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Review

Mixotrophic culture of microalgae: A review

[Cultivo mixotrófico de microalgas: una revisión]

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Abstract

Despite the increasing relevance of microalgae in science and industry in the last few decades, a great part of their application still faces various bottlenecks that prevent their derivatives from making a greater contribution to the economy and society. Among cultivation strategies, mixotrophic culture has emerged as a promising approach to enhance biomass productivity and metabolite synthesis. This review outlines the fundamentals of metabolic mixotrophy in microalgae, focusing on key factors influencing growth and productivity, as well as the coupling of mixotrophic cultivation and phycoremediation applications, such as wastewater treatment, solid waste recycling and flue gas. Additionally, it briefly discusses the sustainable production of value-added biomass for use as biofertilizers, biofuels, in aquaculture, and pigment production within the context of circular economy.

Keywords: *wastewaters, solid waste recycling, bioremediation, phycoremediation, circular economy.*

Resumen

A pesar de la creciente relevancia de las microalgas en la ciencia y la industria en las últimas décadas, gran parte de su aplicación aún se enfrenta a diversos cuellos de botella que impiden que sus derivados contribuyan en mayor medida a la economía y a la sociedad. Entre las estrategias de cultivo, el cultivo mixotrófico ha surgido como un enfoque prometedor para mejorar la productividad de la biomasa y la síntesis de metabolitos a menor costo. Esta revisión aborda los fundamentos de la mixotrofía metabólica en microalgas, centrándose en los factores clave que influyen en el crecimiento y la productividad, así como en el acoplamiento de los cultivos mixotrófico con la ficorremediación, como el tratamiento de aguas residuales, la reutilización de residuos sólidos y el aprovechamiento de gases flue. Además, se analiza brevemente la producción sostenible de biomasa de valor agregado para su uso como biofertilizantes, biocombustibles, en la acuicultura y la producción de pigmentos dentro de un contexto de economía circular.

Palabras clave: *aguas residuales, reutilización de residuos sólidos, biorremediación, ficorremediación, economía circular.*

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1. Introduction

Microalgae are fast-growing, photosynthetic microorganisms capable of fixing CO_2 and releasing O_2 while producing high biomass under diverse cultivation conditions. Their growth does not require arable land, making them a sustainable source of valuable compounds such as lipids, carbohydrates, proteins, pigments, and vitamins (Olguín *et al*., 2022; Acién *et al*., 2021; Chuka-Ogwude *et al*., 2020). Microalgae' biomass can later be processed into pharmaceutical and nutraceutical compounds, cosmetics, food supplements, animal feed, biofuels and biofertilizers, which maintain a potential role in sustainable bioprocesses (Rozenberg *et al*., 2024; Olguín *et al*., 2022; Acién *et al*., 2021).

Currently, large-scale microalgal cultivation is predominantly based on photoautotrophic systems, in which a single microalgal strain is grown in a nutrient medium under exposure to natural or artificial light to drive photosynthesis (Ray *et al*., 2022). This strategy is applicable to both freshwater and marine microalgal genera and is adaptable to diverse geographical regions (Razzak *et al*., 2024; Show, 2022; Acién *et al*., 2021). Despite its widespread application, photoautotrophic cultivation presents several limitations. These include the need for intensive culture management and a high risk of contamination by invasive organisms or pathogens, especially in open systems such as raceways and ponds (Ray *et al*., 2022; Acién *et al*., 2021). Moreover, photoautotrophic cultivation faces intrinsic productivity constraints due to self-shading, photoacclimation and low activity of RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), the enzyme responsible for carbon fixation during photosynthesis (Zuccaro *et al*., 2020). In addition, high water consumption, culture medium formulation, harvesting, and post-harvest treatments increase the overall cost of production (Yin *et al*., 2020; Zuccaro *et al*., 2020; Chen *et al*., 2018). All these factors limit their scalability and reduce their economic and operational viability in industrial contexts (Ray *et al*., 2022).

Mixotrophic conditions involving microalgae is a current strategy adapted to surpass these constraints. Microalgae capable of mixotrophic growth can uptake organic compounds from the culture medium as additional carbon sources, while still performing photosynthesis (EL-Sheekh *et al*., 2012). This metabolic flexibility allows them to achieve significantly higher cell densities compared to strict photoautotrophs, enhancing biomass production under suitable conditions (Chuka-Ogwude *et al*., 2020). This promotes the search and use of novel species, the discovery of new bioactive compounds and, therefore, the expanding of commercial potential of microalgae-based products. Furthermore, these systems can be coupled to the use of wastewaters as source of nutrients, solid waste media and greenhouse gases, including COx, SOx, and NO_X (Olguín *et al*., 2022; Goswami *et al*., 2022; Chuka-Ogwude *et al*., 2020), thus lowering production costs and developing more integrated systems.

This review summarizes current research on mixotrophic cultivation of microalgae for bioremediation approaches, and the potential applications of the resulting biomass as biofertilizers, biofuels, animal feed, aquaculture, and as source of valuable metabolites.

2. Fundamentals of mixotrophic culture

2.1 Mixotrophy in microalgae

Mixotrophy is defined as the regime where microalgae strains can combine autotrophic and heterotrophic metabolism, allow the uptake of organic compounds while use light as the main source of energy (Castillo *et al*., 2021). Thus, inorganic carbon in the form of CO_2 is fixed, and organic carbon dissolved in the culture medium become essential for sustaining mixotrophic growth and cellular metabolism (Patel *et al*., 2020; Zuccaro *et al.*, 2020). Reducing sugars and other organic compounds like fructose, sucrose, glucose, glycerol, acetate and ethanol have been used to induce mixotrophic cultures (Table 1).

Not all microalgae are able to grow under this culture mode or utilize the same substrates. Such is the example of *Nannochloropsis gaditana*, which could grow mixotrophically using glucose and glycerol, but not acetate (Menegol *et al*., 2019). Likewise, the addition of glycerol promoted the growth of *Phaeodactylum tricornutum*, whereas glucose was not utilized as carbon source (Huang *et al*., 2015). In contrast, mixotrophic culture of *Chlorella vulgaris* and *Leptolyngbya* sp. showed higher net biomass when supplemented with sodium acetate compared to dextrose (Silaban *et al*., 2013). This can be explained by the presence of membrane transport proteins that facilitate the assimilation of different carbohydrate molecules (Smith *et al*., 2020). In *Chlorella* sp., glucose can induce an active transport system of hexoses formed by the proteins HUP1, HUP2 and HUP3 (Lee, 2004). Meanwhile, *Galdieria* sp., a red microalga, has developed a unique uptake system of proton symporters, which allows the microalga to use disaccharides, hexoses, pentoses and organic acids (Morales-Sánchez *et al*. 2015). Acetate uptake, on the other hand, is mediated by the mono-carboxylic/proton transporter protein (Perez-Garcia *et al.* 2011). Particularly, acetate has been used to successfully grow *P. tricornutum, Cyclotella cryptica, Tetraselmis suecica* (Smith *et al*., 2020), *Chlamydomonas reinhardtii* (Lacroux *et al*., 2021) and *Chlorella sorokiniana* (Lacroux *et al*., 2021; Lacroux *et al*., 2020).

In addition, the activation of metabolic pathways that allows organic substrates to be utilized is also necessary (Smith *et al*., 2020). As a result, carbon metabolism under these conditions differs from the one observed during photoautotrophic growth, thereby altering intracellular carbon fluxes and their distribution (Smith *et al*., 2020). For instance, when glucose was used to induce mixotrophy, both *C. vulgaris* (Zúñiga *et al*., 2006) and *Asterarcys* sp. (Li *et al*., 2020) showed higher activity of the glycolytic pathway (EMP) and the pentose phosphate pathway (PPP) in contrast to phototrophic ones. In *C. vulgaris*, assimilated glucose is directed into the cytoplasm (EMP), chloroplasts (EMP and PPP) and thylakoids, where it is transformed in either lipids and pigments precursors, or pyruvate, in which case it is further internalized into the mitochondria and enters the tricarboxylic acid (TCA) cycle for respiration. Tanner (2000) reported that only about 1% remains as free glucose, and more than 85% of the glucose is assimilated and converted to oligo- and polysaccharides.

Table 1. Mixotrophic culture of microalgae and cyanobacteria using different organic substrates and concentrations

Culture type	Organic substrate	Concentration ($g\ L^{-1}$)	Biomass yield ($g\ L^{-1}$)	Reference
Arthrospira (Spirulina) platensis	Glucose	2.0	5.29	Li *et al*., (2018)
	Acetate	0.5	2.11	Li *et al*., 2023
Arthrospira (Spirulina) sp.	Glycerol	2.5	1.94	de Morais *et al*., (2020)
Asterarcys sp.	Glucose	10.0	3.71	Li *et al*., (2020)
Chlorella sorokiniana	Glucose	4.0	3.55	Li *et al*., (2014)
	Acetate	1.0	1.70	Cecchin *et al*., 2018
Chlorella sp.	Fructose	1.0	0.37	Lin and Wu (2015)
	Glucose	1.0	0.45	
	Glycerol	1.0	0.45	
	Sucrose	1.0	0.45	
Chlorella vulgaris	Acetic acid	0.05 M	2.37	Yu *et al*., (2020)
Dunaliella salina	Acetate	100 mM	-	Chavoshi and Shariati (2019)
	Glucose	60 mM	-	
Euglena gracilis	Ethanol	-	1.79	Inwongwan *et al*., (2025)
Galdieria sulphuraria	Glucose	-	8.10	Abiusi *et al*., (2022)

Table 1 (continued). Mixotrophic culture of microalgae and cyanobacteria using different organic substrates and concentrations

Culture type	Organic substrate	Concentration (g L^{-1})	Biomass yield (g L^{-1})	Reference
Haematococcus pluvialis	Ribose	1.3	1.15	Pang and Chen (2017)
Nannochloropsis gaditana	Glucose	5.0	1.08	Menegol *et al*., (2019)
	Glycerol	1.0	1.07	
Nannochloropsis oceanica	Glucose	10.0	0.90	Mitra and Misha (2018)
Scenedesmus obliquus	Ethanol	1.84	758.45	Matsudo *et al*., (2016)
Scenedesmus quadricauda	Xylose	4.0	1.03	Song and Pei (2018)
Tetraselmis sp.	Glucose	5.0	2.03	Lari *et al*., (2019)

Instead, *Phaeodactylum* sp. transforms glucose in the cytoplasm using the Entner-Doudoroff and the Phosphoketolase glycolytic pathways (Zheng *et al*., 2013). *P. tricornutum* can also metabolize glycerol through the Calvin cycle, where it is later transformed into ribulose-1,5-bisphosphate (RuBP). In both cases (glucose and glycerol), photorespiration and RuBisCO activity are used by the cell to synthetize amino acids, mainly glycine and serine, from the organic carbon substrate. For glycerol, this process has proven to be modulated by nitrogen availability (Huang *et al*., 2015).

However, the exact mechanism in which photosynthesis and respiration concur is still unclear. Some studies have proposed that both regimes (autotrophic and heterotrophic) function independently (Girard *et al*., 2014; Marquez *et al*., 1993), while others suggest the two metabolic processes interact and affect each other (Li *et al*., 2014; Acién *et al*., 2009; Yu *et al*., 2009; Vonshak *et al*., 2000). These differences are most probably driven by the underlying metabolic pathways developed in each strain, as well as by other environmental factors associated to the culture, as found in *P. tricornutum* by Huang *et al*. (2015). In hindsight, more studies are required to clarify mixotrophic mechanisms and further optimize both growth conditions and the production of high-value metabolites in each strain (Castillo *et al*., 2021).

2.2 Factors affecting biomass production

Microalgae are flexible organisms, capable to adapt to environmental changes and fluctuations in nutrient availability. Nevertheless, optimizing both light conditions and carbon supply is essential for designing efficient mixotrophic systems. By carefully adjusting photoperiods and light intensity, it is possible to alternate between light-driven and organic carbon-based metabolic pathways, thereby enhancing overall growth and metabolite accumulation (Patel *et al*., 2020). For example, different combinations of light intensity and glucose concentration were recorded for optimal biomass production of *Chlorella* sp. (100 µmol photon m^{-2} s^{-1} and 1 % glucose), *Scenedesmus obliquus* (100 µmol photon m^{-2} s^{-1} and 2 % glucose), *C. vulgaris* (80 µmol photon m^{-2} s^{-1} and 1 % glucose) and *Botryococcus braunii* (80 µmol m^{-2} s^{-1} and 1 % glucose) (Gim *et al.,* 2014). Similarly, biomass yield attained by *Chorella protothecoide* at 150 µmol photon m^{-2} s^{-1} (light-sufficient conditions) was comparable to the one obtained at 35 µmol photon m^{-2} s^{-1} (light-deficient conditions) when cultured under continuous light exposure. However, when photoperiod (16h light/8h dark) was applied at a light-deficient conditions, biomass yield decreased. Similar variations were observed while using acetic acid and glycerol as substrates (Patel *et al*., 2019).

Concentration of organic substrates can also affect greatly biomass growth. *Nannochloropsis oculata*, *Dunaliella salina* and *C. sorokiniana* were found to have different optimal glucose concentration under the same culture conditions (Wan *et al*., 2011). Pigment and lipid content in the three species were also greatly influenced by this parameter (Wan *et al*., 2011). This was also observed by Lacroux *et al*. (2021) for *C. sorokiniana* cultures using acetate and butyrate, where biomass yields decreased by 26-48% and 25%, respectively, when increasing initial concentrations from 1.0 g L^{-1} to 2.0 g L^{-1}. Moreover, some organic carbon sources can be non-favorable for certain species, despite being able to grow mixotrophically (Lacroux *et al*., 2021; Kröger and Müller-Langer, 2011; Wan *et al*., 2011). Such is the example of *Nannochloropsis oceanica*; in this case, biomass productivity decreased when glucose was added (Mitra and Mishra, 2018). This also seems true for cyanobacteria like *Arthrospira* (*Spirulina*) sp., where the addition of glycerol affected negatively on biomass production (Markou *et al*., 2019).

Other nutrient sources and concentrations play a critical role. For instance, nitrogen has been described as an important regulator in mixotrophic uptake and metabolization of organic compounds (Li *et al*., 2019). Huang *et al.* (2015) reported that sodium acetate promoted the growth of *P. tricornutum* in nitrogen-replete conditions. However, under nitrogen-limited conditions, growth was slower despite the presence of acetate, indicating a reduced mixotrophic advantage. More recently, Rosa *et al.* (2023) observed a synergistic effect between urea and acetate in *C. reinhardtii* cultures, which enhanced photosynthesis under mixotrophic conditions and subsequently improved cell growth. Interestingly, mixotrophic cultivation of *Arthrospira platensis* also demonstrated enhanced growth and greater tolerance to high ammonium concentrations (Li *et al*., 2019). These studies show that successful medium manipulation for microalgae cultivation can be achieved by screening and selecting appropriate nitrogen sources for each species or strain, which helps to optimize biomass production and improves assimilation of other nutrients.

2.3 Advantages of mixotrophic cultures

Mixotrophic cultivation of microalgae offers several advantages over strictly autotrophic systems, making it particularly attractive for industrial applications. As previously discussed, unlike autotrophic cultures that rely solely on light, mixotrophic systems utilize both light and organic carbon sources, reducing dependence on continuous light and minimizing dark-phase biomass loss. This dual-mode metabolism allows microalgae to overcome key limitations of phototrophic growth, such as photooxidative stress, photolimitation, and photoinhibition (Patel *et al*., 2020; Patel *et al*., 2019; Wang *et al*., 2014; Kröger and Müller-Langer, 2011; Vonshak *et al*., 2000). Additionally, self-shading — a common issue in high-density photoautotrophic cultures due to limited light penetration — is less problematic under mixotrophic conditions, supporting higher biomass densities (Wan *et al*., 2014). This can also translate into significantly higher growth rate and biomass productivity in a shorter time frame.

Mixotrophic cultures also overcome limitations associated with purely heterotrophic systems. While both utilize organic compounds, heterotrophic microalgae require complete darkness for optimal growth and rely exclusively on organic substrates for both carbon and energy (Morales-Sánchez *et al*., 2015). The need for large amounts of sugars can increase production costs, carry more risk of biological contamination, and compromise photosynthetic metabolites (Acién *et al*., 2021; Barros *et al*., 2019; Chen *et al*., 2018). Because mixotrophic systems also utilize light for growth, they require significantly less organic carbon, making the process more cost-effective while still achieving higher yields (Patel *et al*., 2019; Kröger and Müller-Langer, 2011). Notably, mixotrophic microalgae present a highly efficient strategy for wastewater and organic-rich solid waste treatment. These can serve as a valuable supplement to the culture medium, providing nutrients - such as nitrogen, phosphorus, sulphates and potassium - and organic carbon sources to enhance algal growth (Olguín *et al*., 2022; Blanco-Vieites *et al*., 2023; Chuka-Ogwude *et al*., 2020). Heavy metals including copper (Cu), zinc (Zn), cadmium (Cd), and chromium (Cr) present in municipal, agricultural, mining, and industrial effluents can also be removed (Goswami *et al.*, 2022). Additionally, mixotrophic cultivation also facilitates the capture of greenhouse gases such as carbon dioxide (CO_2), sulfur oxides (SO_x), and nitrogen oxides (NO_x), contributing to climate change mitigation (Olguín *et al*., 2022). These applications will be further reviewed in section 3.

Another important advantage is the enhanced synthesis and accumulation of valuable metabolites, as mixotrophy enables the combined exploitation of heterotrophic and photoautotrophic metabolic pathways, mainly photosynthetic and accessory pigments. Several studies have documented a remarkable accumulation of carotenoids in mixotrophic cultures of species like *N. gaditana* (Menegol *et al*., 2019). Accumulation of lipids and fatty acids in mixotrophic cultures of *N. oceanica* (Mitra and Misha, 2018), *Nannochloropsis* sp. (Fang *et al*., 2004), *C. sorokiniana* (Li *et* *al*., 2014) and *Isochrysis galbana* (Yang *et al*., 2024) has also been reported.

The production of biochemical compounds can also be modulated by adjusting light intensity, offering further control over metabolite profiles (Patel *et al*., 2020; Gao *et al*., 2022). In *C. vulgaris*, coupling of glucose or acetate with low light intensity (5000 to 8000 lux) was beneficial to protein accumulation, while the use of high light intensity (12000 lux) was advantageous for carbohydrate and lipid accumulation (Gao *et al*., 2022).

3. Mixotrophic cultivation of microalga for phycoremediation approaches

Since the industrial revolution, various human activities have contributed to water and landfill pollution through the discharge of agro-industrial, domestic, and urban wastewaters (Blanco-Vieites *et al*., 2023; Plöhn *et al*., 2020). These streams typically contain high levels of organic nutrients, nitrogen, and phosphates, which can lead to eutrophication in receiving water bodies (Najar-Almanzor *et al*., 2023) – meaning, the enrichment of nutrients would promote the growth of algae and phytoplankton in natural aquatic ecosystems (Bricker *et al*., 2008). This phenomenon, although problematic and undesirable, gave way to the concept of microalgal bioremediation of wastewater, back in the 1950's (Oswald and Golueke, 1960). Since then, microalgae emerged as a promising biological tool for wastewater treatment due to their ability to reduce biochemical oxygen demand (BOD) and assimilate excess nutrients; this approach has been further extended to bioremediation applications (Figure 1). Moreover, their effectiveness in removing contaminants such as heavy metals, pharmaceuticals and plastics has also been documented (Mohsenpour *et al*., 2021).

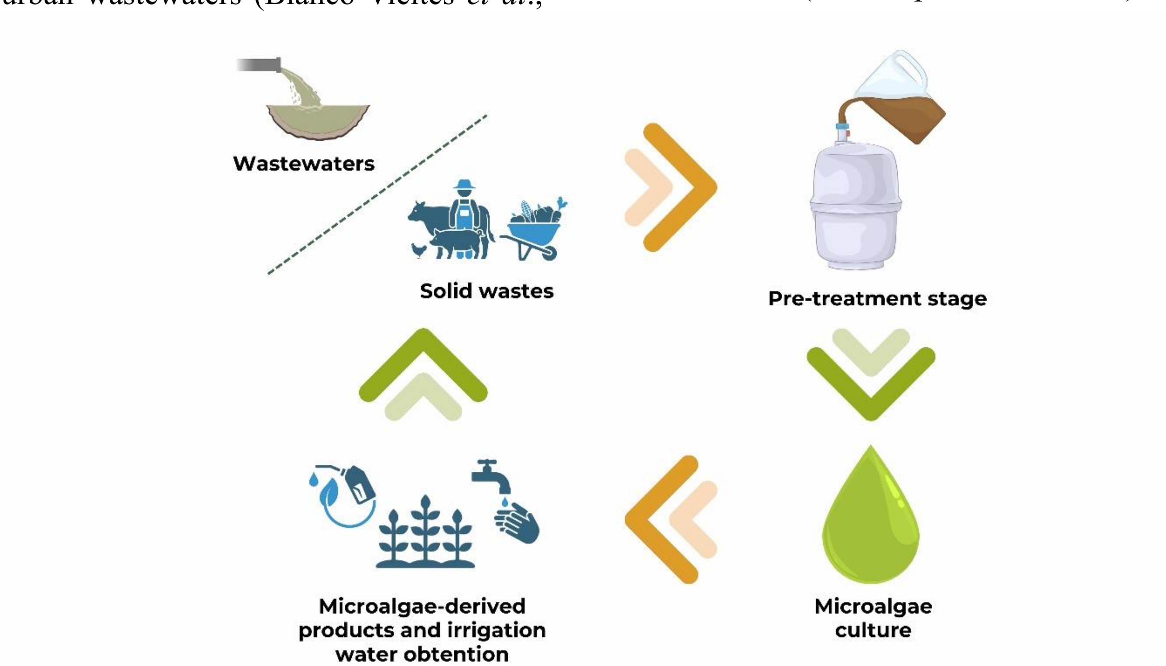

Figure 1. Insertion of microalgal cultures for wastewater and solid waste bioremediation within a circular economy approach.

Microalga can remove contaminants through three main mechanisms: biosorption, biodegradation and bioaccumulation (Najar-Almanzor *et al*., 2023; Al-Jabri *et al*., 2020). During biosorption, functional groups present in microalgal membranes bind to pollutants in the medium, like heavy metals (Najar-Almanzor *et al*., 2023; Plöhn *et al*., 2020). Carbohydrates building or enveloping the cell's wall contain negatively charged functional groups—such as amino, hydroxyl, sulfide, and phosphate - that serve as active sites for binding positively charged metals (Laroche, 2022). This process, known as "extracellular adsorption", is typically rapid and continues until all available binding sites are saturated (Al-Jabri *et al*., 2020). Biosorption is considered a passive process, as it occurs independently of the cell's metabolism (Najar-Almanzor *et al*., 2023; Plöhn *et al*., 2020).

In contrast, bioaccumulation refers to the uptake of both inorganic and organic pollutants through the microalgae's active metabolic processes, either accumulating or metabolizing them (Najar-Almanzor *et al*., 2023). Similarly, biodegradation is when complex compounds are degraded into more simple forms through complete mineralization or enzymatic reactions, and may occur either extracellularly, intracellularly, or as a combination of both (Najar-Almanzor *et al*., 2023). These last two mechanisms can involve the use of mixotrophic conditions.

3.1 Wastewater treatment

Wastewater refers to any water discharge that has been adversely affected in quality by human activity. As global water demand rises and pollution increases, effective wastewater treatment has become essential for protecting public health and the environment (Devi *et al*., 2023; Plöhn *et al*., 2020), and microalgae bioremediation, also called phycoremediation, can serve as a secondary or tertiary phase in the process. Usually, pre-treatment stages are considered to adjust nutritional parameters and control physicochemical variables if needed (Al-Jabri *et al*., 2020). Wastewater composition is often irregular, and can either carry low levels of essential nutrients, or too high concentrations. They can also have undesirable characteristics, depending on their origin, such as dark coloration, high turbidity, extreme pH values, and even contain large solid materials (Devi *et al*., 2023; Plöhn *et al*., 2020; Razzak *et al*., 2017). These circumstances can lead to growth inhibition, poor biomass productivity, low yields and downregulation of high-value metabolites (Devi *et al*., 2023; Al-Jabri *et al*., 2020; Razzak *et al*., 2017).

Many microalgae species have been investigated to treat a great range of wastewaters, including municipal, domestic, industrial, mining aquaculture and livestock wastewaters (Blanco-Vieites *et al*., 2023; Plöhn *et al*., 2020; Al-Jabri *et al*., 2020; Olguín, 2012). Aquaculture wastewaters are usually treated with marine microalgae like *T. suecica*, *I. galbana*, *Dunaliella tertiolecta* (Andreotti *et al*., 2017), and *Navicula* sp., *Nitzchia microcephala* and *Chlorella marina* (Meril *et al*., 2022). Particularly, the use of microalgae for aquaculture bioremediation poses a great perspective in applied circular economy. Because aquaculture wastewaters often contain elevated concentrations of organic and inorganic nitrogen – originated from undigested feed and excreted fecal matter - microalgae can be culture using the generated wastewater and channeled back as feed in the industry

(Tham *et al*., 2023; Milhazes-Cunha and Otero, 2017).

Agricultural activities are also rich in nutrients that can be exploited by microalgae. For instance, *Nannochloropsis limnetica* was grown during a year in wastewater obtained from the greenhouse of tomato production, obtaining even better results than BG11 medium (Guiscafré-Rodea *et al.,* 2018). Similarly, vinasse has been used to culture *Arthrospira maxima* (dos Santos *et al*., 2016) and *Neochloris oleoabundans* (Olguín *et al*., 2015a). In some research, a combination of different wastewater sources can be used to improve biomass growth, as was the case of *C. vulgaris* in nejayote and swine wastewater (López-Pacheco *et al*., 2019).

Several microalgal strains have been documented to mediate the removal of pharmaceutical contaminants from hospital effluents, industrial streams and domestic wastes (Xiong *et al*., 2018). Such is the case of *C. sorokiniana*, capable of removing diclofenac, ibuprofen, paracetamol, metoprolol, carbamazepine and trimethoprim (Wilt *et al*., 2018). Likewise, *Nannochloropsis* sp. uptake played a role in the removal of triclosan, carbamazepine, trimethoprim and sulfamethoxazole (Bai and Acharya, 2017).

Overall, bioremediation of wastewater by microalgae constitutes a cost-effective approach to large-scale cultivation and supports the development of integrated setups near existing industrial sites, eliminating the need for fertile land and helping to improve the ecological health of nearby water sources (Acién *et al*., 2021; Mohsenpour *et al*., 2021). Furthermore, treated wastewater can be subsequently used as irrigation water or to obtain compounds excreted by microalgae, such as exopolysaccharides (Morais *et al*., 2022; Anda-Sánchez, 2017).

3.2 Solid waste recycling

The recycling of solid waste as a nutrient source for microalgae cultivation presents a promising avenue for integrating waste management with sustainable bioresource production. Recovering valuable nutrients from organic and agro-industrial solid wastes—such as livestock manure (e.g., cattle and pig farm waste) (Abu Hajar *et al*., 2017; Olguín *et al*., 2003), food and agricultural residues (e.g. corn silage) (Drosg *et al*., 2020), and discarded fruits and vegetables (González-Ortiz, 2025; Gaytán *et al*., 2023)—not only helps mitigate environmental pollution but also reduces food waste and decreases industrial reliance on global natural resources (Olguín *et al*., 2022; Fernández *et al*., 2021).

Solid wastes require a pre-treatment stage that allows the nutrients to be bioavailable and in optimal concentrations for microalgae to consume (Kim *et al*., 2022). This can involve biodegradation of organic matter by other microorganisms, like fungi, yeast and bacteria. For instance, wheat bran could be used for mixotrophic culture of *C. vulgaris* and *S. obliquus* after being pre-treated by fungi, which allowed for the conversion of the wheat into soluble products that could be up-taken by microalgae (EL-Sheekh *et al*., 2012). Nevertheless, biodegradation can also occur when treating by-products of industrial processes by the same microalgae– this was the case of *N. limnetica*, which was capable of secreting extracellular β-galactosidase to hydrolyze lactose (present in the culture medium) into monosaccharides, prior to absorption and further process through mixotrophic metabolism (Li *et al*., 2024).

One of the most used techniques is anaerobic digestion (AD), a biological degradation of organic matter driven in the absence of oxygen (Chuka-ogwude *et al*., 2020). Biogas can be obtained from AD, along with non-gasified liquid (leachates/effluents) and solid (digestates) by-products (Chuka-ogwude *et al*., 2020). These byproducts are high in nutrients, and therefore are the ones added to microalgae culture medium (Bauer *et al*., 2021; Chuka-ogwude *et al*., 2020). The nutrient composition of AD leachates and digestates is directly connected to the feedstock used for the anaerobic digestion, which, in turn, will have a direct effect on pH value, salinity and turbidity of the cultivation medium (Bauer *et al*., 2021). A dilution rate is needed usually applied based on the concentration of macro-elements like nitrogen in the form of ammonium (Bauer *et al*., 2021).

Mixotrophic cultivation using a digestates and effluents from different livestock has been studied in the last few decades. For example, pre-treated pig manure has been used to successfully stablish microalgal culture with species like *Chlorococcum* sp. (Montero *et al*., 2018), *N. oleoabundans* (Olguín *et al*., 2015b) and *Arthrospira* sp. (Olguín *et al*., 2003).

Agricultural residues and food waste are also high valuable for microalgae culture. The growth of *Lagerheimia longiseta*, *Monoraphidium contortum* and *S. quadricauda* using biocompost of discarded fruits and vegetables as alternative culture medium proved to be comparable of the growth obtained with WC medium (Medeiros *et al.,* 2019). Similarly, the use of food waste leachates for *Arthrospira maxima*'s biomass production was proven by Gaytán *et al*. (2020) and González-Ortiz (2025).

Seawater microalgae can also be grown using solid wastes if the medium is supplemented with essential salts to sustain the culture. *N. oculata* showed that it was capable of growing in a mix of seawater, saline produced water obtained during oil and gas extraction, and liquid digestate from AD of an industrial scale biogas plant, when salinity remained lower than 60 g L^{-1} (Parsy *et al*., 2020).

3.3 Carbon sequestration and flue gas utilization

Efforts have been made to prevent the release of CO_2 into the atmosphere by enhancing microalgal systems to capture it from industrial emissions—also known as flue gases (Olguín *et al*., 2022; Acién *et al*., 2021; Yu *et al*., 2020). This would allow the production of high value-added products such as pigments, proteins, and energy sources like biofuels, with more environmentally friendly characteristics (Li *et al*., 2023; Acién *et al*., 2021). However, low solubility of CO_2 in liquid medium leads to the lower CO_2 fixation efficiency, and a constant supply of flue gases to the culture can lead to pH imbalance and inhibit microalgal growth, limiting the application of this technology (Li *et al*., 2023; Acién *et al*., 2021; Du *et al*., 2019).

In this matter, mixotrophic cultivation has been proposed to enhance carbon sequestration efficiency (Li *et al*., 2023). Recently, Yu *et al*. (2020) developed an open pond, mixotrophic system using *C. vulgaris* cultures, where 5% CO_2 was continuously bubbled during daytime, and acetic acid was added at night to regulate the pH value within neutral range. This treatment resulted in higher growth rate and biomass production, when compared to photoautotrophic cultures, and increased CO_2-fixation in contrast to only adding acetic acid as carbon source (Yu *et al*., 2020). Similar patterns for improved photosynthetic carbon fixation have been reported for *Asterarcys* sp. using glucose (Li *et al*., 2020), and *A. platensis* using acetate (Li *et al*., 2023). To a greater extent, an integrated systems of microalgae-based CO_2 fixation coupled to wastewater treatment was achieved with *C. vulgaris* using seafood processing wastewater (Jain *et al*., 2019). Even though, more research regarding this subject is still necessary, since this integrated approach shows considerable promise.

4. Biomass application

While cleaning wastewaters and recycling solid waste as a cost-effective culture medium, microalgae produce valuable biomass that is suitable for various purposes such as bioenergy, bioactive compounds and biomaterials (Acién *et al*., 2021). Microalgal biomass application depends on its composition. For example, high amounts of proteins and lipids are of great value for livestock and aquaculture feed (Acién *et al*., 2021). Both *Chlorella* sp. and *Arthrospira* sp. are well known for their elevated protein content and safe consumption (Kim *et al*., 2022). Particularly *Arthrospira* sp. has shown a great ability to grow in alternative culture medium in open ponds; due to it, is of great interest in this realm (Olguín *et al*., 2003). Similarly, *Isochrysis zhanjiangensis* was used for treating aquaculture wastewater and could later be used as fish feed (Zheng *et al*., 2011).

On the other hand, a high content of lipids and saturated fatty acids can be used to produce biodiesel or biogas (Al-Jabri *et al*., 2020). *Chlorella* sp. (Jain *et al*., 2019; Lin *et al*., 2015), *M. contortum* (Medeiros *et al*., 2020), *Chlorococcum* sp. (Montero *et al*., 2018), *S. obliquus* (Girard *et al*., 2014) and *N. oleoabundans* (Olguín *et al*., 2015a) are examples of microalgae grown under mixotrophic conditions with this potential application.

Remaining biomass after cell disruption for the extraction of other metabolites may contain some of the essential elements that plants require for their growth, like free amino acids, potassium, phosphorus, chlorine, carbon and sodium (Gaytán *et al*., 2023; Acién *et al*., 2021). Thus, it might be used as crops biofertilizers and bioestimulants in agricultural approaches, as proposed by Gaytán *et al.* (2023) for *A. maxima* biomass grown in food leachates.

One of the highest-valued products obtained from microalgae are pigments. Microalgal cell produce these photosynthetic compounds to harvest light (e.g. chlorophylls, phycocyanin, phycoerythrin) and protect themselves from light-stress conditions (carotenoids). These portray bioactive activities and can be used for health and cosmetic applications (Acién *et al*., 2021). Using mixotrophy, some species have been documented to increase pigment production and accumulation, as reported by Morowvat and Ghasemi (2016) for *D. salina*; they were able to obtain 8.12 mg of β-carotene g^{-1} DW using glucose. In other cases, the production of metabolites such as phycocyanin did not show statistical differences between photoautotrophic and mixotrophic conditions, but the increase in biomass productivity and concentration per area (m^{-2}) derived into higher pigment recovery (Abiusi *et al*., 2022). Likewise, *A. platensis* grown in whey boosted its chlorophyll production, as well as other secondary metabolites, including phenolic compounds and flavonoids (Herrera-Peralta *et al*., 2022). Mining wastewater also promoted phycocyanin production in *A. maxima* (Blanco-Vieites *et al*., 2023).

This variety of microalgae-derived products, obtained from revalorization of both water and solid wastes, shows the great extent of possibilities and industrial processes that can benefit from this technology. Furthermore, as previously mentioned for aquaculture phycoremediation, this highlights the circular economy potential, where residues serve as raw materials for new products. Even though a big, transitional leap still needs to be taken - based on further research – the potential of these systems lays a solid foundation for their integration into sustainable and economically viable industrial frameworks.

5. Conclusion

Mixotrophic cultivation of microalgae has emerged as a highly promising strategy for integrating wastewater treatment with the sustainable production of biomass and high-value bioproducts. Taking advantage of the unique capabilities of microalgae to grow in diverse waste streams—while simultaneously removing pollutants and assimilating carbon dioxide—positions them as a key component in phycoremediation and circular bioeconomy initiatives. Current research highlights the potential of mixotrophic systems to revalorize solid and liquid waste residues as nutrient sources, supporting microalgae-based biorefineries that produce biofertilizers, biofuels, animal feed, aquaculture inputs, and valuable metabolites. However, to achieve the full potential of these systems at industrial scale, further work is needed to optimize strain selection, to optimize wastewater or waste pre-treatment to ensure the consistency and suitability of waste-derived media, and to assess long-term biomass productivity under variable environmental and wastewater conditions. Further advances in these areas are critical for developing economically viable and environmentally sustainable microalgal technologies.

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