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# Microalgae Growth under Mixotrophic Condition Using Agro-Industrial Waste: A Review

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## Abstract

Microalgae has a great potential to produce biofuels and bioproduct but the cost is still too high mainly due to the biomass production. Mixotrophic cultivation has been pointed as microalgae cultivation mode for biomass/bioenergy production with lower cost and able to make remediation of organic waste. The proposals of this work was to make a review of microalgae growth under mixotrophic condition using agro-industrial waste. Agro-industrial by-products and wastes are of great interest as cultivation medium for microorganisms because of their low cost, renewable nature, and abundance. However biotechnological technologies are necessary to develop the production of microalgae on a large scale.

**Keywords:** microalgae, mixotrophic growth, biomass, agro-industrial waste, biorefinary

## 1. Introduction

Microalgae are a variety of autotrophic, prokaryotic or eukaryotic organisms, where their single-cell structure allows solar energy to be easily converted into chemical energy. This biochemical conversion is being used commercially to obtain the biomass, consequently, in the insertion in products with commercial application. The most used microalgae cultivation techniques are opens aerated lagoons and closed photobioreactors [1–4].

Due to the advantages that microalgae offer over many other species, researchers and entrepreneurs have shown great interest in the development of production processes for biofuels, functional foods and bio-products from different species. Compared to terrestrial crops, these microorganisms have photosynthetic efficiency, growth rate and higher biomass production, consequently mass cultivation for commercial microalgae production can be carried out efficiently [5]. In addition, the cultivation of microalgae does not require arable soil, and can be grown in saline, brackish and wastewater and in harsh conditions, not competing with the production of food that is currently a major challenge for the production of first and second biofuels generation [6]. Therefore, competition for arable land with other crops, especially for human consumption, is greatly reduced.

Although most microalgae grow exclusively through photosynthesis, some species are mixotrophic and use extracellular organic carbon when a light source is not available [7]. Microalgae can be a source of several important compounds, including hydrogen and hydrocarbons, pigments and dyes, food and feed, biopolymers, biofertilizers, insecticides, nutraceuticals (foods capable of providing health benefits) and pharmacological compounds, in addition to being a potential biomass for production of biofuels [8].

Although the production of microalgae does not directly compete with food production and can be grown in harsh conditions, economic viability does not yet exist in many of the processes of industrial interest. However, the improvement and mastery of technologies capable of making inserted industrial processes viable become essential. Despite of the microalgae have a wide potential for production and applications, there are many obstacles to the biodiversity of these algae, such as mastery of technologies for production, genetic improvement research of strains more resistant to pathogens and economic viability in large-scale production [9–10]. According to Georgianna and Mayfield [11], although promising, the success of inserting microalgae in the production of various products depends mainly on two important factors: high productivity and quality of biomass, as well as cost-effective production.

One of the viable solutions to reduce the costs of microalgae biomass production is to explore different forms of energy metabolism, highlighting the photoautotrophic, heterotrophic and mixotrophic for commercial production. Understanding these forms of metabolism allows the application of efficient crop strategies aimed at increasing the production of biomass and bioproducts on a large scale with cost optimization to couple the agroindustry waste treatment [7]. Microalgae are able to eliminate a variety of pollutants in wastewater mainly nitrogenated, phosphates and organic carbons [12].

Mixotrophic cultivation is a preferable microalgae growth mode for biomass production [13]. Compared to photoautotrophic and heterotrophic metabolism, mixotrophic cultures have been demonstrated many advantages, such as less risk of contamination, reduced cost and high biomass productivity. Even susceptible to contaminations, the use of photobioreactors minimizes this risk, but increases the cost of the process, which can be offset by the high biomass yield that can reach 5–15 g/L, being 3–30 times higher than those produced under autotrophic growth conditions [14, 15].

The use of waste for microalgae mixotrophic growth has been researched, mainly with the objective of expanding and diversifying in an alternative way the control and combating the inappropriate disposal of these in the respective industries, combined with the perspective of minimizing the operational costs of producing microalgae in large scale that are still considered high. The waste generated by the agribusiness has a high load of organic matter with high concentrations of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), ammonia, phosphates, suspended solids harmful to the environment, in addition to dissolved components such as sugars, fat and proteins originating from food, contributing to environmental pollution [16].

According to Patel et al. [7], research involving the mixotrophic cultivation of microalgae using organic matter as a source of carbon points to the production of high yields of biomass and biocomposites of industrial interest when compared to systems involving photoautotrophic and heterotrophic metabolisms. In this sense, recent studies have been carried out using agroindustrial waste to grow microalgae in a mixotrophic regime in order to minimize the cost of the biomass production process and treat the effluent adding value to the process, suggesting a microalgae biorefinery system [17].

Patel et al. [18] cultivated *C. protothecoides* UTEX-256 under mixotrophic conditions using dairy waste as source of carbon. The high CO<sub>2</sub>-emitting dairy industry obligated to treat waste and improve its carbon-footprints. In general, biochemical treatment was effective to remove respectively 99.7 and 91–100% of organic and inorganic pollutants and produce biomass and lipids fractions.

Xio-Bo Tan et al. [19] demonstrated that *Chlorella pyrenoidosa* (FACHB-9) cultivated under mixotrophic conditions using anaerobic digestate of sludge with an optimal addition of acidified starch wastewater improved biomass and lipids production by 0.5-fold (to 2.59 g·L<sup>-1</sup>) and 3.2-fold (87.3 mg·L<sup>-1</sup>·d<sup>-1</sup>), respectively. In addition, 62% of total organic carbon, 99% of ammonium and 95% of orthophosphate in mixed wastewater were effectively removed by microalgae.

Wang et al. [20] utilized glucose recovered from enzymatic hydrolysis of food waste as culture medium in mixotrophic cultivation of *Chlorella sp.* to obtain high levels of lipid and lutein. The algal biomass was 6.9 g L<sup>-1</sup> with 1.8 g L<sup>-1</sup> lipid and 63.0 mg L<sup>-1</sup> lutein using hydrolysate with an initial glucose concentration of 20 g L<sup>-1</sup>. Furthermore, lipid derived from microalgae biomass using food hydrolysate was at high quality in terms of biodiesel properties.

Due to the success of mixotrophic microalgae growth, the use of agro-industrial by-products stands out, adding value to production processes and reducing costs. The nutritional characteristics, availability and low cost of obtaining evidence the possibility of using the by-products in the cultivation of microalgae. This work reviews the mixotrophic cultivation system of microalgae using waste from agribusiness as a source of organic carbon, pointing out the benefits of this strategy as a solution to the environmental problems caused by these effluents, adding value to an industrial process for the production of biomass and biocompounds as biorefinery.

## 2. Microalgae

Microalgae is a generic term used to refer a widely diverse group of photosynthetic microorganisms [21]. There are several species of microalgae, which are found in aquatic environments of fresh water, brackish and saline [22]. Microalgae in general, have varying microscopic sizes, perform photosynthesis, use carbon dioxide as a nutrient source for growth, in addition to playing a fundamental role in ecosystems [23–25]. It is estimated that there are about 800 thousand species of microalgae, of which about 40 to 50 thousand are of scientific knowledge, which makes it an almost unexplored resource, demonstrating the great biodiversity of these algae [26–27]. In addition, most species are not yet known and very few are used for any purpose.

The basic composition of microalgae is based on carbohydrates, lipids, proteins, ash and nucleic acids, in addition to chlorophyll and other protective pigments and light capture that provide high photosynthetic capacity, allowing conversion of up to 10% of energy in biomass [28]. In conventional plants, this percentage is higher when compared to other conventional plants, whose conversion is limited to a maximum of 5% [29]. The predominant elements in the biomass of microalgae are carbon, nitrogen and phosphorus and some metals such as iron, cobalt, zinc is also found [28].

## 3. Application of microalgae

In recent years, several researches have been carried out seeking to develop technologies for the elaboration and diversification of products based on microalgae.

The growing expansion of these products is part of a wide range of utilities inserted in the most different commercial niches, expanding the possibilities of use and adding value to the market. According to Hu et al. [30], the global algae market is expected to be worth of about \$ 1.1 billion by 2024.

Microalgae are inserted in a wide variety of species, distinguishing one from the other due to their biological structure. In this sense, these microorganisms offer potential possibilities for CO<sub>2</sub> biofixation, remediation, effluent treatment, production of biofuels, high-value products including pharmaceuticals, food and nutraceuticals [31].

According to Rizwan et al. [32], microalgae can be a source of antioxidant compounds, carotenoids, enzyme polymer, lipid, natural dye, polyunsaturated fatty acid, peptide, toxin and sterols, which are widely used in industry. In addition, they are used for the synthesis of antiviral, antimicrobial, antiviral, antibacterial and anticancer drugs [33].

Commercial microalgae cultivation systems are operated to produce mainly pigments and metabolites for nutritional supplements [34]. The algae that have technical and economic viability of production are *Spirulina* (*Arthrospira*) for supplements with a high protein content, *Haematococcus* as a source of astaxanthin and *Dunaliella salina* for the production of pro-vitamin A [31]. *Spirulina* represents 60% of all biomass produced on a large scale [35]. This species can be easily grown in tropical regions and is well adapted to extreme environments, being relatively less susceptible to contamination than other microalgae, making it the most favorable choice for large-scale production [36].

*Spirulina* consists mainly of proteins (50–70%), being widely used in human nutrition to combat malnutrition [37]. This species is rich in essential amino acids, beta-carotene, minerals, essential fatty acids, vitamins, polysaccharides, among others. *Chlorella* accumulate high concentrations of carotenoids (astaxanthin, lutein,  $\beta$ -carotene, violaxanthin and zeaxanthin), antioxidants, vitamins, polysaccharides, proteins, peptides and fatty acids [5, 38]. In addition to all the benefits mentioned, the bioactive compounds of microalgae can have a biological, immune, antiviral and anti-cancer properties, being highly active [39].

Global warming has been worrying environmentalists across the planet. Although there are different ways of capturing CO<sub>2</sub>, the biological method stands out as a potentially attractive alternative. The requirements for producing and obtaining biomass from microalgae are basically CO<sub>2</sub> and a source of light, be it natural or artificial [2]. Carbon dioxide can be converted into organic matter by performing photosynthesis using sunlight as an energy source [40–42]. Microalgae are more efficient for fixing CO<sub>2</sub> and have a higher productivity rate (ton/ha/year) when compared to terrestrial plants. In addition, CO<sub>2</sub> biofixation can be combined with other processes, such as the treatment of organic waste, being advantageous in terms of economic viability and environmental sustainability. Microalgae can also be grown in nutrient-rich organic effluents, salt and brackish water, reducing the use of fertile land and fresh drinking water [43].

Studies involving the mixotrophic cultivation of microalgae using industrial residues from agro-industry as a source of organic carbon have been carried out to minimize the cost of biomass production, treat the effluent and promote CO<sub>2</sub> biofixation [7]. In this sense, expanding the ways in which these residues are used, avoiding their incorrect disposal, minimizes the effects of environmental pollution and adds value to industrial processes, encouraging a cleaner and more sustainable bioeconomy.

Currently, fossil fuels represent the main source of energy in the world, but unsustainable and directly related to the pollution of air, land, water and climate change. The burning of fossil fuels consolidated to increase the atmospheric

concentration of CO<sub>2</sub> being directly associated with global warming. Allied to this, the future oil scarcity is a major challenge for scientists, motivating a constant search for technologies capable of producing clean and sustainable fuels [44]. Among many biomasses, microalgae represent a promising source for the production of clean renewable energy, as they are capable of fixing CO<sub>2</sub> by performing photosynthesis with efficiency and productivity superior to that of conventional oilseeds and terrestrial plants used in the production of biodiesel and bioethanol. Among the available biomass sources, microalgae have been evaluated and investigated as generation third biomass, being researched to produce different types of biofuels, among which are biodiesel, bioethanol, bio-oil, char, hydrogen and synthesis gas [45]. Recent research involving the production of biofuels has been focused on third generation biomass, since the first and second raw materials are based on terrestrial cultures that compete with food production and can lead to food crises [46]. Algae biofuels are not yet obtained on a large scale due to the high cost of the process justifying the development of new technologies that can bring economic viability [47].

Bioremediation and biofuel production from waste resources by microalgae platform is mainly important to utilize abundantly available solar energy biofixing CO<sub>2</sub> and treat effluents through the mixotrophic growth of microalgae [7]. Algal bioremediation is a good strategy to produce biomass for biofuels production while remediating wastes, also improving carbon-footprint through carbon capturing and utilization technology.

A microalgae biorefinery enables to integrate fractionation and conversion processes to transform biomass into bioproducts such as food, feed, chemicals, and bio-energy as optimization of the use of the microalgae for reducing waste production, and maximizes process profit. After lipid transesterification for biodiesel, the residual biomass can be used to produce other biofuels such as methane, bio-oil and ethanol or biocompounds for food and pharmaceutical industry [48].

## 4. Cultivation systems of microalgae

There are currently four cultivation technologies in use for the production of commercial microalgae including open ponds and raceway ponds (open systems), photobioreactors and fermenters [49]. In open systems, microalgae are grown in open areas, including tanks, lakes, and ponds, deep channels, among others. In closed systems, crops are grown in transparent bioreactors, exposed to sunlight or artificial radiation for photosynthesis and fermenters.

### 4.1 Opens systems

Natural and artificial lakes and ponds, where most of the systems commonly used are large, shallow ponds and tanks, represent open pond systems. The main advantages of these systems are the ease of construction and operation when compared to photobioreactors and the possibility of operating hybrid processes involving the cultivation of algae associated with the treatment of wastewater. However, the disadvantages are inefficient light distribution, losses through evaporation, diffusion of CO<sub>2</sub> into the atmosphere, contamination and the requirement for large areas of land [50]. Open ponds are currently in use for wastewater treatment and production of *Dunaliella salina*, characterized as a hybrid process. These systems are used by Ognis Australia Pty Ltd. to produce  $\beta$ -carotene from *Dunaliella salina* in Hutt Lagoon and Whyalla. In terms of surface area used, these are among the largest algae production systems in the world.

Closed or artificial ponds (circular ponds and raceway ponds) are more efficient than open systems for producing microalgae, since control over the production environment is much better than open ponds or extensive ponds. The cost of raceway ponds is higher than that of open lagoon systems, but lower than that of photobioreactors. These systems are the most used due to their potential to produce large quantities of biomass for commercial application. Raceway ponds are commonly used to grow *Chlorella sp.*, *Spirulina platensis*, *Haematococcus sp.* and *Dunaliella salina*, with a biomass production rate of 60–100 mg of dry biomass/L/day [50]. Raceway ponds are used to produce *Spirulina* at Earthrise Nutraceuticals in the USA and Cyanotech Corp. in Hawaii [49].

## 4.2 Closed systems

Photobioreactors were designed to overcome the problems associated with open growth systems. It has been shown that cultivation of microalgae in these systems are capable of producing large amounts of biomass as they allow an effective control of process parameters, such as pH, temperature, CO<sub>2</sub> concentration, level of contamination, among others. However, photobioreactors are much more expensive than open ponds and raceway ponds. Commercial photobioreactor productions include the production of *H. pluvialis* in Israel and Hawaii and *C. vulgaris* in Germany. Production costs are very high, reaching \$ 100/kg [49]. As a result, biofuel production based entirely on photobioreactors is generally considered unlikely to be commercially viable [6, 49, 50].

Closed fermenters are used for the production of heterotrophic algae, where sugars or other simple carbon sources are used for growth instead of CO<sub>2</sub> and light. Open fermenters similar to the fermenters used in the production of ethanol in industries are not suitable for the growth of algae, since these microorganisms have slow growth when compared to yeasts and bacteria. In the USA, India and China  $\omega$ -3 fatty acids are produced from *Thraustochytrids* by heterotrophic fermentation through sugars and O<sub>2</sub>. As it is a high value-added product, it is sold for 100 \$ /kg, which justifies the high cost of the process [49].

## 5. Growth metabolism

Microalgae have different growth metabolisms, which characterizes their versatility. Cultivation conditions define the metabolic route for the production of biocompounds, including proteins, carbohydrates, pigments and fatty acids. Although the production of microalgae has traditionally been photoautotrophic, these microorganisms have different forms of energy metabolism including heterotrophic and mixotrophic, which use an organic carbon source for the growth and production of biomass. The understanding of these metabolisms allows the diversification of current growth systems aiming at increasing the production of biomass and certain specific metabolites.

### 5.1 Photoautotrophic

Photoautotrophic growth is the most common way to cultivate microalgae through photosynthesis. In these systems, high concentrations of CO<sub>2</sub> are sequestered, but productivity is low when compared to heterotrophic and mixotrophic systems that provide high yields of biomass and secondary metabolites [51, 52]. Through photobioreactors is possible to obtain the maximum cell density of 40 g/L, while in outdoor open-pond or raceway-pond cultures, the cell concentration is

usually lower than 10 g/L. This significantly increases the energy consumption of cell harvesting and the cost of biomass production [53]. Usually scale-up of microalgae cultivation in wastewater is fulfilled phototrophically, which may be hindered by inefficient illumination and the low biomass density, leading to poor removal of nutrients [54].

For photosynthesis, light is used as an energy source and CO<sub>2</sub> is used as a carbon source [55]. The carbon source is essential for growth and the higher its concentration, the higher the productivity. Nitrogen sources in the culture medium are also essential for the synthesis of proteins, nucleic acids and other biocomposites necessary for cell growth and survival [56] and the concentration must be compatible with the amount of carbon in the medium [6]. The type of cultivation, the nutrients, the carbon source, the salinity of the medium, the irradiance and the temperature vary according to the chosen species and also considerably influence the success of the microalgae production.

## 5.2 Heterotrophic

Microalgae can grow in the absence of light in culture media assimilating organic carbon. Studies show that some species, including *Chlorella*, *C. protothecoides*, *C. vulgaris*, *C. zofingensis*, *C. minutissima*, *Tetraselmis* and *Neochloris* [57] are able to grow in both autotrophic and heterotrophic conditions [56]. According to Hosoglu et al. [58], microalgae of the *Chlorella Beyerinck* genus are those that have the greatest potential for large-scale heterotrophic production, with emphasis on the species *C. protothecoides* that has been widely studied.

In heterotrophic crops, microalgae acquire carbon and energy from organic sources via oxidative phosphorylation, consuming O<sub>2</sub> and releasing CO<sub>2</sub>. According to Behrens [59], the cost of producing the kg of dry biomass produced in photoautotrophic conditions can be 5.5 times higher than in heterotrophic conditions. In heterotrophic systems there are no light limitation problems, since microalgae grow in the absence of light using organic carbon as a carbon and energy source reaching high concentrations of biomass reaching up to 100 g/L, which considerably facilitates the harvesting process [60]. In heterotrophic processes, 18% of the energy obtained can be converted into adenosine triphosphate (ATP) whereas in photoautotrophic cultures this percentage is only 10% [61]. However, despite the advantages, the cultivation of microalgae under heterotrophic conditions, due to the use of organic carbon, requires reactors and techniques of greater complexity and high cost to avoid contamination caused by other microorganisms.

## 5.3 Mixotrophic

Under mixotrophic conditions, microalgae grow both photoautotrophically and heterotrophically, being able to assimilate organic compounds as a carbon source and use inorganic carbon as an electron donor [62]. In this metabolism, the energy is captured through the catalysis of external organic compounds through respiration and the light energy is converted into chemical compounds via photosynthesis, being a promising solution in the processes of environmental remediation, being able to treat the effluents of the agribusiness and produce biomass rich in metabolites of industrial interest.

In photoautotrophic crops, self-shade caused by the high density of cells makes light penetration difficult, causing photoinhibition and may be the limiting factor to its propagation leading to low biomass yields. Under heterotrophic conditions, not all microalgae species are able to grow and the strict use of only organic substrates as a source of carbon and energy makes the process more prone to contamination.



Thus, an alternative to maximize production can be through mixotrophic cultivation. Therefore, the cells would multiply with autotrophic metabolism until reaching the maximum cell density, at which point a source of organic carbon is added to stimulate, also, heterotrophic growth. Thus, due to the high cell density in the medium, problems with contamination of microorganisms would be less likely to happen. For the success of this technique, the cultivated species must be able to grow in heterotrophic conditions without microbial contamination and the organic compound to be added, as well as its ideal concentration must be known.

Reducing use of light in mixotrophic processes decreases the demand for energy, which minimizes the operational cost in processes with artificial light. According to Perez-Garcia and Bashan [63] in these systems there is a better control capable of regulating the growth rate of the species, which minimizes the risk of contamination by photosynthetic microorganisms. Due to the significant heterotrophic contribution of the organic fraction, the reactor design does not require a maximized area to expose the microalgae to light to perform photosynthesis, decreasing the process cost.

According to Patel et al. [7], mixotrophic cultivation has advantages over photoautotrophic and heterotrophic metabolism. In this process, in addition to the higher growth rates reducing the microalgae growth cycle, there is also growth in the dark phase mediated by respiration, potentiating the production of biomass. There is also a comparatively prolonged exponential growth phase, great flexibility to change the metabolism from heterotrophic to photo-autotrophic and vice versa, prevention of photo-oxidative damage caused by O<sub>2</sub> accumulated in closed photobioreactors and reduction in substrate uptake photoinhibition. It has been shown that microalgae cultivated under mixotrophic conditions can present, under controlled conditions, higher biomass productivity when compared to photoautotrophic and heterotrophic cultures [64].

## 6. Growth microalgae using waste from agro-industry

The advancement of agro-industry has generated a large amount and variety of waste causing serious environmental problems and is one of the sectors that generate more waste rich in organic matter. According to Dahiya et al. [65], approximately 1.3 billion tons of foods are wasted each year during its production, handling, storage, processing, distribution or consumption. The composition of food processing residues is extremely varied and depends on both the nature of the raw material and the production technique employed.

Due to the scarcity of available areas close to large urban centers for the disposal of industrial and urban waste, the vast majority of companies do not carry out treatment and/or correct disposal of this material, which contributes to the increase in environmental pollution. Pollution is due to high concentrations of organic matter and heavy metals causing contamination, eutrophication of water bodies, death of aquatic organisms and local vegetation, ecological imbalance and health problems for the population. In this sense, the use of agro-industrial waste as a source of organic carbon for the cultivation of microalgae is presented as an option for the bioremediation of these effluents added to the production of biomass rich in biocompounds with different applications.

A lot of studies have evaluated the mixotrophic growth of microalgae using glucose, glycerol and acetate as a source of organic carbon and observed that the biomass yields were higher, which could decrease the cost of the process. Liang et al. [66] investigated *C. vulgaris* strain under autotrophic, mixotrophic and heterotrophic growth conditions. Mixotrophic growing on glucose with light produced the highest

lipid productivity compared with other growth modes. Garcia et al. [67] studied the mixotrophic growth of tricornutum UTEX-640 using acetate, lactic acid, glycine, glucose and glycerol. The best results were obtained using with urea, which resulted in maximum biomass and eicosapentaenoic acid productivities significantly higher than those obtained for the photoautotrophic control, which suggest the possibility of using mixotrophy for the mass production of microalgae. Cheirsilp & Torpee [68] cultivated *Chlorella sp.*, *Chlorella sp.*, *Nannochloropsis sp.* and *Cheatocecos sp.* under mixotrophic condition using glucose as source of organic carbon. They observed that the biomass and lipid production of all tested strains in mixotrophic culture were notably enhanced in comparison with photoautotrophic and heterotrophic cultures.

Due to the success of the microalgae mixotrophic cultivation method, recent studies have been evaluating the possibility of using residues from the agro-industry as source of organic matter as a strategy to treat the effluent, produce biomass and reduce the cost of the process.

Hu et al. [69] evaluated the mixotrophic cultivation of *Chlorella sp.* UMN271 utilizing swine manure as nutrient supplement for evaluate the nutrient removal efficiencies by alga. The results showed that addition of 0.1% (v/v) acetic, propionic and butyric acids, respectively, could promote algal growth, enhance nutrient removal efficiencies and improve total lipids productivities. They concluded that *Chlorella sp.* grown on acidogenically-digested manure could be used as a feedstock for high-quality biodiesel production.

Li et al. [70] investigated the effects of autotrophic and mixotrophic growth on cell growth and lipid productivity of green microalgae *Chodatella sp* and Piggery wastewater served as nutrient sources for mixotrophic growth. The specific growth rate, biomass production, and lipid productivity obtained with mixotrophic growth were until 5.6 times higher than those obtained with autotrophic growth. The mixotrophic cultivation simultaneously assimilated 99.7% ammonia nitrogen and 75.9% total phosphorus from piggery wastewater, which reduced the required nutrient for the culture of microalgae, thereby reducing the cost of biomass for diverse application.

León-Vaz et al. [71] cultivated *C. sorokiniana* microalgae under mixotrophic conditions utilized Oxidized wine waste lees among other agro-industrial wastes as carbon source. The fed-batch strategy and the medium optimization, with nutrient supplementation, have been found to be very effective in enhancing biomass and neutral lipid productivity, suggesting that this is a promising strategy for production of microalgal biomass. The algal biomass concentration was 11 g L<sup>-1</sup> with a lipid content of 38% (w/w).

Bhatnagar et al. [72] evaluated mixotrophic growth of *Chlamydomonas globosa*, *Chlorella minutissima* and *Scenedesmus bijuga* under medium supplemented with different organic carbon substrates and wastewaters. The mixotrophic growth of these microalgae resulted in 3–10 times more biomass production relative to phototrophy. Poultry litter extract as growth medium recorded up to 180% more biomass growth compared to standard growth medium, while treated and untreated carpet industry wastewaters also supported higher biomass, with no significant effect of additional nitrogen supplementation.

Andrade & Coosta [73] determined the effects of molasses concentration and light levels on mixotrophic biomass production by *Spirulina platensis*. Molasses concentration was the main factor influencing maximum biomass concentration (X<sub>max</sub>) reached 2.94 g L<sup>-1</sup> and  $\mu_{max}$  0.147 d<sup>-1</sup>. Molasses, suggesting that this industrial by-product could be used as a low-cost supplement for the growth of *Spirulina platensis*, stimulated the production of biomass.

Melo et al. [74] evaluated the growth, nutrients and toxicity removal of *Chlorella vulgaris* cultivated under autotrophic and mixotrophic conditions using corn steep

liquor, cheese whey and vinasse as source of organic matter. The results demonstrated that corn steep liquor toxicity was totally eliminated and cheese whey and vinasse toxicity were minimized by *C. vulgaris*. They demonstrated that the mixotrophic cultivation of *C. vulgaris* is able to increase cellular productivity and could be an alternative to remove the toxicity from agroindustrial by-products.

Hugo et al. [75] studied the growth of forty microalgae strains under mixotrophic conditions using sugarcane vinasse as source of organic matter. *Micractinium sp.* Embrapa|LBA32 and *C. biconvexa* Embrapa|LBA40 presented expressive growth in a light-dependent manner even in undiluted vinasse under non-axenic conditions. Microalgae strains presented higher biomass productivity in vinasse-based medium compared to autotrophic medium. This research showed the potential of using residues derived from ethanol plants to cultivate microalgae for the production of energy and bioproducts.

Mitra et al. [76] cultivated *Chlorella vulgaris* under mixotrophic/heterotrophic conditions using dry-grind ethanol thin stillage and soy whey as nutrient feedstock. The results showed the biomass yields from thin stillage, soy whey and modified basal medium after 4 days of incubation at mixotrophic conditions in the bioreactor were 9.8, 6.3 and 8.0 g.L<sup>-1</sup> with oil content at 43, 11, and 27% (w/w) respectively. This research highlights the potential of these agro-industrial co-products as microalgal growth media with consequent production of high-value microalgal oil and biomass.

Salati et al. [77] cultivated *Chlorella vulgaris* using cheese whey, white wine lees and glycerol as carbon sources under mixotrophic conditions. The mixotrophic biomass production was 1.5–2 times higher than autotrophic growth. Furthermore, it gave much higher energy recovery efficiency, i.e. organic carbon energy efficiency of 32% and total energy efficiency of 8%, suggesting the potential for the culture of algae as a sustainable practice to recover efficiently waste-C and produce biomass.

Piasecka et al. [78] studied the growth of *Tetradismus obliquus* by supplementation with beet molasses in photoheterotrophic and mixotrophic culture conditions. The highest protein content was obtained in the mixotrophic growth suggesting this metabolism promising for protein production.

Tsolcha et al. [79] evaluated a mixed cyanobacterial-mixotrophic algal population, dominated by the filamentous cyanobacterium *Leptolyngbya sp.* and the microalga *Ochromonas* under non-aseptic conditions for its efficiency to remove organic and inorganic compounds from second cheese whey, poplar sawdust, and grass hydrolysates. Nutrient removal rates, biomass productivity, and the maximum oil production rates were determined. The highest lipid production was achieved using the biologically treated dairy effluent (up to 14.8% oil in dry biomass corresponding to 124 mg L<sup>-1</sup>), which also led to high nutrient removal rates (up to 94%). Lipids synthesized by the microbial consortium contained high percentages of saturated and mono-unsaturated fatty acids (up to 75% in total lipids) for all the substrates tested, which implies that the produced biomass may be harnessed as a source of biodiesel.

Grupta et al. [80] cultivated the *Chlorella* microalgae under mixotrophic conditions using a raw food-processing industrial wastewater. About 90% reduction in TOC and COD were obtained for all dilutions of wastewater. Over 60% of nitrate and 40% of phosphate were consumed by microalgae from concentrated raw wastewater. The degradation kinetics also suggested that the microalgae cultivation on a high COD wastewater is feasible and scalable.

Yeesang et al. [81] evaluated *B. braunii*, a microalgae rich in oil under mixotrophic cultivation using molasses, a cheap by-product from the sugar cane plant as a carbon source and under photoautotrophic cultivation using nitrate-rich wastewater supplemented with CO<sub>2</sub>. The mixotrophic cultivation produced a high amount of

biomass of 3.05 g L<sup>-1</sup> with a high lipid content of 36.9%. The photoautotrophic cultivation in nitrate-rich wastewater supplemented with 2.0% CO<sub>2</sub> produced a biomass of 2.26 g L<sup>-1</sup> and a lipid content of 30.3%. They showed that these strategies could be promising ways for producing cheap lipid-rich microalgal biomass as biofuel feedstock and animal feeds.

Gélinas et al. [82] studied the mixotrophic growth and lipid production of *Chlorella consortium* using residual corn hydrolysate and corn silage juice as source of organic and compared to heterotrophic conditions. Maximum microalgal biomass of 0.8 g/L was obtained with 1 g/L of residual corn hydrolysate whatever the trophic strategy. Under mixotrophic conditions, the use of residual corn hydrolysate led to an increase of 21% and 22% in comparison with the biomass produced with glucose or silage juice, respectively. This increase varied between 11% and 28% under heterotrophic condition. They observed that at the end of the experiment, algae exposed to silage juice decreased significantly. Residual corn hydrolysate represented an interesting and efficient alternative as an organic carbon source. However, silage juice needs additional treatments to be implemented as a culture medium.

Nur et al. [83] studied palm oil mill effluent (POME), one of the wastewaters generated from palm oil mills, as source of organic carbon for mixotrophic microalgae growth. The aim of this research was to identify the growth of *Chlorella vulgaris* cultured in POME medium under mixotrophic conditions in relation to a variety of organic carbon sources added to the POME mixture. The research was conducted with 3 different carbon sources (D-glucose, crude glycerol and NaHCO<sub>3</sub>) in 40% POME. They showed that *C. vulgaris* using D-glucose as carbon source gained a lipid productivity of 195 mg/l/d.

Manzoor et al. 2020 presented the growth of *Scenedesmus dimorphus* NT8c cultivated mixotrophically on sugarcane bagasse hydrolysate, a low-value agricultural by-product. Under mixotrophic conditions the *S. dimorphus* NT8c showed higher growth rates compared to photoautotrophic cultivation and the biomass productivity was 119.5 mg L<sup>-1</sup> d<sup>-1</sup>, protein contents was 34.82% and fatty acid contents was 15.41%. They concluded that mixotrophically-cultivated microalgae are able to increase the biomass and lipid productivity. However, the concentrations of supplementation need to be studied because higher level of organic carbon can result in unfavorable levels of turbidity and bacterial growth, reducing microalgal biomass productivity.

## 7. Conclusions

Microalgal biomass represents a sustainable alternative to fossil consumption and bioproducts for food and pharmaceutical industry. Microalgae can grow under photoautotrophic, heterotrophic or mixotrophic modes where the latter two trophic modes require organic carbon to grow efficiently. Actually, researchers have highlighted the role of low cost-efficient agro-industrial by-products used as supplements in algal culture media. However, supplementation of organic carbon contributes significantly to a higher cost of microalgae production and this can compete with human and animal alimentation. Agro-industrial by-products and wastes are of great interest as cultivation medium for microorganisms because of their low cost, renewable nature, and abundance.

Faced with this scenario, biotechnological technologies are necessary to develop the production of microalgae on a large scale and expand the range of utilities that, in the short or long term, contribute to the improvement of industrial processes. In addition, mixotrophic microalgae growth is a great strategy to reduce environmental

pollution generated by residues rich in organic matter and can reduce the cost of the industrial process. However, more investments, development and greater knowledge of the metabolism of these microalgae and their effectiveness in the generation of new bioproducts are increasingly necessary.

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