cox) and particulate matter emissions. Finally, various new technologies and potential future strategies were offered for efficient and sustainable biomass and energy production.

1 Introduction

Microalgae are essential feedstock materials for the production of various biofuels, including biogas, biochar, bioethanol, biohydrogen, biobutanol, and biodiesel. Among these important energy resources, biodiesel is the most prominent and commercially viable alternative to conventional diesel fuel, suitable for use in most direct-injection and compression–ignition engines.[1–4] The primary advantages of biofuels over hydrocarbon-based liquid fuels include their renewable nature, inherent biodegradability, non-toxicity and their capacity to substantially reduce greenhouse gas emissions: an essential factor in climate change mitigation and control.[5–7]

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Microalgae are ubiquitous plants and they grow rapidly on non-arable land, salt-water environment and waste water habitats compared to terrestrial crops. The application of micro-algae in biofuel production does not result in needless competition with food production. They are generally referred to as one of the ancient plants with rapid growing rate. They are phototrophic plants that require carbon dioxide, water and mineral salts in the presence of adequate sunlight for their optimum growth. Microalgae are capable of fixing CO_2 from the atmosphere, released gases and from dissolved organic compounds.[8] Microalgal biomass are known to contain about 50% of carbon by dry weight.[9] It produces complex oil and the oil yield that are obtainable from the plant species is largely higher than those of other crops for biofuel production and usually in the range of 25–60%.[10,11]

Interestingly, *Chlorella* micro-algae offer sustainable biomass for biofuel production because of the following innate characteristics of the micro-algae strains. These are: ability to thrive under multiple cultivation systems (such as open ponds, freshwater, brackish water bodies and even polluted sources), high photosynthetic efficiency rates, high CO_2 fixation rates contributing to carbon sequestration efforts, rapid growth rates and biomass yield producing substantial biomass rich in carbohydrates, proteins, lipids and other valuable compounds and they can tolerate wide ranges of water quality, nutrient levels, salinity and chemical compositions.[12–14]

Finally, this review paper emphasizes the current research gaps in biofuel production from *Chlorella* microalgae species and various recommendations were suggested on how future research studies can address these gaps. Such gaps include biomass and total lipids production challenges due to inverse relationship that exist between the duo because lower lipids are usually accumulated by micro-algae strains with higher biomass growth rate. Furthermore, techno-economic feasibility, optimum integration of waste water and flue gas as low-cost nutrients and carbon dioxide sources, limited applications of genetic engineering, scalability and commercialization, culture contamination, efficient algae lipids transesterification process and process optimization are other important barriers towards sustainable micro-algae biofuels development.

1.1 The genesis of biofuel development from micro-algae

Biofuels are produced from renewable biomass resources such as edible and non-edible vegetable oils and animal fats.[15] According to Rozaq *et al.*,[16] compression engines that are fueled with diesel are associated with the release of harmful pollutants such as particulate matter as well as oxides of nitrogen, sulphur and carbon (Nox Sox and Cox) due to the presence of polycyclic-aromatic-hydrocarbons (PCAHs) and nitro compounds such as nitrophenols and nitro-polycyclic aromatic hydrocarbons (NPAHs). Unfortunately, this causes global warming and the inhalation of these hazardous pollutants can trigger cancer and other respiratory disorders in humans. Biofuels therefore offer sustainable solutions to all these fossil-fuels induced human and environmental catastrophes.

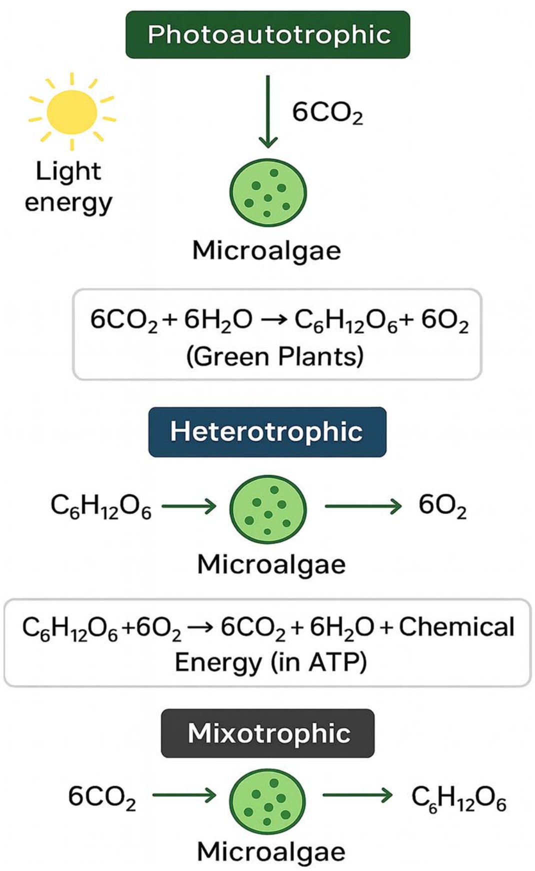

Fig. 1 Pictorial chart for basic micro-algae cultivation modes.

The first generation of biodiesel was produced through the modification of edible vegetable oils from palm oil, soybean oil, rapeseed oil, and sunflower oil.[17,18] Nevertheless, the reliance on these feedstocks presents significant challenges, particularly with rising future energy demands. Since these materials are edible and widely used in the food sector, their use in energy production creates competition between food and energy industries. This competition can lead to increased commodity prices, and therefore result in resource allocation shortages.

However, in order to limit the dependence of biodiesel feedstock materials on edible sources and thereby reducing competition and cost of these edible vegetable plant oils, various non-edible biomass materials such as tallow plant oil, waste cooking oil, grease oil, lard oil and *Jatropha curcas* plant oil were investigated as alternative biomass resources.[19,20] These non-edible plant oils constitute the biomass materials for the second-generation biofuels. Particularly, the use of waste cooking oil as a second-generation biodiesel feedstock helps us to address the various environmental problems arising from indiscriminate waste disposal by recycling. Therefore, the second-generation feedstock materials enhance energy security, food preservation, circular economy development and reduction in soil, water and air pollution issues.[21,22] Regrettably, these materials typically contain higher levels of free fatty acids (FFA) and impurities, which result in lower yields of fatty acid esters (biodiesel). Furthermore, additional physical and chemical treatments are often required to ensure the produced esters meet international biodiesel quality standards.[23,24] Therefore, the overall production cost of second-generation biodiesel tends to be higher than that of the first-generation alternatives.

Meanwhile, the third generation of biodiesel feedstocks was derived from microorganism-based oils or lipids, with micro-algae emerging as a particularly promising candidate owing to their comparatively higher oil content and rapid biomass production rate.[25] Microalgae exhibit several environmental benefits, such as a high CO_2 capturing and sequestration capacity and the ability to grow in low-quality or non-arable cultivation media.

Finally, the fourth generation of biodiesel feedstocks has been proposed through the genetic engineering of microorganisms used in third-generation production systems. Genetic modification in this context aims at enhancing biomass growth rate, increase oil content, and improve adaptability to various growth media.[26] However, the development of this generation remains in its preliminary stages. Therefore, further research is required to identify the optimal genetic configurations that can maximize fuel-supporting potential.

Table 1 Summary of major differences, advantages and limitations of various cultivation modes

Growth mode	Phototrophy	Heterotrophy	Photo-heterotrophy	Mixotrophy
Substrate or carbon source	Inorganic (CO_2)	Organic (glucose glycerol, acetate)	Organic (glucose)	Inorganic and organic sources
Energy source	Sun light	Oxidation of organic carbon sources	Sunlight	Sunlight and oxidation of organic carbon sources
CO_2 fixation	Fixes CO_2	Does not fix CO_2	Does not fix CO_2	Fixes CO_2
Major reactions	Light and dark reactions (Calvin cycle)	Electron transport chain, oxidative phosphorylation, glycolysis, krebs cycle	Light, photo-phosphorylation and citric acid cycle	Electron transport chain, tricarboxylic acid cycle (TCA)
Major intermediate products	ATP, NADPH	Pyruvate, acetyl-CoA, tricarboxylic acid (TCA), hexose sugars	ATP generation through photo-phosphorylation	Glyceraldehyde-3-phosphate, pyruvate, acetate and glucose
Major end products	Glucose and oxygen	ATP, CO_2, water, organic acids	CO_2, water, organic acids	Fatty acids
Typical micro-algae species	*Chlamydomonas*. *Scenedesmus* cyanobacteria	*Crypthecodinium*, *Schizochytrium*, *Prototheca*	*Chlorella vulgaris* ESP-31	*Chlorella vulgaris*, *Chlorella protothecoides*
Major advantages	Renewable biomass source. Reduction in atmospheric CO_2. Cost effective. Higher pigments and phytochemicals synthesis. Land use economy	Increased growth rate, biomass and lipid synthesis than phototrophic. Easy scale-up for biofuel and waste-water treatments. Simplified culture conditions	Lower cost. Allows micro-algae cultivation in areas with limited CO_2 supply such as shallow-water basins and deep oceans. Production of high-biomass	Enhanced cell growth rate, biomass and lipid synthesis than heterotrophy. Ability to adapt to varying light and nutrients availabilities. Higher conversion efficiency
Major limitations	Sunlight dependency may limit growth rate, biomass and lipids production	Higher cost of organic nutrients. Prone to culture contamination and substrates inhibition	Process inhibition by sunlight or organic substrates	Higher cost of biomass production. Requires sterile medium. Complex metabolic pathway

2 Modes of *Chlorella* micro-algae cultivation

Microalgae can be cultivated through various metabolic modes; notwithstanding, autotrophic and heterotrophic growth remain the primary methods for large-scale production. In autotrophic cultivation, microalgae utilize light energy and carbon dioxide (CO_2) to drive photosynthesis, forming organic matter (eqn (1)). In contrast, heterotrophic microalgae derive both energy and organic carbon from external organic compounds such as glucose or waste sugars (eqn (2)).[27,28] The pictorial representation of the major modes of micro-algae cultivation is shown in Fig. 1. While Table 1 represents the major differences, advantages and limitations of different micro-algae cultivation modes.

$$CO_2 + 2H_2O \xrightarrow[\text{green plants}]{\text{light}} (CH_2O) + O_2 + H_2O \tag{1}$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{chemical energy (in ATP)} \tag{2}$$

2.1 Photoautotrophic growth mode

Photo-autotrophy is a dual-stage micro-algae metabolic process in which inorganic carbons are being assimilated into organic compounds in a process that is known as carbon fixation. Absorption of light energy occurs in the first stage to produce adenosine tri-phosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) by electrons transfer. This stage is often referred to as the light-capturing or photochemical reaction. The second stage (dark stage) involves the reduction of carbon dioxide (CO_2) to organic compounds (carbon) by ATP and NADPH that were synthesized in the photochemical reactions stage.[29] During this stage, enzyme rubisco, RubP (ribulose bisphosphate) carboxylase or oxygenase reacts with the atmospheric CO_2 to form two molecules of an intermediate product (3-phosphoglyceraldehyde (PGA)). The PGA undergoes reduction to form glyceraldehyde-3-phosphate. Carbohydrates are produced from the regeneration of RuBP from G3P. Phototrophic pathway mode of micro-algae cultivation is mainly influenced by sunlight intensity, availability of CO_2, temperature and water. Other important factors include minerals and the physiological conditions of the plants.

2.2 Heterotrophic growth mode

In the heterotrophic micro-algae metabolic route, energy is majorly obtained by the micro-algae through the oxidation of simple sugars, such as glucose, to generate adenosine triphosphate (ATP) through a series of chemical reactions collectively known as glycolysis.[30] This process is marked by the conversion of glucose into pyruvate (an important metabolic bi-molecule), and the energy that is released from the reactions is used to form ATP—a high-energy molecule—and NADH (nicotinamide adenine dinucleotide in its reduced form).

According to Perez-Garcia *et al.*[31] glycolysis involves a sequence of ten enzyme-catalysed reactions with three important primary steps: These are:

1. Substrate-level phosphorylation, where ATP is directly synthesized.

2. Conversion of glucose-phosphate to fructose-phosphate, which is a key regulatory step.

3. Synthesis of two phosphate-containing molecules, which helps to drive the energy yield. These steps play a central role in harvesting energy from glucose to support cellular functions in micro-algae.

2.3 Photo-heterotrophic growth mode

In photo-heterotrophy, micro-algae use sunlight as energy source to convert organic substrates such as glucose, acetate and glycerol to useful metabolic products. However, photo-heterotrophy is different from mixotrophy because why the former applies light energy for absorption and metabolism of only organic compounds such as glucose and acetate, the latter metabolizes atmospheric inorganic carbon dioxide (CO_2) by using light energy.[32,33]

2.4 Mixotrophic growth mode

Mixotrophic micro-algae cultivation mode is a two-stage nutrition mode where the micro-algae specie is supplied with surplus organic carbon nutrient but compelled to assimilate carbon dioxide (CO_2) autotrophically due to organic matter depletion and produce oxygen through photosynthesis.[34] Mixotrophic micro-algae exhibit significant reduction in light inhibition and enhanced growth rates compared to autotrophic and heterotrophic modes. This mode is independent of growth substrates and phototrophism. Mixotrophic *Chlorella* micro-algae have higher biomass productivity with a growth rate that is equivalent to the sum total of photoautotrophic and heterotrophic pathways due to the synergistic effects between the two co-existing processes in which the oxygen from photosynthetic process is used in the oxidation of glucose during heterotrophic process and the CO_2 that is produced during this oxidative process serves as inorganic carbon source during photo-autotrophic process.[35]

3 Methods of micro-algae cultivation

3.1 Indoor or *in situ*

This involves pilot-scale cultivation of micro-algae strains in the laboratory in different conical flasks such as erlenmeyer flask (EMF) and fernbach flasks (FBF) using modified blue-green algae (BG-11 nutrients) or bolds-basal nutrient medium (Fig. 2). This method helps in the understanding of the dynamics of the effects of surface area to volume ratio (V/S) which is pivotal in the eventual design of large photo-bioreactors owing to their influence on micro-algae growth.[36] Fundamentally, the V/S ratio influences penetration of light, CO_2 diffusion and nutrients availability for micro-algae growth with a larger surface area relative to volume ratio (lower V/S) flasks offering better light penetration through the cells and enhanced carbon assimilation during photosynthesis. Nayana *et al.*[37] critically investigated the effects of surface area to volume ratio (V/S) on the cell growth and biomass productivity of five micro-algae species of commercial importance under different V/S ratios of 1 : 1, 1 : 1.2, 1 : 1.28, 1 : 1.3, 1 : 1.6, 1 : 2.22, 1 : 4.16 and 1 : 6. The micro-algae species were *Chlorella* sp., *Dunaliella* sp., *Chroococcus* sp., *C. saipanensis* and *H. pluvialis*.

The *H. pluvialis*, *C. saipanensis*, and *Dunaliella* sp. micro-algae species have cell concentrations of 176.08 ± 3.9 cells per mL, 2529.6 ± 167.01 cells per mL, and 900 ± 36.7 cells per mL respectively during cultivation in a 50 mL conical flask with V : S of 1 : 6. Interestingly, *Chroococcus* sp. and *Chlorella* sp. showed optimum growth in 500 mL flasks, with a surface area to volume ratio of 1 : 4.16 yielding 3963.13 ± 339.95 cells per mL and 1147 ± 67.73 cells per mL, respectively. Moreover, an appreciable cell growth rate was shown by *Chlorella* sp. in a 500 mL flask with V : S of 1 : 1.6. The authors therefore concluded that, V : S ratios of 1 : 6 and 1 : 4.16 are representative of tubular and flat panel bio-reactors design respectively. Additionally, Xu *et al.*[38] posited that the kinetics of micro-algae growth in a volumetric-flask reactor are usually influenced by the micro-algae surface area and volume ratio with large-sized micro-algae showing slower growth rate in comparison to smaller-sized species. Schade and Meier[39] describes the kinetics of growth rates in relation to nutrients absorption and V/S ratio. The authors submitted that large, slow-growing cells are more sensitive to changes in the V/S ratio whereas the small, developing cells are nutrients-saturated.

Larger flask volumes enable random micro-algae movement and thereby prevent the culture's light from self-shading. However, lower volumes result in tight packing of the algae cells and those on top will hinder light penetration in the culture and thereby decreasing biomass productivity.

3.2 Outdoor or *ex situ*

This micro-algae cultivation system is typically designed for the growth of phototrophic organisms using solar or artificial light sources in an open, closed or semi-closed system. The open or race way ponds (Fig. 3) and industrial waste water sludge (Fig. 4) are examples of an open micro-algae cultivation system which depends on sunlight energy and inorganic carbon dioxide for micro-algae metabolism and growth.

3.2.1 Open ponds or raceway ponds. The first commercial micro-algae production method was carried out on a natural open pond or raceway ponds. During this process, the growth culture with the required nutrients and inorganic carbon dioxide is continuously pumped around the pond surface and the pond is illuminated directly by sunlight. This method is highly economical in terms of operational and equipment costs when compared to closed bio-reactor systems. Notwithstanding, there is a limited control dynamics on growth parameters most especially sunlight and carbon dioxide. The method also suffers from likely contamination from other microorganisms and water losses due to atmospheric evaporation.[40]

3.2.2 Closed or semi-closed photo-bioreactors. This involves the use of different closed or semi-closed containers such as tubular or cylindrical reactors for large-scale micro-algae cultivation. Examples include vertical flat plate (VFP) (Fig. 5a), thin-layer cascade (TL), flat panel airlift (FPA), tubular reactors (Fig. 5b), horizontal bioreactors (Fig. 5c) and stirred-tank photobioreactors. This system contains sophisticated growth monitoring tools for efficient control of various growth conditions such as temperature, acidity or alkalinity and substrates levels (organic and inorganic). According to Skoneczny *et al.*,[41] the total

Fig. 3 Open race way pond.

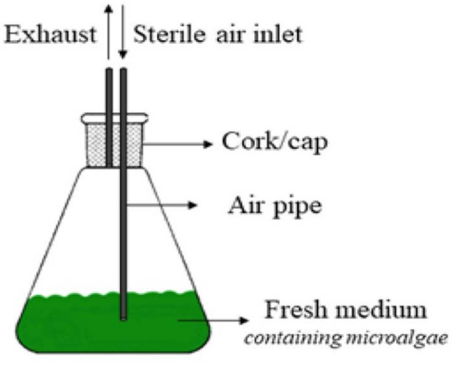

Fig. 2 Micro-algae cultivation in a laboratory flask.

Fig. 4 Micro-algae growth on a sludge water.

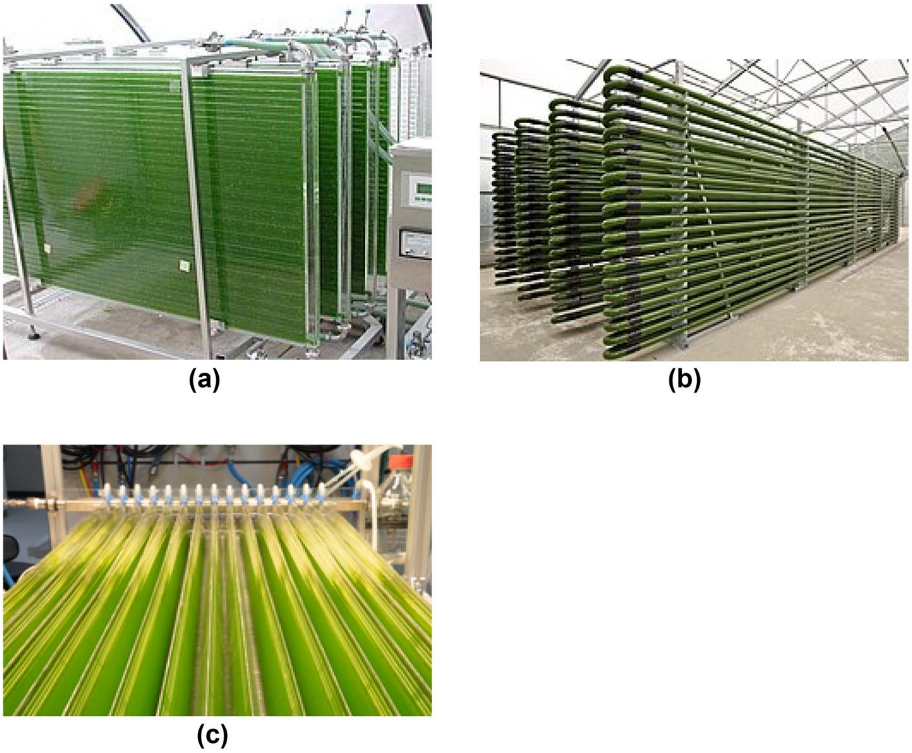

Fig. 5 (a) Vertical flat plate bioreactor, (b) tubular bioreactor, (c) horizontal photobioreactor.

micro-algae biomass productivity in a photobioreactor is influenced by reactor shape, size and thickness, dilution rate, nitrogen concentration and influx rate and artificial light source.

The major differences, advantages and limitations of each cultivation method are presented in Table 2. While Table 3 describes the effects of cultivation modes and methods on total biomass and lipid productivity of different *Chlorella* micro-algae species.

Finally, strain selection, type and concentration of organic and inorganic carbon sources, illumination (light intensity and

Table 2 Summary of major differences, advantages and limitations of various cultivation methods

Cultivation methods	*In situ* (laboratory)	Open ponds or raceway ponds	Sludge water	Photobioreactors
Major differences	Performed in the laboratory using volumetric flasks	Large-scale pond system consisting of a closed-loop channel	Complex mixture that contains micro-organisms, nutrients, heavy metals and other mineral elements	Large, enclosed and illuminated system for cultivation of photosynthetic microbes
Major advantages	It gives room for growth control and nutrients optimization. Production of micro-algae biomass and other high valued products	Highly economical to construct and maintain. Lower cost of energy requirements owing to relative abundance of solar energy. Simple, functional design and operation for easier scale-up. Supports the use of non-arable lands	It provides sustainable remedy for waste water treatment. Enhances micro-algae biomass production at lower cost. Enhances carbon sequestration and GHG emission reductions	Increased biomass production. Possibility of growth parameters control and optimization. Lower CO_2 and water loss. Lower likelihood of contaminants
Major limitations	High cost of maintaining laboratory environment. Contamination and difficulties in pure culture maintenance. Uncertainties in scale-up and nutrients optimization for optimal growth	Lower cell densities and biomass productivity. Vast land mass requirement. Poor diffusion of light across the pond depth and surface area. Incessant pond contamination and water loss due to evaporation. Poor control over growth parameters due to high environmental dependency	Micro-algae cell growth inhibition can occur due to waste-water components. Different challenges in micro-algae harvesting. The process may lead to high initial capital and operational costs requirements	Requires higher initial equipment and maintenance costs. Light cannot be efficiently and evenly distributed in large PBR (50–100 L volume). Poor light penetration due to the presence of micro-algae biofilms

photoperiod), availability of nutrients, inoculum conditions, and process operation (such as batch, semi-batch, and continuous culture) are the key parameters that influence the growth performance and biomass productivity of microalgae under different modes and methods of cultivation.

4 The super-critical fluid extraction (SFE) process

Different methods are available for micro-algae cell disruption. These are solvent extraction, ionic liquids technique, direct saponification, high-pressure homogenization and ultrasound/microwave assisted extraction system.[50,51]

Super critical extraction process involves the use of solvents in their supercritical conditions. That is temperatures and pressures that are above their critical states. The super critical fluid possesses both gas and liquid characteristics and thus enables excellent solvating power, high diffusivity, low viscosity and higher mass transfer rates. The two most frequently employed supercritical solvents are carbon dioxide (CO_2) and water (H_2O). However, CO_2 offers superior super critical fluid characteristics because it is non-toxic, non-flammable, absence of chemical residual problems and low critical temperature.[52] Moreover, CO_2 is very easy to remove by reducing the pressure without leaving any residual particles behind, it is colourless, odourless, inert and cheap with critical temperature and pressure of 31 °C and 74 bar, respectively.

The major steps in SFE process are transport by diffusion of the solid materials to the surface, dissolution of the solutes in the supercritical fluids, diffusion into the particle by the super critical fluid and desorption (Fig. 6). The advantages of the process are absence of residual solvent in the extract, solubility changes due to changes in pressure and temperature, non-destructive process owing to low temperature operation, simple and efficient separation process and environmentally friendliness.[53] The various factors that affect extraction rate in a SC-CO_2 equipment are particle size, shape and porosity of the materials.

During extraction, the solvent (SC-CO_2) with the co-solvent such as ethanol which are added to increase the miscibility of the SC-CO_2 solvent diffuses into the material matrix and reduces the mass transfer resistance. Thereafter, soluble materials are dissolved into solvent and transported further by diffusion mechanisms. The soluble compounds diffused into the solid surface and subsequently to the bulk of the fluid phase. Finally, the solute and the bulk of the fluid phase are then transported to the extractor where they are separated.

The basic components of the SFE extraction process are extraction solvent chamber, reciprocating CO_2 pump for fluid pressurization, an oven that houses the extraction vessel, pressure maintenance device, sample pressure cell, inlet and outlet valves, vent valve, cooler, a CO_2 recycling system, a modifier pump, pre-heater coil and a collecting vessel. During SFE, the carbon dioxide solvent migrates into the supercritical phase where it then behave as though it is in a gas or liquid state and act as an efficient solvent and thus maintain the chemical composition of the compound especially if the material is heat sensitive (labile) and reduce solvent residue formation.

5 Comparison of *Chlorella* micro-algae lipid contents with different oil crops

The total lipids contents that can be obtained from the extraction of 100 g of the micro-algae seed by supercritical fluid and solvent extraction techniques are shown in Table 4. Moreover, by using supercritical fluid extraction technology, the *Chlorella protothecoides* micro-algae plant has higher triglycerides contents than

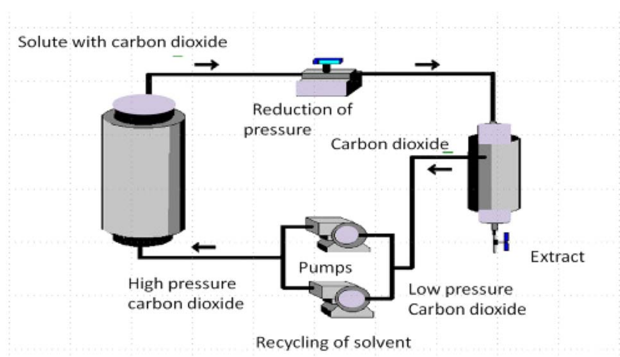

Fig. 6 Annotated diagram of super-critical fluid extraction process.[54]

Table 3 Effects of cultivation modes and methods on *Chlorella* micro-algae biomass and total lipid productivities

Micro-algae specie	Cultivation mode	Cultivation method	Nutrient source	Biomass productivity (mg L^{-1} day^{-1})	Lipid productivity (mg L^{-1})	References
C. vulgaris	Mixotrophic	*In situ* (laboratory)	Modified BG-11 medium	4.86 ± 0.15	47.6 ± 2.31	42
C. vulgaris	Photo-autotrophic	Photo-bioreactor	Bolds basal medium	250	56.2 ± 2.9	43
C. sorokiniana	Mixotrophic	2-Litre-photobioreactor	Optimized bolds-basal medium (BBM)	52.8	64.3	44
C. pyrenoidosa	Photo-heterotrophic	Open ponds	Paddy soaked waste water	260 ± 0.02	549.4 ± 1.41	45
C. scenedesmus	Heterotrophic	*In situ* (laboratory)	BG-11	92.3	56	46
C. vulgaris	Mixotrophic	*In situ*	Industrial sludge water	3.22 ± 0.04	26.66 ± 0.5	47
C. protothecoides	Heterotrophic	*In situ*	Modified basal medium	14.47	20.70	48
C. sorokiniana	Mixotrophic	Photo-bioreactor	BG-11 and Knop's medium	200	17.3	49

all the other seed crops from literature studies (Table 4). This value is higher than rapeseed and soybean seed materials with 35.8% and 19.9% oil yield from super critical fluid extraction process according to Walker *et al.*[55] and Friedrich and List[56] respectively (Table 4). Conversely, the results from Mouahid *et al.*[57] and Rai *et al.*[58] showed that the *Jatropha curcas* and the sunflower seeds have higher values of oil contents than the *Chlorella vulgaris* micro-algae plant from both growth media. The variations in oil content of crops are majorly due to individual plants genetic make-up, cultivation and growth mode as well as water and nutrients availability. The supercritical CO_2 process offers better oil yield characteristics than the Soxhlet technique due to increased solubility of the oil seed materials in a mixture of CO_2 and ethanol. A supercritical fluid has lower viscosity and superior solute diffusivity than polar solvents and thus increases mass transfer rates with higher yield within a short period of time. The advantages of SC-CO_2 process are absence of residual solvent in the extract, solubility changes due to changes in pressure and temperature, non-destructive process owing to low temperature operation, simple and efficient separation process and environmental friendliness. The various factors that affect extraction rate in a SCF equipment are nature of supercritical solvent (CO_2 or water (H_2O)), extraction temperature and pressure, type of co-solvent (methanol, ethanol, tertbutyl alcohol), particle size, shape and porosity of the materials. However, the growth medium (photoautotrophic, heterotrophic and mixotrophic), cultivation methods (open pond, volumetric flasks, photobioreactor systems) and micro-algae strain types are the important factors that affect micro-algae oil yield.

Table 4 Comparison of *Chlorella* micro-algae lipid content with different oil crops

Seed crop	Extraction method	Extraction solvent	Oil yield (%)	References
C. protothecoides	SFE	SC-CO_2	68.7%	25
Rapeseed	SFE	SC-CO_2	35.8	55
(*Brassica napus*)	Soxhlet	MeTHF	30	55
	Soxhlet	*n*-Hexane	18.22%	59
C. vulgaris	SFE	SC-CO_2	47.6 ± 2.31	42
Soybean	SFE	SC-CO_2	19.9	56
(*Glycine max*)	Soxhlet	*n*-Hexane	18.8 ± 0.1	60
Physic nut	SFE	SC-CO_2	60	57
(*Jatropha curcas* L.)	Soxhlet	*n*-Hexane	48.2 ± 0.12	61
Sunflower	SFE	SC-CO_2	54.37	58
(*Helianthus annus*)	Soxhlet	*n*-Hexane	37.50	62
Photoautotrophic *C. protothecoides*	Soxhlet	*n*-Hexane	14.57 ± 0.16	63
Heterotrophic *C. protothecoides*	Soxhlet	*n*-Hexane	55.20 ± 0.28	63
Photoautotrophic *C. vulgaris*	Soxhlet	*n*-Hexane	34.01	64

6 Biodiesel properties

Biodiesel fuel performance is dependent on the physical and chemical properties of the fuel. The two major properties that define a fuel are the cold flow and the critical properties.

Cold flow properties are associated with flowability and low temperature application of the biofuel. This property is usually characterized by cloud point, pour point, filter plugging point and low temperature filterability test point. Biofuels from *Chlorella* micro-algae species are usually associated with better cold flow characteristics compared to other biofuel biomass resources (Table 5). This improved low-temperature performance of biodiesel from *Chlorella* microalgae is attributed to its higher concentration of unsaturated fatty acids. According to Chandravati *et al.*,[65] unsaturated fatty acids exhibit lower crystallization temperatures compared to saturated fatty acids, which crystallize at higher temperatures. This lower crystallization temperature is primarily due to the presence of weak hydrogen bonding, particularly in methyl esters with *cis*-configurations.[66,67] In contrast, saturated methyl esters have

Table 5 Comparative analysis of *Chlorella* micro-algae biodiesel critical properties with vegetable oil and other previous studies

Biofuel	Pour point (°C)	Density @15 °C (kg m^{-3})	K.V. @40 °C (mm^2 s^{-1})	Cetane index	HHV (MJ kg^{-1})	I.V. (g I_2/gester)	A.V. Mg kOH g^{-1}	References
Jatropha oil	4	918	35.4	23–40	33/38.6	101	28.0/11	82
Biodiesel	2	880	4.84	51.6	37.2/39	104	0.4/0.24	83
Soybean oil	—	893 ± 13	12.8	—	37.16	119	0.96	84
Biodiesel	0	877	4.3	52.5	38.31	118	0.3	85
Sunflower oil	+3	920	36.3	37	39.6	135.4	0.72	86 and 87
Biodiesel	−3.74	880	4.68	50.54	43.90	125.2	0.32	88
Canola oil	—	906.2	36.3	36.55	36.99	113.5	2.7	89
Biodiesel	−9	861.9	4.7	49.4	33.65	106.9	0.49	90
WCO oil	+5	918.4	45.34	42	35.82	92.5	2.896	91
Biodiesel	+3	886.0	4.027	53	38.034	57.3	0.38	92
C. vulgaris biodiesel	ND	0.858	4.21	56.01	38.85	94.09	0.49	77
C. vulgaris biodiesel	−4.33 ± 0.333	0.858	4.967 ± 0.033	51.33 ± 0.333	43.33 ± 0.333	ND	ND	93
C. Protothecoides biodiesel	−12	0.864	5.2	ND	41	ND	0.374	63
Diesel fuel	−35 to 15	815	2.54	54.01	42–48	Very low	0.5	94 and 95
ASTM biodiesel	−15 to 16	880	1.9–6.0	47 min	Close to diesel	Report	0.5	71
EN biodiesel	Min	860–900	3.5–5.0	51 min	35 MJ kg^{-1}	130 max	0.5	70

stronger intermolecular forces, resulting in higher crystallization temperatures.[68] Poor cold flow characteristics can lead to abnormal fluid behavior due to fuel particles crystallization and gum formation, which may cause clogging of fuel pipes, fuel pumps, and injectors.[69] When biodiesel is used in cold climates, phase separation can occur, leading to either liquid–liquid separation (emulsion formation) or the formation of solid waxy substances due to liquid–solid phase transitions. Such phenomena can cause fuel filter clogging and poor engine performance. The solidified waxes increase fuel viscosity, thickening the biodiesel and exacerbating filter and injector blockages. Therefore, careful consideration of cold flow properties is essential, especially for biodiesel that is intended for use in low-temperature environments.

Additionally, viscosity, density, cetane index, calorific (HHV) and iodine values are the major biofuel critical performance indicators. *Chlorella* micro-algae biodiesel has viscosity values within the American biodiesel standard requirement (Table 5). A viscosity value in the range of 1.9 to 6.0 is recommended by both the American and European biodiesel standards.[70,71]

Viscosity is a scalar numerical measure of a fuel's resistance to flow, and it plays a critical role in influencing fuel injection, lubricity, atomization, and combustion characteristics.[72] It is an essential parameter for assessing fuel flow behavior. Elevated kinematic viscosity can cause soot deposits to accumulate on fuel injectors, engine pistons, rings, and valves. Additionally, it may contribute to increased smoke emissions due to inefficient fuel injection.[73]

Density is an important property of biodiesel, primarily influencing the fuel injection rates. It is largely dependent on the composition of ester components and the purity of the fuel. Density tends to increase with shorter carbon chain lengths and higher number of double bonds, with saturated fatty acid esters generally exhibiting higher density than their unsaturated counterparts.[74]

However, the degree of unsaturation has a more significant impact on density than the straight chain saturation factor (SCSF).[75] The *Chlorella* micro-algae biodiesel has moderate density values as observed from Table 5 compared to other biomass sources. These values result in increased fuel mass, better fuel atomization, enhanced fuel injection rate and improved combustion and emission performance.

The iodine value refers to the amount of iodine (in grams) absorbed by 100 grams of a given oil or biodiesel. It is a key indicator of the degree of unsaturation of the oil. Generally, biodiesels with lower iodine values are more combustible and efficient as fuels, but they may exhibit poor cold flow properties.[76] *Chlorella* micro-algae biodiesel has relatively lower iodine value according to Sharma *et al.*[77] (Table 5) and thus a more combustible and efficient fuel. When biodiesel contains a high proportion of unsaturated fatty acids, heating can lead to the polymerization of the glycerides, resulting in carbon deposits formation. This, in turn, can degrade the lubrication properties of the fuel.[78] In order to address these concerns, the EN 14214 biodiesel standard recommends a maximum iodine value of 120 g I_2/100 g of biodiesel. Cetane index is a critical indicator of biodiesel's combustion and ignition qualities. *Chlorella* micro-algae biodiesel is associated with higher cetane values (Table 5). This will unarguably result in shorter ignition delay, enhanced fuel combustion and performance characteristics and lowered carbon oxides (Cox) emissions.[79]

The heating value (or calorific value) refers to the amount of heat energy that is released during the complete combustion of a unit mass of biodiesel. A higher heating value promotes more complete combustion, which can lead to lower emissions; however, it may also result in a reduction in the total energy released per unit volume of the biodiesel.[80]

According to Chuah *et al.*[81] the heating value is primarily influenced by the molecular weight (that is., carbon chain length) and the degree of unsaturation of the fuel. An increase in unsaturation tends to raise the heating value, while an increase in chain length generally reduces it.

7 Recent advances in biodiesel production

Zhang *et al.*[96] critically examined biodiesel production using heterogeneous, functionalized organic–inorganic hybrid porous coordination polymer-based catalysts (OIHPCPs) through transesterification and esterification reactions. Their study systematically reviewed the application of OIHPCPs catalysts in the conversion of liquid biomass to biodiesel, covering pristine metal–organic frameworks (MOFs), functionalized MOFs (including acidic, basic, bifunctional, and enzymatic types), MOF derivatives, unconventional MOFs (UMOFs), and biomass-derived polymer catalysts. Therefore, OIHPCPs are regarded as highly promising catalysts for efficient biodiesel production due to their high porosity, uniform pore size distribution, tractable functional groups, and excellent catalytic activity and stability.

Meanwhile, Wang *et al.*[97] designed a highly efficient and recyclable bifunctional acid heterogeneous catalyst (FS-B-L-PILS), for the one-pot conversion of crude *Euphorbia lathyris* L. oil into biodiesel. The catalyst was synthesized by integrating acidic poly-ionic liquids (PILS) with a magnetic Fe_3O_4–SiO_2 (FS) carrier, resulting in a material with mesoporous structure (5.7 nm), abundant Brønsted and Lewis acid sites, strong superparamagnetic magnetization (19.7 emu g^{-1}), favourable hydrophobicity (81.9°), and excellent structural stability. The catalyst efficiently converted high-acid-value feedstock (25.60 mg KOH g^{-1}) into biodiesel in a single step using tetrahydrofuran (THF) as a biomass-derived co-solvent, achieving a biodiesel yield of 98.1%. The synergistic interaction between Brønsted and Lewis acid sites was identified as the main contributor to its high activity. Owing to its magnetic nature, FS-B-L-PILS could be rapidly and easily separated by an external magnetic field and retained a 91.2% yield after five reuse cycles, indicating strong reusability. Kinetic analysis revealed that the transesterification followed a quasi-first-order kinetic model with an activation energy of 64.6 kJ mol^{-1}, while thermodynamic studies indicated an endothermic and non-spontaneous reaction process. Furthermore, DFT calculations supported the proposed catalytic mechanism, confirming the catalyst's active role in facilitating biodiesel formation. The resulting biodiesel met both ASTM D6751 and EN 14214 standards, demonstrating the catalyst's efficacy in practical and sustainable biodiesel production from low-cost, high-acid feedstocks.

Moreover, Huang *et al.*[98] developed a photothermal catalyst composed of a graphene-like biomaterial that was uniformly anchored with neighbouring single atoms of potassium. Life-cycle and cost analyses were performed for biodiesel production from various acidic oils using this catalyst. The system achieved a high biodiesel yield of 99.6% at room temperature. The high yield was attributed to strong local photothermal effects at the solar interface and the presence of associated super-alkali sites of the potassium atoms. The produced biodiesel satisfied both European and the American biodiesel standards. The catalyst also showed excellent acid resistance and reusability, with virtually no loss in activity.

Interestingly, Zheng *et al.*[99] designed an efficient acid–base bifunctional heterogeneous catalyst, mesoporous zinc fluoride (MP-ZnF_2), for the one-pot conversion of low-quality, non-edible *Koelreuteria integrifoliola* oil (KIO) into biodiesel. The catalyst was synthesized through a fluorolytic sol–gel method using P123 as a template agent, resulting in a material with a large specific surface area (62.9 m^2 g^{-1}), uniform mesopores (12.15 nm), and abundant stable acid (1.22 mmol g^{-1}) and base (0.56 mmol g^{-1}) sites. These physicochemical features were identified as key factors contributing to its high catalytic efficiency in simultaneous esterification and transesterification reactions. Under optimized conditions determined by response surface methodology (103 °C, 6.2 h, 10.2 wt% catalyst loading, and a 29 : 1 methanol-to-oil molar ratio), MP-ZnF_2 achieved a maximum biodiesel yield of 97.15%. Kinetic studies revealed that the reaction followed pseudo-primary kinetics with a low activation energy of 49.24 kJ mol^{-1}, affirming its superior activity. Furthermore, MP-ZnF_2 exhibited excellent reusability (maintaining >90% yield after six cycles), strong water tolerance (89.25% yield at 8 wt% water), and adaptability to various feedstocks (yields >90%). The produced biodiesel complied with ASTM D6751 and EN 14214 standards. Economic analysis also demonstrated good affordability (≈$13.31 kg^{-1}), highlighting MP-ZnF_2 as a sustainable, cost-effective catalyst for green biodiesel production under mild conditions, supporting the advancement towards carbon-neutral energy systems.

He *et al.*[100] developed a novel ternary S-scheme heterostructure photocatalyst (g-C_3N_4–TiO_2@Mt (CTM)) using a theory-guided experimental strategy to precisely tailor the microenvironment for photocatalytic biodiesel production. By incorporating inexpensive and biocompatible montmorillonite (Mt), the optimized CTM-2 exhibited outstanding performance, achieving a 98.5% biodiesel yield with excellent stability (>90% activity retention after 5 cycles). Through a combination of theoretical calculations, *ex*/*in situ* X-ray photoelectron spectroscopy (XPS), and X-ray absorption near-edge structure (XANES) analyses, the study revealed that CTM-2 enhances reactant enrichment and facilitates directional charge carrier migration. The built-in interface electric field (IEF) within the heterostructure accelerates charge transfer while creating a favourable microenvironment for substrate adsorption. This synergistic effect significantly promotes the enrichment of electronegative oleic acid (OA) carboxyl groups, thereby increasing local substrate concentration and boosting photocatalytic biodiesel conversion efficiency.

Finally, Sun *et al.*[101] conducted a comprehensive review of recent advances in catalytic strategies for biomass-to-biofuel conversion. The study systematically explores the fundamental mechanisms of major catalytic pathways such as photocatalysis (PC) and electrocatalysis (EC) and their synergistic hybrid approaches, including photothermal catalysis (PTC), photo-enzymatic catalysis (PENC), and photo-electro-catalysis (PEC). These integrated methods provide innovative avenues for converting biomass into high-value biofuels. The authors emphasize that catalyst design, reaction condition optimization (temperature, solvent, light source, voltage), and biomass feedstock characteristics play critical roles in determining conversion efficiency. Key reaction pathways such as esterification, transesterification, hydrogenation, and decarboxylation were discussed in depth, along with strategies for synthesizing gaseous biofuels like hydrogen (H_2) and syngas from lignocellulosic biomass. Through biochemical transformations, glucose can be efficiently converted into levulinic acid (LA) *via* glycolysis and the tricarboxylic acid (TCA) cycle. Subsequently, LA can be transformed into γ-valerolactone (GVL) and alkyl levulinates through esterification, both of which serve as high-quality fuel precursors compatible with conventional diesel and gasoline. This significantly enhances energy density, combustion efficiency, and economic feasibility in real-world fuel applications. Notably, the conversion of LA to GVL can proceed efficiently without an external hydrogen source under PC or EC conditions. In syngas production, solar-driven photocatalytic and photothermal technologies are highlighted for their ability to lower energy consumption, enhance C–C bond cleavage, and preserve C–O and C–H bonds, improving product selectivity and yield. Although direct lignocellulose photodecomposition can generate hydrogen, enhancing H_2 yield from lignin remains a critical challenge for future research. The authors therefore concluded that advanced catalytic strategies most especially those leveraging on renewable energy inputs offer a promising pathway towards sustainable biofuel production, contributing to a greener and more resilient energy ecosystem.

8 Recent advances in sustainable micro-algae biomass production

The following are the new technologies in micro-algae cultivation towards higher biomass and total lipid productivity.

8.1 Hybrid cultivation system

This is majorly a two-stage micro-algae cultivation system or an inter-play between outdoor and in-door cultivation systems towards improved and cost-effective algae biomass and total lipids production. During this process, a particular micro-algae strain is initially grown in a photo-bioreactor under desired growth parameters and nutrients supply for rapid biomass growth and then transferred to an open pond under nutrient (nitrogen) deficient conditions for higher lipids accumulation.[102,103]

This technology addresses the high cost of photo-bioreactor design and operation and the contamination risks that are encountered in raceway ponds. Examples of hybrid systems are tubular bioreactors and open ponds, airlift reactors and open raceway ponds. These systems have potentials for the use of waste-water as a growth medium and thereby encouraging nutrients and contaminants removal as well as in biofuel production. According to Bora *et al.*[104] hybrid cultivation technology offers inherent advantages such as lower culture contamination risks, increased biomass production and better carbon sequestration tendencies. The system is economical and can be employed in large scale micro-algae cultivation for biofuel production with enhanced biomass yield, higher lipid productivity and optimal recovery of resources from waste water.[105,106]

Swan *et al.*[107] demonstrated the viability of two-phase cultivation method in biomass production for biodiesel synthesis using effluents from diary outlets.

8.2 Microbubble-assisted photobioreactor (M-PBR)

Bubble-column photobioreactors (PBRs) are reactor vessels that are cylindrical in shape with height-to-diameter ratios typically greater than two. They offer cost-effectiveness, a high surface-area-to-volume ratio, and efficient heat and mass transfer advantages.[108,109]

During operation, gas is introduced through a sparger at the base to provide mixing and to facilitate CO_2 transfer, and perforated plates are often incorporated at larger scales to break up and redistribute bubbles. M-PBRs are easy and energy-efficient bubble column reactor systems with simple designs that can be easily scaled-up. Micro-bubbles enhance large gas–liquid (CO_2 and liquid culture nutrients) contact area and algae mixture homogeneity and thereby promote better carbon dioxide absorption and nutrients assimilation by the micro-algae.[110] This technique boosts photosynthesis and biomass daily productivity for biofuel production.

8.3 Nanotechnology-enhanced photobioreactor (N-PBR)

The addition of nanoparticles to micro-algae culture in photobioreactors can enhance biomass yield and total lipids accumulation in micro-algae cells. This is attributed to improved carbon dioxide absorption and enhanced solar energy conversion in photobioreactors. Nanoparticles in small concentrations can inhibit the growth and development of other microorganisms that are present in the algae culture and thereby reducing competition for nutrients among different microbes that coexist with algae in the culture.

8.4 Genetic engineering

Micro-algae strains can be genetically modified to greatly adapt to unfavourable environmental stresses such as high temperature or inadequate rainfall that may adversely affect biomass and lipids production. The important technologies include genome editing, gene expression and repression, and synthetic promoters' application These key technologies are used to increase lipids accumulation and improve the quality of micro-algae lipids for biofuel synthesis.

8.5 Smart farming technologies and automation

In recent advances, farmers can make rational decisions with the aid of digital technologies such as artificial intelligence (AI), internet of things (IoT) and data analytic tools in regards to micro-algae specie selection, growth variables optimization (temperature, soil profile, rainfall, humidity, sunlight intensity), nutrients requirement and proportions, fertilizer, bio-reactor design variables, micro-algae harvesting and storage ability among others. These digital instruments enhance biomass and total lipid productivity, increase cultivation efficiency, promotes economy of cultivation through waste reductions, efficient resource allocation and improve overall algae-farming profitability and for sustainable biofuel production.

9 Conclusions

1. *Chlorella* micro-algae contain lipid-rich strains that are ideal for sustainable biodiesel production.

2. The micro -algae is effective in carbon capture and industrial CO_2 mitigation and they are also viable in nutrients and heavy metals removal during waste water treatment and biomass growth.

3. The daily biomass productivity of *Chlorella* micro-algae depends on strain type, nutrient source and method of cultivation with typical values ranging from 1.5 to 10 mg L^{-1} day^{-1}. The lowest value is found in the photoautotrophic culture in the vicinity of 1.5 to 2.5 mg L^{-1} day^{-1}. While heterotrophic and mixotrophic modes have values around 5–7 mg L^{-1} day^{-1} and 8–10 mg L^{-1} day^{-1} respectively.

4. *Chlorella* micro-algae have higher total lipids productivity in the range of 15–70 mg L^{-1} depending on the route of nutrient metabolism with mixotrophic growth mode having the highest lipids accumulation of 45–70 mg L^{-1}. While photoautotrophic and heterotrophic modes have lipids contents of 15–30 mg L^{-1} and 35–68 mg L^{-1} respectively. This high lipid presence greatly enhances sustainable biodiesel production from the *Chlorella* micro-algae species.

5. Enzymatic activities, redox balance and metabolic fluxes are the major parameters that influence carbohydrates and lipids production in *Chlorella* micro-algae. For instance, the absence of sunlight and the availability of organic carbon compounds such as glucose can shift heterotrophic metabolic pathways from rapid PUFAs synthesis towards excess lipids accumulation.

6. Mixotrophy and heterotrophy favour excess sugar conversion into lipids for biodiesel synthesis. While photoautotrophy enhances higher carbohydrate synthesis for bi-hydrogen, bioethanol and biogas production.

7. The close cultivation technique is sine qua non for enhanced micro-algae biomass and total lipid productivity for liquid fuel synthesis. However, the open cultivation system is

economical for better waste water treatment because of accommodation of large volumes of wastewater.

8. Micro-algae growth in photobioreactors is primarily influenced by light intensity, its duration and exposure impacts, nutrients availability and transport mechanisms and reactor/cultivation temperature.

9. Hybrids cultivation technology offers inherent advantages such as lower culture contamination risks, increased biomass production and better carbon sequestration tendencies.

10. Finally, *Chlorella* micro-algae biodiesel has higher cetane index and calorific value between 50–56 and 38–43.33 MJ kg^{-1} respectively. This results in better ignition quality (shorter ignition delay period), enhanced fuel combustion (more complete combustion) and performance characteristics, lower engine fuel consumption and lowered carbon oxides (Cox) emissions.

10 Recommendations for further studies

1. Further studies should focus towards scale-up and commercial application of *Chlorella* micro-algae biofuels in Africa.

2. More research towards technically and economically friendly micro-algae cultivation methods because high costs of production usually threaten its commercialization and economic sustainability in Africa.

3. Advancement of future research for efficient and sustainable micro-algae cultivation methods such as hybrid system, nano-technology enhanced photobioreactor (N-PBR), flat-plate photobioreactor system (FP-PBR) and genetic engineering.

4. More research should focus on the growth of micro-algae in sludge water as it provides sustainable remedy for industrial and agricultural waste water treatment and enhances micro-algae biomass production at lower cost. It is also effective in carbon sequestration and GHG emission reductions.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Abbreviations

Acetyl-COA	Acetyl-coenzyme
ADP	Adenosine diphosphate
ASTM	American Society for Testing and Materials
ATP	Adenosine tri-phosphate
BBM	Bolds basal medium
CTA	Citric acid cycle
EN14214	European Committee for Standardization
FAMEs	Fatty acid methyl esters
FP-PBR	Flat plate photobioreactor
HCS	Hybrid cultivation system
HHV	Higher heating value
Modified BG-11	Modified blue green algae medium
MOFs	Metal–organic frameworks
M-PBR	Microbubble-assisted photobioreactor
MUFAs	Monounsaturated fatty acids
NADH	Nicotinamide adenine dinucleotide
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
N-PBR	Nano-technology-enhanced photobioreactor
OIHPCPs	Organic–inorganic hybrid porous coordination polymer-based catalysts
PGA	Phospho-glyceric acid
PG-aldehyde	Phosphor-glyceride-aldehyde
Pi	Inorganic phosphate
PILS	Poly-ionic liquids
PUFAs	Poly unsaturated fatty acids
RubP	Ribulose bisphosphate
SC-CO_2	Supercritical carbon dioxide
SFE	Supercritical fluid extraction
TAGs	Triacylglycerols
TCA	Tricarboxylic acid cycle
UMOFs	Unconventional metal organic frameworks
V/S	Surface area relative to volume ratio

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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