

Enhancement of the productivity of microalgae biomass combined with waste water treatment to produce sustainable biofuel and bioproducts.

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Abstract

Microalgae are considered as a feedstock of third-generation biofuel without competing with land crops, displays promising viable alternative energy source to substitute fossil fuels. Wastewater streams are the prerequisites of organic and inorganic compounds for the cultivation of the microalgae culture converting CO₂ into fuel products in a lucrative way to minimize environmental pollution and greenhouse effect. Studies on the mixotrophic mode of microalgae demonstrated the potential to accumulate metabolites (lipids, starch, etc.) and reported domestic wastewater purification by *Chlorella minutissima* sp. The real interest in microalgae anaerobic digestion lies in its ability to mineralize nitrogen and phosphorus. It results in a flux of phosphate and ammonium as substrate that can be further processed to produce fertilizers. Many species produce amounts of lipids as high as 50–60% of their dry weight including *Botryococcus braunii* sp., *Chlorella vulgaris* sp., and *Nitzschia laevis* sp.

Microalgae can also be used as a substrate for dark fermentation producing hydrogen by photobiological pathways. In addition, CO₂ is generated as a by-product in this pathway can be recycled as a carbon source for further microalgae cultivation and exploring its effect on different wastewater *Tetrademus obliquus* sp. indicated microalgae capabilities achieving significant biomass. Production of biofuel is impeded by many commercialization challenges like deficiency of energy and cost-intensive processes for algae growth. But, the promising features of microalgae have pitched the idea of establishing a sustainable bio-refinery and reinforce the objectives of resource-efficient bio-economy.

Keywords: Microalgae; Waste water treatment; Biofuel; Environmental pollution; CO₂ sequestration.

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I. Introduction

Burgeoning industries venture and expanding population are responsible for creating abundant solid, liquid and gaseous waste barely investing in monitoring and eco-friendly degradation of baneful chemical (G Yadav, et al., 2019). New data from World Health Organization (WHO) suggests that 90% of the total population is breathing high pollutants air, estimating death of 7 million people every year. In addition to air pollution, intense ecological disturbance due to climate change, global warming (greenhouse effect) and municipal waste has called for urgent need of actions to be taken, for reclaiming the detrimental environment (Air pollution, 2021). Municipal sewage/sludge comprise major source of mistreated waste water which, greatly harm the aquatic ecosystem and fresh water streams. Therefore, finding solutions from waste water (domestic, industrial and agricultural) treatment to rapidly rising CO₂ concentrations a biological approach, is required in a cost-effective way over conventional methods like precipitation, evaporation and carbon adsorption on the edge of recycling valuable nutrients and renewable technologies. Also, increasing population dominated by limited renewable and nonrenewable resources triggers up the need of economy to bend over alternative production chains in the energy, biomasses and biofuels. Thus, in face of preserving environment and biodiversity, microalgae culture has shown potential for waste water treatment owing to its greener application, significant biomass production, CO₂ fixation and biofuel generation (MI Khan, et al., 2018). Emphasizing on the photosynthetic efficacy, this study aims to provide some insights into the third-generation feedstock microalgae for generation of biomasses and biofuels. Though, numerous studies have revealed purification techniques of waste water combined with microalgae and large-scale production of biomass. Present study is an attempt to discuss the critical comparison, enhancement and evaluation of micro algal culture in treating waste water to achieve cost-effective integrated biorefinery applications. Further signifying the microalgae-based biomass

production through different chemical, biological and heat based conversion, Fig 1 provides basic understanding and an outlook on how microalgae is represented as The Third Generation Feedstock.

Photosynthetic organisms growing alone or symbiotically in aquatic habitat with a wide range of temperature tolerance, light intensities, pH and salinities are known as algae. Marine ecosystem is dominated by microalgae symbiotically associated with cyanobacteria collectively called as phytoplankton capable of performing photosynthesis simultaneously utilizing the greenhouse gas CO₂ photo autotrophically (MS Parker, et al., 2008). This process of converting sunlight as a source of energy and inorganic carbon captured from atmospheric CO₂ is demonstrated in Fig 2 giving a brief account of overall process inside the algal cell. Yet, microalgae are also able to take up dissolved components from atmosphere and heterotrophically carry out metabolic pathways demonstrating a mixotrophic mode of nutrition. Thus, being the rich source of carbon through fixing atmospheric CO₂, microalgae serve as promising machinery to produce biofuels, supplements and other pharmaceutical products. Beside carbon as a key element the metabolism of microalgae is also regulated by nitrogen, hydrogen, sulfur, oxygen and phosphorus which add up 99.9% of the total biomass (T L da Silva, et al., 2021).

II. Efflux from different industries and variation in components present in wastewater.

Wastewater is the polluted form of water which is generated from any combination of domestic, commercial, industrial and any inflow or sewer infiltration (G Tchobanoglous, et al., 1991). It is a mixture of complex organic (hydrocarbons, lipids etc.), inorganic (sodium, calcium, potassium, magnesium, chlorine, Sulphur, phosphate, bicarbonate, ammonium salts) and heavy metals as well as man-made compounds (C Alcántara, et al., 2015). Waste water has a great potential to growth of these microalgae and eliminate groups of harmful chemicals. Most of all impurities may be removed by Biological processes using microorganism. At the present Microalgae biofuels are affiliated to the third generation of biofuels, which are considered as an alternative energy source of green renewable fuel for sustainable energy production in the future. Various species of microalgae, like *Botryococcusbraunii*, *Nannochloropsis* sp., *Dunaliellaprimolecta*, *Chlorella* sp., and *Cryptocodiumcohnii*, produce bulk amount of hydrocarbons and lipids (SR Medipally, et al., 2015). Some of the species of microalgae are mentioned in Table no. 1 considering major components in the biomass production besides total amount of biomass produced. Different types of wastewater efflux garnishing the algal productivity have also been indicated. A colonial green algae *Botryococcusbraunii*, has the ability to produce a huge number of hydrocarbons as compared to its biomass, and it is also able to produce different types of commercially important compounds like carotenoids and polysaccharides etc. Microalgae oil content production level is 20-50% but some species are able to produce up to 80% of its biomass. Other than biofuel, microalgae is also able to produce several bioactive compounds and they have many applications in pharmaceuticals, nutraceuticals, chemical and food industries (A Rahman, et al., 2020).

III. Cultivation of microalgae on waste water.

Microalgae cultivation using wastewater offers to resolve three big problems of world first wastewater management second atmospheric carbon fixation and third biofuels production (E Posadas, et al., 2015). Microalgae have potential to 40-50 % higher biomass production among all terrestrial crops and able to 80-100% pollutant remove from waste water. during production of biomass average rate of fixation of atmospheric CO₂ concentration is 1.83 kg CO₂/kg of biomass. the microalgae biomass carries valuable metabolites including amino acids, omega-3-fatty acids, pigments, and hydrocarbons etc. these compounds are extracted and use for biofuels production through biological fermentation or transesterification(S Sriram and R Seenivasan, 2012).The cultivation systems of microalgae could be classified into three major type: Phototrophic Cultivation, Heterotrophic Cultivation, Mixotrophic Cultivation respectively (S R Medipally, et al., 2015) (M Molazadeh, et al., 2019).

3.1 Phototrophic Cultivation.

In Phototrophic Cultivation Microalgae are able to photosynthesis for their blooming. They fix the environmental CO₂ in the presence of sunlight so it has a significant role in reducing CO₂ in the environment. They are widely used for biomass production due to low cost and simpler management but abiotic factors in which light has a main limiting factor in production. In this cultivation , microalgae is largely cultivated from two types -open system and close system (enclosed photobioreactors) although sometimes these groups might show overlap (M Molazadeh, et al., 2019) (F Javed, et al., 2019).Fig 3 shows the structural representation in the form of Flow diagram mainly concerning towards a comparative approach on the basis of sources, cell density and operating of different cultivation system. However, species associated with photobioreactors with certain parameters have been indicated in Table no. 2.

3.1.1 Open system

The open systems generally established in outdoors included ponds, deep channels, lake, lagoons, man-made tanks etc. It was the first microalgae cultivation systems proposed. It is widely used in commercial-scale algae production as well as waste water treatment. But a limited number of algae genera *Spirulina*, *Chlorella*, and *Dunaliella* are used in open pond cultivation because of extreme environmental conditions like having a high alkalinity, high nutrient concentrations and high salinity and climate changing throughout the year etc (S R Medipally, et al., 2015). Open system classified generally in two terms -non stirred and stirred.

Non-stirred ponds are open and around one-half meter or less in depth. In South-East Asia more than 30 tons per year production occur from non-stirred ponds and lakes. For commercial production mainly *Dunaliella salina* microalgae species are used (M Molazadeh, et al., 2019). Stirred ponds are open and shallow and the water is flowed with a paddlewheel. In Stirred pond- proper light, aeration improves the biomass production capacity. The most common example of stirred ponds is raceway ponds and circular ponds. Raceway ponds also called HRAP, (high rate algal ponds) (R RNarala, et al., 2016). The raceways are made up with single as well as multiple channels in a closed loop. Raceway ponds are commonly used for industrial waste water management and for *Chlorella* spp., *Arthrospira platensis*, *Hematococcus* spp., and *D. salina* culturing. At present, raceway ponds are in contribution to microalgae cultivation because of less expensive and easy to manage than closed system and other system. However, raceways also suffer from many drawbacks like low productivity due to algal contamination, poor mixing efficiency, shading effect etc (S R Medipally, et al., 2015).

3.1.2 Closed System

It is also called enclosed photobioreactors. Usually, they are located indoors and made up of several transparent tubes. Closed systems reduced many of the problems of open systems such as avoid of extreme environmental conditions, minimization of water loss through evaporation and reduction of contaminating species and also show control on many parameters like pH, dissolve oxygen, irradiation of system. Close system able to utilize large number of microalgae species for biomass production and able to exhibit high control. Hence various types of photobioreactors have been designed for algae production (F G Acién, et al., 2017).

3.1.3 Tubular Photobioreactor

Tubular photobioreactors, are primarily used for industrial purposes. Use of wide range of algae species including, *Porphyridium*, *Haematococcus*, *Arthrospira*, *Chlorella*, *Dunaliella*, *Tetraselmis*, and *Phaeodactylum* species. On the basis of applications, various types of tubular photobioreactors are formed like horizontal, vertical, helical tubular reactors. The algae and culture media are continuously circulated through the tubes to a reservoir using an airlift or mechanical pump. But its main downside is high level of dissolved oxygen, unfavorable pH and CO₂ gradient of system (R RNarala, et al., 2016) (S R Medipally, et al., 2015).

3.1.4 Flat-plate photobioreactor

Flat-plate photobioreactors are most appropriate for large-scale microalgae cultivation in indoor and outdoor. It is able to reduce tubular photobioreactor's negative side by lowering the accumulation of dissolved oxygen (J Carvalho, et al., 2014).

3.1.5 Plastic Bag Photobioreactor

Plastic Bag Photobioreactor are large transparent bag which are 0.5 meter in diameter and filled aerators. Generally, they are dangled vertically but sometime placed in plastic cage and come to direct contact with sunlight. Moreover, microalgae culture is mixed regularly using diffused air pump. Main disadvantage constitutes continuous attention and maintenance issues and poor mixing problems (J Carvalho, et al., 2014) (L Xu, et al., 2009).

3.2 Heterotrophic Cultivation

In heterotrophic cultivation microalgae utilize organic carbon source (mostly glucose) for blooming and do not require sunlight for essential metabolic reactions like phototrophic microalgae. In this type, cultivation biomass production is comparably higher than phototrophic microalgae production because of high level control on cultivation procedure and elimination of light necessity. Additionally, microalgae are metabolically propitious for large amounts of lipid accumulation which can be converted into biodiesel. Currently heterotrophic marine algae cultivation are used for polyunsaturated fatty acids production (V S Mohan, et al., 2015). However, heterotrophic cultivation also exhibits some limitations i.e. limited number of microalgae application. Glucose is the primary organic source for heterotrophic microalgae growth and release the CO₂. (T L da Silva, et al., 2021) (S R Medipally, et al., 2015). Some of the species encouraged to produce lipids in heterotrophic type of cultivation system are indicated in Table no. 3.

3.3 Mixotrophic Cultivation

Microalgae are able to do photosynthesis as well as utilization of organic sources for blooming. So these microalgae do not possess limiting factor for their metabolic activities. In the absence of sunlight they use

organic source as food and reduces the impact of biomass loss while in the presence of sunlight they don't use organic matters for growth. Therefore, based on these properties they also play role in CO₂ fixation and significant role in microalgae biofuel production. Like heterotrophic it also faces the same problem of limited number of microalgae including *Spirulina platensis* sp. (cyanobacteria) and *Chlamydomonas reinhardtii* sp. (green algae)" (S A Razzak, et al., 2017).

IV. Co₂ capture, effect and challenges in algal biomass production.

The long-term removal of atmospheric CO₂ to mitigate climate change is called CO₂ sequestration. It is a natural capture of CO₂ through biological, physical or chemical processes occurring within ecosystems but can be artificially devised to large scale ocean water, saline aquifers, ocean fertilizers etc (R Sedjo and S Brent, 2012). Biological conversion/fixation of CO₂ can be acquired using microalgae or macroalgae concentrating over effective large scale production. Thus, acting as carbon source CO₂ is effective on biomass and lipid accumulation. Microalgae convert CO₂ into glucose (energy) to determine cell growth by the process of photosynthesis. However, the increasing concentration of CO₂ enhances the mechanism of mass transfer allowing declining in cell pH, this declination inhibits cell growth. Hence, potentially trapping bicarbonate from ponds is one of the attractive alternative approaches producing algal biomass (R Kothari, et al., 2021). It is further believed that algal biomass reduces the CO₂ emission resulting from respiration besides CO₂ sequestration. This green technology utilizing algae as a source of feedstock for biofuel and biogas by extracting nitrogen, phosphorus, CO₂ and other nutrients from wastewater/ sewage is an alternating approach replacing harmful chemicals and bad odor issues (S A Razzak, et al., 2017). Certain studies show that *Auxenochlorella protothecoides* cultivated in wastewater potentially increases biomass and lipid content (G Chen, et al., 2015). However, confirmation of both positive and negative impact of CO₂ sequestration on the saturation point of biodiesel conversion has also been reported. While comparing CO₂ fixation through agricultural, aquatic plants and natural forest cover, energy-conserving structure of microalgae have shown potential to fix CO₂ on the scale higher than 10×. Recently, life cycle assessment (LCA) studies evaluated that extensive energy input is required for microalgae cultivation and harvesting processes. For this, energy in the form of electricity generated from ignition of natural gas and coal is required, but the emission of considerable amount of CO₂ terminates the entire productivity of biofuel from cultured microalgae through CO₂ bio-fixation. It is quite a considerable concern over drawbacks of CO₂ capture technologies and effects of associated flue gas to grow microalgae. Thus, this fluctuation in biomass production and total lipid content depending on increasing and decreasing demand of CO₂ (CO₂ sequestration) is represented in Table no. 4. However, cost effective and eco-friendly approach needs to be further diagnosed thoroughly for sustainable biofuel production (R Kothari, et al., 2021).

1. Applications future prospects and strategies to enhance microalgae biomass production.

Microalgae biofuels are used in several purposes such as – biofuel production, bio gas formation, pharmaceutical companies, electricity generation and waste water treatment etc. Biomass pretreatment thermochemically yields bio-oil and residue as intermediary products. As a result, bio-oil must be transformed into biofuel under various circumstances. Intermediary Residue are transformed into a gaseous fuel or a solid, nutrient-rich bioproduct using biochemical or thermochemical processes. Transesterification (chemical, enzymatic), biochemical conversion (fermentation, anaerobic digestion), thermochemical conversion (gasification, pyrolysis, liquefaction), and hydro processing are examples of conversion processes. Pyrolysis, gasification, and hydrocracking (the use of high-pressure, high-temperature catalysts and hydrogen to produce hydrocarbons) have the potential to produce biomass-based petroleum equivalents, such as biocrude, bio gasoline, and biodiesel fuels, (Y Ghasemi, et al., 2012) that are nearly indistinguishable from and even have advantages over their petroleum-based counterparts. Some of the major applications and uses of biomass are illustrated in Fig 4.

There are some added advantages with biofuel. Biofuel lowers emissions of unburned hydrocarbons, carbon monoxide, sulfates, polycyclic aromatic hydrocarbons, and nitrated polycyclic aromatic hydrocarbons in conventional diesel engines. Algal biofuels come in a wide range of types. Algal biomass may be used as a solid biofuel to create heat, steam, and electricity, or it can be transformed into gaseous biofuels like biogas and biohydrogen. Starch-rich algae can readily be fermented into liquid biofuels like bioethanol and biobutanol. Existing technologies can convert algae oils to diesel, gasoline, and jet fuel (Algae to Biogasoline and Beyond, 2021). Currently, biodiesel is blended (2–10%) with crude oil in current engines without any changes due to the same viscosity, vapor pressure, density, and octane/cetane number. Algae can generate 267 liters of ethanol (assuming ideal conditions). Algae-based biofuels production's commercial success will ultimately be determined by economics. In algae production, harvesting has the most financial impact on cost. Harvesting includes costs like: drying algae, Chemicals, electricity, and personnel costs for all operations, as well as infrastructure and capital expenditures. The cost of producing algal oil is determined by a variety of parameters, including the yield of biomass from the culture system, the oil content, the size of production systems, and the

cost of extracting oil from algal biomass. The future economic viability of using algal oil as a biofuel largely depends on the price of petroleum oil. To Enhance the economic viability of microalgae biofuels following can be done a) biorefinery: strategy for high-value co-products b) design of sophisticated photobioreactors; c) selection of cost-effective biomass collection and drying methods (Y Ghasemi, et al., 2012)(Y Christi, 2007).

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Figures

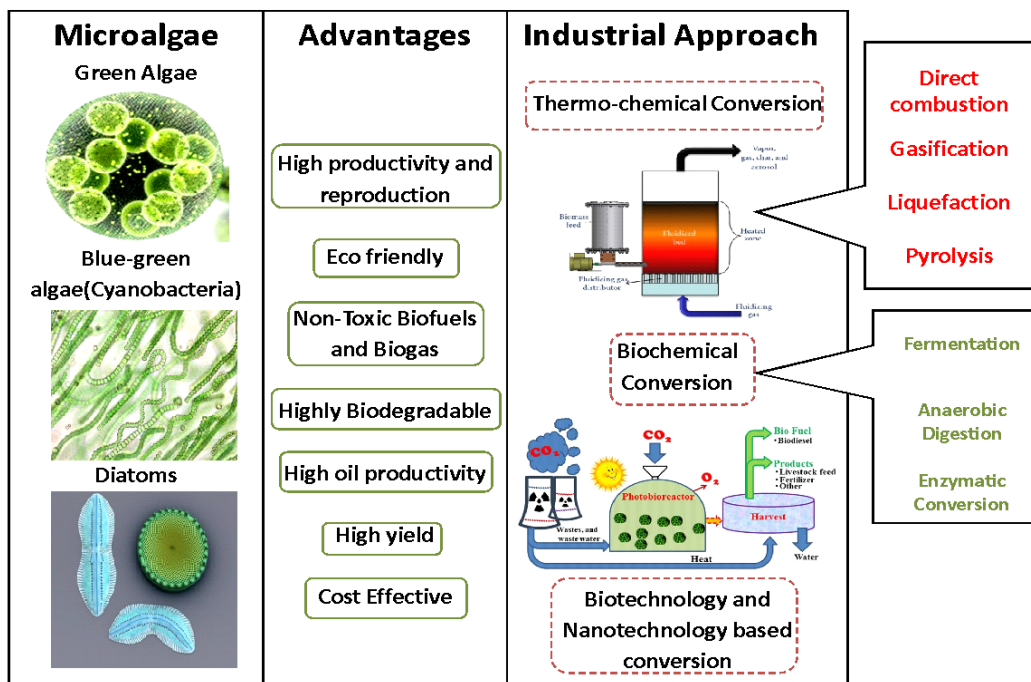


Fig.1 Microalgae: The Third Generation Feedstock- types, advantages and industrial process of conversion (MJ Borah, et al.,2018) (“Diatoms Stock Photos”, 2021)

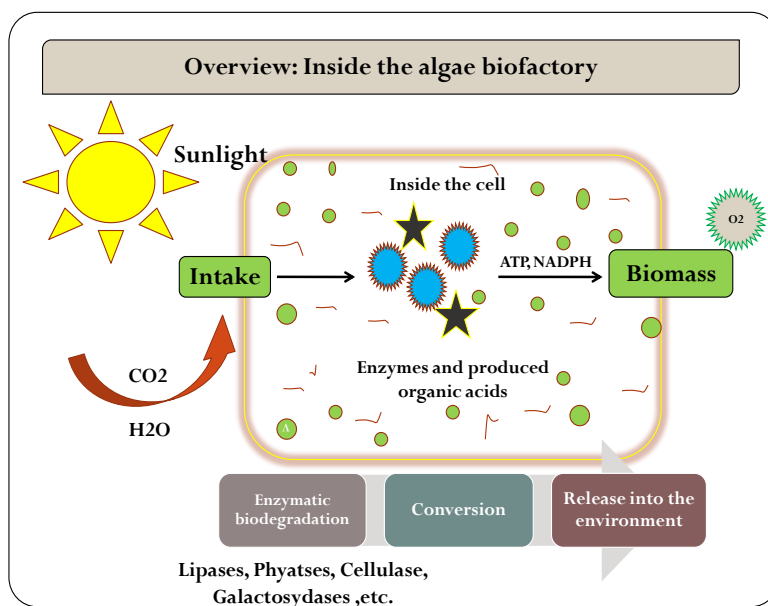


Fig.2Structural illustration of the algal bio factory representing three different phases.

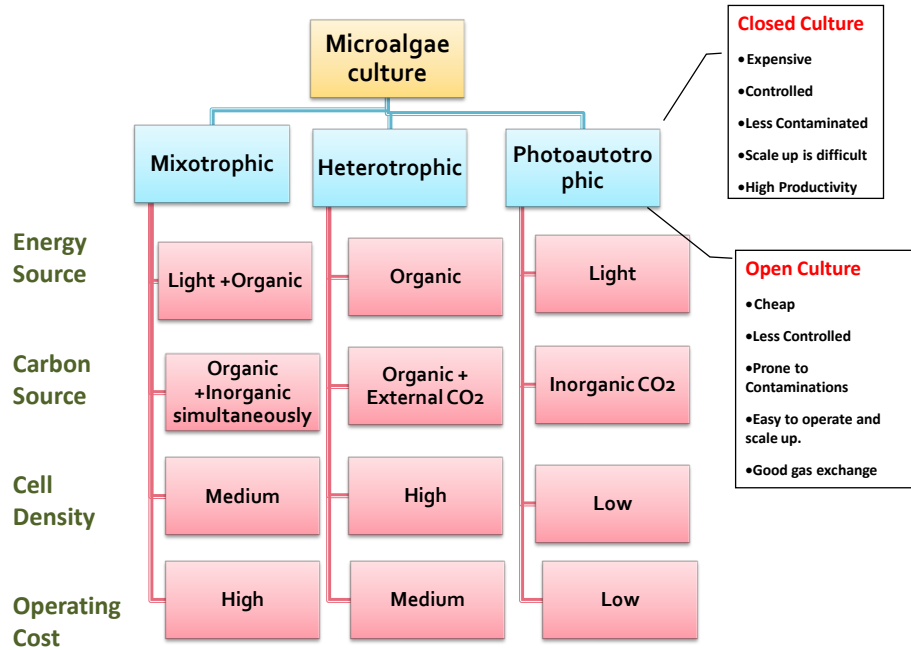


Fig.3 Comparison between three different types of microalgae cultivation system, representing energy and carbon source along with cell density and operating cost (F Javed, et al., 2019).

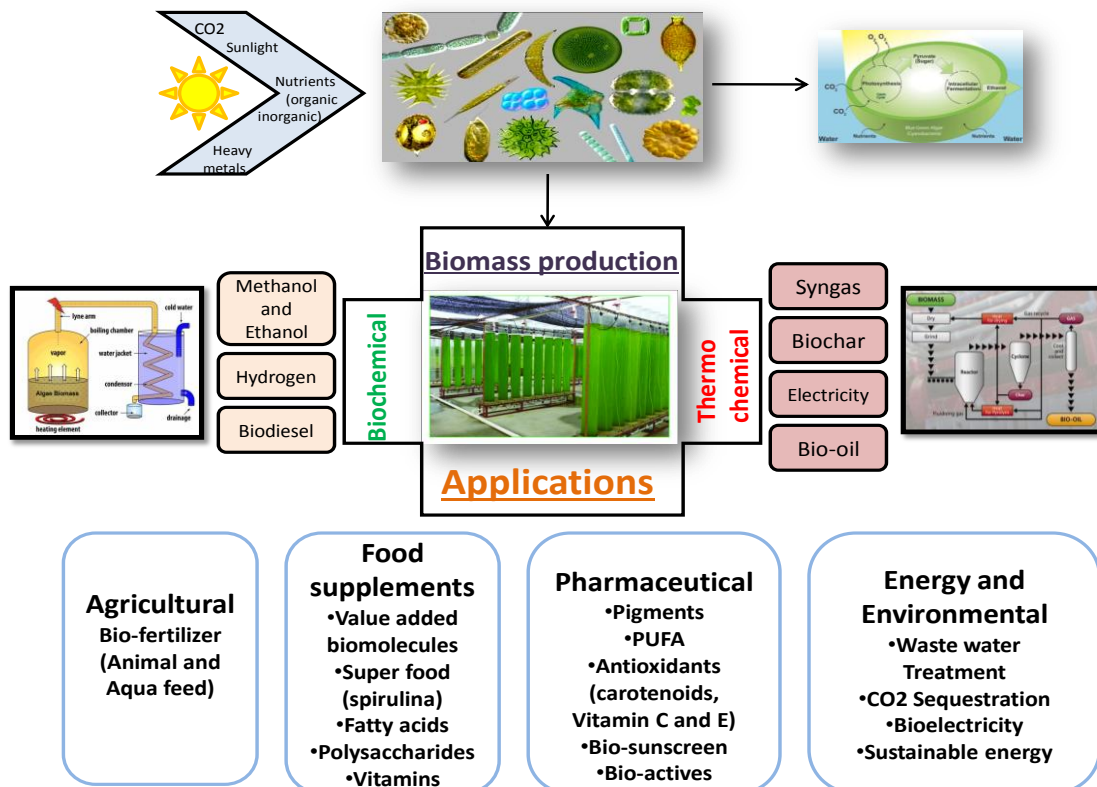


Fig.4 Applications of large-scale production of microalgae biomass (“Algae to Biogasoline and Beyond”, 2021) (“Types of microalgae”, 2021) (“Production of ethanol in alge”, 2021) (“Production of Biomass through pyrolysis”, 2021) (“Photobioreactor”, 2021).

Tables

Table 1: Comparison of different types of algal species on the basis of maximum amount of Carbon and Temperature tolerance considering total biomass and lipid productivity in various wastewater sources (R Kothari, et al., 2021) (Z Arbib, et al., 2013) (R W Gaikwad, et al., 2016) (C Viegas, et al., 2015).

Algae Species	Algae Type	Wastewater efflux	Biomass production (Mg L ⁻¹ day ⁻¹)	Lipids Productivity (Mg L ⁻¹ day ⁻¹)	Maximum CO ₂ Tolerance (%)	Maximum Temp. Tolerance (°C)
Botryococcusbraunii sp.	Green	Treated sewage (secondarily)	35	-	-	30
Chlorella sp.	Green	Centrate municipal wastewater	231.4	18.7	50	50
Chlamydomonas sp.	Green	Piggery wastewater	-	-	15	35
Scenedesmus sp.	Green	Centrate municipal wastewater	247.5	35.5-40.5	80	30
Chlorococcum littorale sp.	Green	Municipal wastewater	1000	50-55.7	70	42
Synechococcus elongates sp.	Cyanobacteria	Municipal wastewater and activated sewage	-	-	60	60

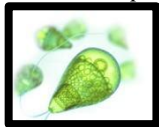
Table 2: Different species showing productivity rate at varying parameters of aeration and types of system cultivated on (F Javed, et al., 2019) (HX Chang, et al., 2016) (SP Guillaume and P C Hallenbeck, 2009).


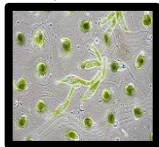



Photoautotrophic cultivation (Photobioreactors)			
Microalgae species	System/Culture type	Aeration and Flux	Productivity (g/L/day)
Chlorellavulgaris	Membrane system- Hydrophilic polyvinyl chloride (PVC) / silica	Aeration rate: 2 L/min Flux: 1.5-8.63 L/m ² /h	0.08
Scenedesmus	-	Aeration rate: 4 L/min Flux: 12 L/m ² /h	-
Hasleaostrearia	-	Flux: 3-10 L/m ² /h	-
Chlorellasorokiniana	Inclined tubular system	-	1.47
Chlorella	Flat plate system	-	3.8

Table 3: Species showing total lipid content and products cultivated with batch culture type (F Javed, et al., 2015).

Heterotrophic cultivation			
Microalgae species	Culture type	Product	Lipid content (%)
Chlorellaprotothecoides sp.	Batch	Biodiesel	57.8
Cryptocodininmcohnii sp.	Batch	Docosahexaenoic acid	56
Chlorellaprotothecoides sp.	Batch	Biodiesel	46.1

Table 4: Effects of increasing CO₂ capture on the Biomass production and total lipid content (R Kothari, et al., 2021).

Microalgae species	CO ₂ sequestration	pH	Temp.(°C)	Lipid content	Biomass (g/L/day)
Dunaliella sp. 	Increases ↑	8.55	26.5 ± 1.5	-	Increases ↑

Chlorella sp.*(10-15 %) 	Increases ↑	7.5-9.0	28± 1.5	-	Increases ↑
Chlorella vulgaris sp. *(13-15%) 	Increases ↑	-	28± 1.5	-	Increases ↑
Scenedesmus sp. 	Increases ↑	7.5	28± 1.5	-	Increases ↑
Scenedesmus dimorphus sp. *(15%) 	Increases ↑	7.0-8.0	-	-	Increases ↑
Synechococcus sp. 	Increases ↑	8.55	26.5 ± 1.5	Average	Decreases ↓

Shagun Sharma, et. al. "Enhancement of the productivity of microalgae biomass combined with waste water treatment to produce sustainable biofuel and bioproducts." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 17(1), (2023): pp 39-47.