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Review

Phycoremediation Processes for Secondary Effluent from Sewage Treatment Plants Using Photosynthetic Microorganisms: A Review

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Abstract: Taking into account the worrying scenario of water scarcity, it is essential to enable more efficient technologies for wastewater treatment. Wastewater may be treated by conventional biological processes that remove pathogenic organisms, particulate and soluble organic compounds, and other components. However, secondary effluents from treatment plants may still contain toxic elements or high concentrations of inorganic nutrients (mainly nitrogen and phosphorus), which enable the growth of photosynthetic microorganisms in water bodies, resulting in eutrophication. In this context, cultivation of photosynthetic microorganisms in secondary wastewater from sewage treatment allows the removal of nutrients from such wastewater, reducing the possibility of eutrophication. Moreover, microalgal biomass, produced in this tertiary wastewater treatment, may be harvested by different methods with the potential for different applications, such as fertilizer and biofuel.

Keywords: microalgae; tertiary wastewater treatment; sewage treatment; cleaner water and sanitation; sustainable cities and communities

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1. Introduction

The scarcity of water is a consequence of a lack of balance between the availability and demand. The growing demand comes from a rapidly rising urban population, irregular distribution of water resources, severe dry periods, and pollution of surface and groundwater. Government authorities, as well as public service managers, need to provide new sources or find new strategies to improve water resources management in a sustainable way [1–3].

Water has been used by human beings for several purposes, including irrigation, navigation, recreation, generation of electricity, and the most quality-demanding requirement: industrial and cosmetic supply. Moreover, human beings may interfere in water bodies by discharging domestic or industrial wastewater or by releasing fertilizers and pesticides. In this sense, waterbodies may be polluted by organic matter or pathogenic microorganisms. In fact, domestic wastewater (sewage) is one of the main pollutants in water bodies [4,5].

This review brings an important approach for photosynthetic microorganisms-based treatment of secondary wastewater, presenting in simple language the latest specialized studies regarding wastewater properties and the great potential of microorganisms for its tertiary treatment. The study presents wastewater characteristics from different treatment stages and how photosynthetic microorganisms can be applied successfully in the tertiary treatment. Besides the reduction in eutrophication that is the main advantage of phycoremediation, other biotechnological advantages are also described. The research still comprises different types of photobioreactors for photosynthetic microorganism cultivation and the main biomass-harvesting methods, in addition to potential uses for the biomass produced in the tertiary treatment. Furthermore, the study presents a discussion regarding environmental issues and the main challenges to making this technology feasible.

2. Wastewater Properties and Treatment

Wastewater production is inherent to humanity as a result of industrial and agro-based processes, and it is also composed of residues from daily hygiene and alimentation activities. Thus, this residue must be considered and treated in order to maintain both health and environment under secure conditions. The primary and secondary treatments of wastewaters aim to remove particulate and organic materials. They are well documented in the literature and applied in all plants of wastewater treatment. On the other hand, tertiary treatments, including phycoremediation, are not yet applied in all industrial wastewater and sewage treatment plants to improve the quality of the effluent and mitigate environmental problems such as eutrophication. Some relevant aspects may be considered to highlight the importance of wastewater tertiary treatment by phycoremediation, including the characteristics of wastewater and the treatment processes, as described below.

2.1. Characteristics of Wastewater

Wastewater arriving at a treatment plant originates from three main sources: domestic sewage (bathroom, kitchen, laundry), infiltration, and industrial effluents [4,6]. These sources have to be characterized in terms of quality and quantity [4].

Wastewater compounds depend on the type of source, because they vary significantly depending on lifestyles, customs, discharged substances, etc. Additionally, some of these compounds undergo chemical and biological degradation in the piping or during storage [6].

Physical attributes, as well as chemical and biological properties, define the quality of wastewater [5,6]. Approximately 99.9% of domestic wastewater is composed of water, and the remaining 0.1% consists of organic and inorganic compounds, suspended and dissolved solids, and microorganisms [4].

Physical parameters include temperature (depending on the seasons), color (black appearance due to biological reactions), odor (dissolved impurities), and turbidity. Temperature is a very important parameter, since it influences microbial activity, gas solubility, and liquid viscosity [4]. High temperature can also affect other parameters such as pH, conductivity, gas saturation, and alkalinity [6,7]. Among the chemical characteristics are suspended or dissolved solids (organic and inorganic), mineral compounds, organic matter (heterogeneous mixture of various organic compounds), biochemical oxygen demand (BOD: biodegradable fraction of organic compounds), chemical oxygen demand (COD: amount of total oxygen to oxidize the organic compounds), and alkalinity (buffer capacity of the medium) [4,8]. The main components of wastewater are proteins, carbohydrates, lipids, urea, and low concentrations of synthetic organic chemicals. The most commonly found inorganic components comprise the following: chloride, hydrogen ions, and elements such as phosphorus, nitrogen, sulfur, and some heavy metals [9]. Among the organic xenobiotic compounds found are the following: detergents, soaps, perfumes, and preservatives. They are resultant of the use of chemical substances in industries and residences. The alkalinity, hardness, and pH of the water determine the buffering capacity [6].

The measurements of turbidity and suspended solids enable gaining information about particles or colloids that can generate obstruction of installations or filters. This obstruction must be taken into account, because the association of colloids and surfactants may stabilize the solid phase. It reduces the efficiency in pretreatment (solid matter settlement). BOD and COD enable knowing if oxygen may be depleted as a consequence of organic matter deterioration throughout transport and storage, generating the production of sulfur [6].

Regarding wastewater's biological characteristics, it contains different types of microorganisms. The effectiveness of wastewater treatment depends on the concentration of the species needed for the treatment [7]. Different microorganisms may be present in wastewater, including bacteria, archaea, algae, fungi, protozoa, helminths, and viruses; it is worth mentioning that, from this group, bacteria is important for helping to stabilize organic matter [4]. The organic matter contained in wastewater may be decomposed by aerobic bacteria, generating carbon dioxide, ammonia, phosphate, etc. These products may be used by the algae, liberating oxygen, which is released and, again, used by the bacteria to oxidize additional waste material [10].

2.2. Primary and Secondary Treatment of Wastewater

Sewage is conducted to the wastewater treatment unit for screening and primary treatment, which consists of physical–chemical processes, such as flocculation, primary decantation, and rotary sieve. The secondary treatment consists in a conventional biological process, which removes or diminishes pathogenic organisms, nutrients (e.g., nitrogen and phosphorus), metals, dissolved inorganic solids, and particulate and soluble organic matter [5,11,12]. Generally, sewage treatment is divided into aerobic or anaerobic processes. In respiration processes, organic matter is oxidized and carbon dioxide and large amounts of sludge are produced (aerobic treatment). On the other hand, in anaerobic treatment, two groups of microorganisms perform degradation of soluble and insoluble organic matter. Acidogenic bacteria convert organic matter to different compounds such as hydrogen, acetic acid, and carbon dioxide. Methanogenic microorganisms, in turn, convert these compounds to methane gas [13]. Alternatively, some methanogenic microorganisms ferment acetic acid or methanol to methane gas and carbon dioxide [14]. Aerobic processes are of vital importance for reducing the waste transported by water, eliminating the soluble organic compounds in less time than in anaerobic processes [10].

There are some strategies (aerated lagoon, trickling filters, rotating biological contactors, stabilization pond, anaerobic digestion, etc.) to remove the nutrients from wastewater [7]. Nonetheless, these processes may be very expensive and produce high amounts of sludge [15]. Chudoba [16] classified the organic compounds produced by microorganisms in wastewater treatment into three categories: (i) compounds excreted by the interaction of environment and microorganisms; (ii) compounds originated by the microbial metabolism from the substrate; and (iii) compounds that are released by the degradation and lysis of microorganisms.

It is also important to mention that secondary effluents from treatment plants may still contain high concentration of nutrients (NH_4^+, NO_3^-, PO_4^{3-}) [15,17,18] or toxic elements (copper, lead, arsenic, cadmium, chromium, etc.). Even if these toxic elements may be present in small concentrations, not affecting humans, they may have phytotoxic effects [19]. Compounds with nitrogen that are discharged to the environment may be mineralized, nitrified, and denitrified under natural processes [20].

These effluents should not be poured directly into watercourses such as lakes, rivers, or seas, because they contain ammonium, nitrates, and phosphates, which have significantly contributed to eutrophication in natural water bodies, requiring greater attention to causing pollution when accumulation takes place [11,18,21–23].

2.3. Eutrophication Issue

Eutrophication (also known as over-enrichment with nutrients) is an environmental matter that can be caused by human activities [24]. It takes place when nutrients are released into the water by way of manure, sewage, and fertilizers. As a consequence, excessive growth of aquatic plants and algae takes place. This phenomenon may induce modifications in the composition and diversity of aquatic plants and also affect the structure of an ecosystem and the food web [5,11,25,26].

Eutrophication may be caused by natural causes, such as the aging of a lake over thousands of years. However, in 2008, it was estimated that 30 to 40% of water bodies world-wide were affected by anthropogenic high nutrient concentrations, and eutrophication is believed to have significantly increased around 1950 [27].

Therefore, special attention should be addressed to the treatment of wastewater to reduce nutrients, principally nitrogen and phosphorus [5]. It could allow, at a later stage, the reuse of water for non-potable purposes. Reuse of water offers the potential for exploiting a resource [1], and it is increasingly recognized as a sustainable water management strategy, taking into account stability, reliability, economy, and distribution to meet different user requirements [28].

2.4. Phycoremediation as a Tertiary Treatment of Wastewater

In order to reduce the global problem of eutrophication, bioremediation could be a more sustainable alternative, which can be performed using photosynthetic microorganisms (phycoremediation). This process consists in removing the excess of nutrients or other toxic components, such as heavy metals and organic contaminants, through the application of eukaryotic algae (e.g., microalgae or seaweeds) or prokaryotic microorganisms such as cyanobacteria [26].

This pioneering approach, cultivating photosynthetic microorganisms in secondary effluents, contributes to the decrease in or removal of the extra nutrients in wastewater with valuable resources instead of downloading it to the environment, where ecological sustainability is characterized by two major challenges, wastewater and greenhouse gases. Moreover, it brings the advantages of the production of valuable biomass in addition to carbon dioxide bio-fixation and cost effectiveness [7,15,17,29].

In this context, photosynthetic microorganisms can be used in tertiary treatment of wastewater to remove contaminants such as ammonia, nitrates, and phosphates [30]. The growth of photosynthetic microorganisms, carrying out phycoremediation for a tertiary treatment of wastewater, allows a purification of the water. the nutrients supplied do not have an additional cost, and the type of nutrient is a significant aspect of determining the cost of biomass production [31,32].

In this context, nutrients are essential substances for optimizing microorganisms' growth. Besides carbon, nitrogen and phosphorus are the most important nutrients for the growth of photosynthetic microorganisms. Concentrations of total nitrogen and total phosphorus in wastewater differ significantly depending on the type of sewage [33]. In most cases, the presence of nitrogen and phosphorus in wastewater means that they may be used for the growth of photosynthetic microorganisms. However, some wastewaters with high concentrations of nutrients may have low growth or, conversely, wastewaters with low concentrations of nutrients may have intense growths of algae [34]. As it can be seen in Table 1, wastewater contains enough of these nutrients to meet growing needs.

Table 1. Chemical characteristics of secondary wastewater from different urban wastewater sources.

Parameter	**Concentration**				
Reference source	[35]	[36]	[23]	[37]	[22]
$Na^{2+}\left(mgL^{-1}\right)$	28.98			19.33 ± 1.41	
$NH_4^+\_N\left(mgL^{-1}\right)$	12.76	0.4 ± 0.1		3.84 ± 4.01	27.4
$K^+\left(mgL^{-1}\right)$	7.99			0.62 ± 0.07	
$Mg^{2+}\left(mgL^{-1}\right)$	2.63				
$Mn^{2+}\left(mgL^{-1}\right)$			<0.05		
$Zn^{2+}\left(mgL^{-1}\right)$	0.71		<0.05		
$Cu^{2+}\left(mgL^{-1}\right)$	0.71		<0.05		
$NO_3^-\_N\left(mgL^{-1}\right)$	0.2	8.5 ± 0.4	0.9	1.29 ± 0.46	<1

Table 1. *Cont.*

Parameter	Concentration				
NO_2^-_N(mgL^{-1})			0.14		<1
PO_4^{-3}_P(mgL^{-1})	2.00	1.69 ± 0.4	11.5		11.8
$Cl^-(mgL^{-1})$	1.7	75 ± 0.4			
$SO_4^{-2}(mgL^{-1})$	4.21	8.0 ± 0.5			
Total carbon (mgL^{-1})		22.6 ± 1	38.43		
Total inorganic carbon (mgL^{-1})		14.6 ± 0.1	38.36		
Total organic carbon (mgL^{-1})		8.1 ± 0.2	0.4		
pH		7.3	7.6	7.68 ± 0.14	9.3
Total nitrogen (mgL^{-1})		8.7 ± 0.5	11.9		
Total phosphorus (mgL^{-1})		1.71 ± 0.3		3.02 ± 0.71	
Organic matter $(mgC.kg^{-1})$			0.11		
Mineral matter $(mgC.kg^{-1})$			0.47		

Another consideration that must be taken into account is the nutrients balance: if only one nutrient is in excess, addition of the other component in wastewater may be necessary to balance the proportion of nutrients so that there is no limitation on cell growth [38].

Generally, microalgae have the following approximate chemical composition: $C_{106}H_{181}O_{45}N_{16}P$ [39]. McGinn et al. [40] reported that during the balanced growth of microalgae, the elemental proportions of C:N:P of microalgae biomass assume, in molar terms, predictable ratios of 106:16:1, which is called the Redfield stoichiometry, although deviations may be found in this proportion. When growth becomes limited by only one nutrient, the absorption of the others will diminish, leading to the corresponding accumulation in the extracellular environment. Additionally, Wang et al. [41] indicate that the N:P mass ratio in the effluent fractions to be used in the range of 6.8–10.0 can be understood as optimal in the production of microalgae. They also observed that the microalgae were efficient for the removal of dissolved metals (Al, Ca, Fe, Mg, and Mn) in the wastewater from the secondary treatment. In fact, microalgae such as *Chlorella vulgaris* have the capacity to adsorb heavy metals [42], which could be useful in wastewater treatment plants (WWTP), since even in the sewage, metals may be present [43].

Photosynthetic microorganisms assimilate inorganic nitrogen in the form of NO_3^-, NO_2^-, NH_4^+, and, only in some cases, N_2. However, the preferred form is NH_4^+, since NO_3 and NO_2 have to be reduced to NH_4^+ to be incorporated by glutamine synthetase enzyme systems. After assimilation, nitrogen plays an important role in the constitution of biomolecules such as nucleic acids, amino acids, and pigments. Phosphorus, in its turn, is an important component of nucleic acids, phospholipids, and ATP, and the main form of assimilation by photosynthetic microorganisms is orthophosphate (PO_4^{-3}) [44,45].

When secondary wastewater contains residual organic compounds, they also may contribute to the microalgal growth. These microorganisms can assimilate organic nitrogen in the form of urea or amino acids [44], and, by mixotrophic metabolism, they can assimilate carbon in the form of organic compounds. In fact, Matsudo et al. [46] and Matsudo et al. [47] have shown that the cyanobacterium *Artrospira platensis* and the green microalga *Scenedesmus obliquus* have their growth increased by the addition of acetate and ethanol, respectively, in the culture medium.

Several studies evaluate the use of green microalgae in wastewater treatment [48–51], such as *Chlorella vulgaris* [50,51], *Monoraphidium contortum* [52], *Chlamydomonas incerta* [53], *Actinastrum* sp. [54], *Pediastrum* sp. [55], *Ankistrodesmus* sp. [56], *Coelostrum* sp. [57], *Chaetomorpha linum* [58], *Neochloris oleoabundas* [59], *Dictyosphaerium* sp., *Micractinium* sp., *Mucidosphaerium* sp., *Pseudotetracystis*, *Tetracysti* sp. [60], *Desmodesmus* sp. *L02*, *Coccomyxa* sp.

L05, *Chlorococcum* sp. *L04, Chlorella* sp. *L06, Tetradesmus* sp. *L09*, and *Scenedesmus* sp. *L08*, and the last six strains were collected from the effluent of an anaerobic reactor, used for municipal wastewater treatment [61]. Wu et al. [62] also isolated nine green microalgae species of genus *Desmodesmus* from wastewater. Other microalgae, including cyanobacterial species, that can be found in wastewater treatment plants are *Euglena* sp. [63], *Micractinium inermum* sp. [64], *Spirulina maxima*, and *Synechocystis* sp. [65].

In addition, wastewater from sewage treatment plants may require dilution to reduce turbidity or to avoid inhibition phenomena, which may occur due to the presence of organic matter [66] or presence of ions [67]. Turbidity is caused by suspended matter or impurities that reduce the transparency of the water and, therefore, limit the light input (which is necessary in the case of cultivation of photosynthetic microorganisms). These impurities may be represented by clay, slime, organic and inorganic matter, soluble organic colored compounds, and microorganisms [66].

3. Parameters Related to Growth of Microorganisms Applied in Phycoremediation, Types of Bioreactors, and Biomass-Harvesting Methods

Concerning the utilization of photosynthetic microorganisms in phycoremediation processes, there are important factors that must be considered. In this context, it is fundamental to evaluate growth parameters, types of bioreactors, and harvesting processes, as described below.

3.1. Growth Factors of Microorganisms Applied in Phycoremediation

As indicated above, growth of the photosynthetic microorganisms depends on several factors (light, temperature, nutrients, etc.) even in a culture with artificial medium [7]. Carvalho et al. [68] and Carvalho et al. [69] describe how these parameters may influence the growth of photosynthetic microorganisms. Some studies that used photosynthetic microorganisms in wastewater are detailed below.

Mutanda et al. [70] report that residues from domestic wastewater treatment could lead to higher growth rates of *Chlorella* sp. in shaken flasks with the addition of sodium nitrate in a batch process, in comparison with wastewater without sodium nitrate.

Li et al. [71] evaluated the viability of growth of *Chlorella* sp. in municipal wastewater at high concentration, produced from activated sludge, for the treatment of wastewater with simultaneous energy generation. Two culture media with wastewater were tested: autoclaved and untreated media. It was found that after completing a batch culture of 14 days, the microalgae were able to remove ammonia at 93.9%, total nitrogen at 89.1%, total phosphorus at 80.9%, and chemical oxygen demand (COD) at 90.8% from the untreated medium. The content of fatty acids in dry biomass was 11.04%, yielding up to 0.12 g-biodiesel L^{-1} of microbial suspension. It is possible to expand the system using continuous operating, which may provide up to 0.92 g L^{-1} d^{-1} of biomass productivity [71].

In Table 2, other examples of photosynthetic microorganisms used in tertiary wastewater treatment are shown. It is possible to observe the importance of green algae in this kind of study, mainly because of their fast growth rate and high nutrient uptake per unit biomass, in addition to the fact that they are natural colonizers of ponds, as already pointed out by Tang et al. [72]. On the other hand, another important factor for removal of nutrients is the use of adequate reactors, which increases the uptake of nutrients by biomass [73].

Table 2. Comparison of different studies of photosynthetic microorganisms in tertiary wastewater treatment.

Photobioreactor	Microorganisms	Process	Light/ Dark Cycle	Temperature	Additional Parameters	Reference
Closed cylindrical photobioreactor. Total volume 30 L.	Mixed culture (microalgae, bacteria, protozoa, and metazoan). *Chlorella* sp., *Scenedesmus* sp., and *Stigeoclonium* sp. (dominant generos)	Semi-continuously (fed once a day)	12/12 h	25~29 °C.	Microalgae digestate diluted in secondary effluent at a ratio of 1:50 and operated at 8 days of hydraulic retention time (HRT) and solids retention time (SRT).	[74]
Aerated bioreactors made of transparent polyethylene terephthalate (3 L).	*Scenedesmus obliquus Chlorella vulgaris*	Semi-continuous		25 °C	Free and immobilized cells.	[15]
Erlenmeyer. Total volume 2 L. Working volume of 1.3 L.	*Synechococcus nidulans Chlorella vulgaris Botryococcus braunii Chlorella minutissima*	Batch	12/12 h	25 °C	Cellular adaptation was evaluated by Neubauer counting chamber.	[35]
11 L BioFlo Fermenter.	*Chlorella vulgaris Botryococcus braunii*	Batch	12/12 h	25 °C	Supply 5% CO_2.	[35]
Serum bottles. Total volume 500 mL. Working volume of 200 mL.	*Chlorella vulgaris Scenedesmus obliquus Ourococcus multisporus*	Batch	12/12 h	27 °C	Supply 15% CO_2. Optical density measurement at 680 nm.	[36]
Stirred tank reactor.	*Botryococcus braunii*	Batch	12/12 h	25 °C	Optical density measurement at 600 nm.	[23]
Acrylic tanks. Total volume 3.5 L. Working volume of 2 L.	*Chlorella vulgaris*	Batch	16/8 h	24 ± 1 °C	pH = 7.1. Initial density = 3.0×10^6 cells mL^{-1}. Air provided CO_2.	[75]

3.2. Types of Bioreactors for Phycoremediation

As mentioned above, for an efficient nutrient removal, biomass production, and carbon dioxide bio-fixation, it is necessary to pay attention to bioreactor design [7,17]. There is a wide variety of bioreactors used for photosynthetic microorganism cultivation [69]. However, after studies have been conducted to treat water with photosynthetic microorganisms, one of the main current challenges is the scheduling of successful laboratory-scale experiments for commercial-scale production. Particularly in the cultivation of photosynthetic microorganisms, Grobbelaar [76] reported that there are several problems around this, which include it being difficult to stagger due to the geometry of the photobioreactor and external environmental variables that are difficult to control, such as temperature and contamination. In addition, the harvesting process demands a lot of effort because of the large amount of biomass to be produced and relatively low concentrations of photosynthetic microorganisms after the tertiary treatment. In fact, the final biomass concentration is another field that deserves effort to maximize, contributing to diminishing the cost of the harvesting process.

In this sense, it is imperative that laboratory-scale experiments, followed by pilot-scale work, should be evaluated whenever possible to support a full-scale operation. Generally, the algal culture bioreactors are classified as open and closed systems [77].

Open photobioreactors (Figure 1) are the most commonly used for the cultivation of photosynthetic microorganisms. The ponds are provided with a paddle to allow the circulation of algae and nutrients. They are relatively inexpensive to build, and the operation is easy (minimal maintenance). However, this type of reactors presents some restrictions because of the possibility of contamination (monocultures are almost impossible); environmental conditions, which are difficult to control throughout the year; a high rate of water evaporation and diffusion of carbon dioxide; and a relatively low viable cell density, due to the shadowing effects (dark zones). Consequently, extensive areas of land are needed for deployment [7,12,17,78–80]. Some of the disadvantages of this kind of photobioreactor could be minimized by covering them. Such a procedure could avoid liquid outflow during rainy seasons, and, if appropriately covered, both the water and CO_2 lost from the tank could be at least partially recovered after treatment of the gases outputted from the system.

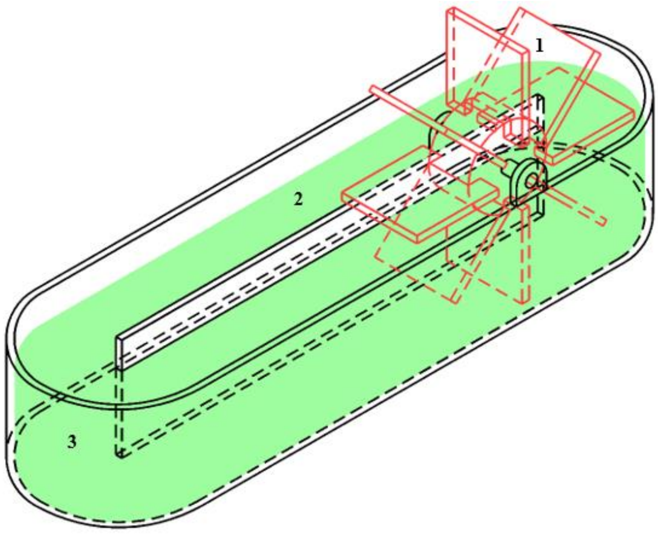

Figure 1. Scheme of open photobioreactor: (1) paddle wheel; (2) pond area; (3) baffle.

Numerous studies show advantages of closed systems for microalgae cultivation, which include maximizing the surface/volume ratio, less loss of liquids by evaporation and gas, and higher cellular concentrations, besides the possibility of achieving monocultures. The tubular photobioreactor is usually adopted for the large-scale cultivation of autotrophic microalgae [7,17,78–80]. Despite their advantages, such photobioreactors have some limitations that need to be taken into account in an evaluation for industrial use. They are more expensive than open photobioreactors; oxygen can be accumulated in the system, leading to a possible inhibition of cellular growth; and as consequence of the minor water evaporation, there is a high increase in temperature in the cultivation medium during the day, which needs to be controlled to avoid a possible loss of the culture viability.

The tubular photobioreactor (TPBR) is an arrangement of transparent tubes commonly composed of transparent plastic or glass, which collects sunlight for photosynthesis. The microorganism suspension is continuously pumped and distributed from a reservoir (degassing column) to the solar collector and returned to the column. The photobioreactor is usually operated continuously throughout the period of microbial growth. The reservoir receives a continuous aeration to eliminate the oxygen produced during photosynthesis [17,81–83]. Continuous cultivation is important in commercial ventures to determine optimum nutrient concentration per unit time relative to the dilution rate or crop harvest in order to avoid wastage of resources. TPBR are distributed in four categories: serpentine

(Figure 2); manifold; helical; and fence arrangement with manifold photobioreactors [77], where the tubes may be positioned horizontally or vertically for maximal solar capture [7].

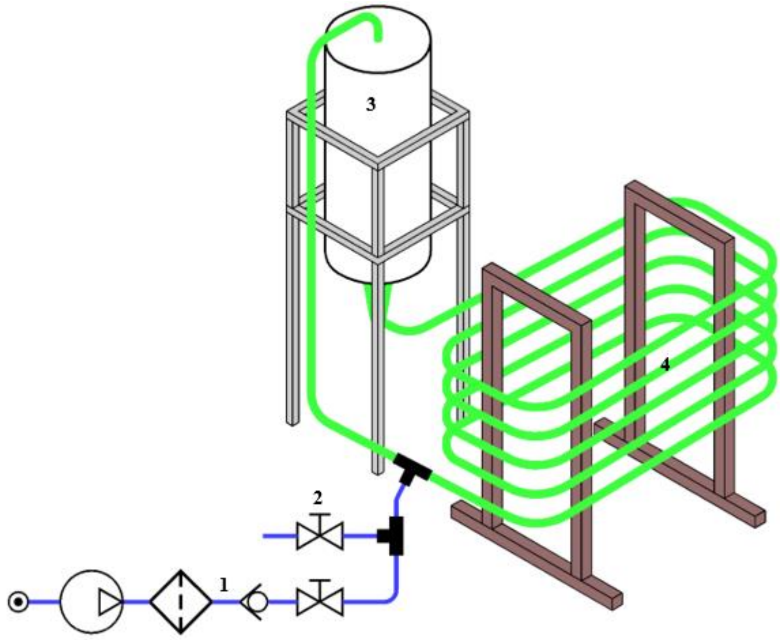

Figure 2. Scheme of tubular photobioreactor, serpentine category: (1) air system; (2) sampling system; (3) degasser; (4) glass tubes.

One example of cultivating microalgae in closed system was with *Botryococcus braunii* (variety A), in a batch photobioreactor for tertiary treatment and the accumulation of value-added components in biomass. The concentration of protein was 12.5%, and the concentration of total lipids was 17.85% [23]. These values are lower than those acquired in biomass of *Botryococcus braunii* produced in synthetic medium: 32.1~34.1% of protein and 32.6~36.9% of total lipids [84]. However, nitrogen and phosphorus were satisfactorily removed from medium.

3.3. Separation Methods for Harvesting Biomass from Tertiary Wastewater Treatment

The main steps for effective biomass production are cultivation, harvesting, and processing [85]. The tertiary treatment of wastewater can be a source of photosynthetic microorganism biomass, which could be used as fertilizer and/or source of fatty acids for biodiesel production.

After cultivation, an important factor to take into account is how to harvest biomass, considering the small size of the cells (in some species of algae) and the relatively diluted culture in some cases (<0.5 g L^{-1} in open photobioreactors and 1.5 g L^{-1} for tubular photobioreactors) [17]. Therefore, effective techniques are required that allow resourceful harvesting of biomass from culture [86], depending on the characteristics of culture [7].

Filtration, centrifugation, sedimentation, flocculation (organic and inorganic), and flotation, or a combination of these operations, are the methods for harvesting biomass [7,17,87].

3.3.1. Filtration

Filtration is a method for solid–liquid separation and, in some cases, it is the first operation that takes place for harvesting biomass. The principle is bringing together algae biomass into a screen of designed aperture [88]. When the volume of medium to filter is small, a vacuum filtration system is used, and depending on the size of the cells, microfiltration or ultrafiltration membranes can be used [7].

On the other hand, when the volume is large, rotary vacuum drum filters (RVDF) may be employed. Chamber filter press may also be used for harvesting microorganisms. These filters can be used in vacuum or pressure operation, possessing an advantage in continuous processes where sterility and containment are not severe. The material of these filters may be canvas, nylon, dacron, metal, or fiberglass [87].

3.3.2. Centrifugation

Centrifugation is another downstream operation, being useful in processes where it is necessary to maintain sterile conditions. It may be fast, efficient, and universal. However, it is not economically feasible in large-scale processes because it requires high energy rates [7]. Algal biomass is separated by a gravitational force greater than the force of gravity [88] using a centrifuge as a sedimentation tank [87]. The supernatant is removed by means of a tube, and solids are retained in the container (batch processing) or eliminated constantly or intermittently (continuous operation) [88]. The amount of biomass collected by sedimentation depends on the sedimentation rate, residence time of the biomass (can be higher depending on flow-rate reduction) and settling distance (may be reduced by reducing the flow rate). Three types of centrifuges are used depending on the size of the cell: tubular bowl, disc-stack bowl, and scroll-discharge decanter [87]. However, this process may have some limitations such as breakdown of cell structure, due to gravitational force, and large volumes of medium require more time and increase operational costs [89,90].

3.3.3. Sedimentation

Another process to collect biomass is gravity sedimentation. It is an operation employed for several species of algae. Although it is rudimentary, it has energy efficiency [7]. This technique allows the separation of a feed suspension into a more concentrated substratum and a clear supernatant and can be carried out by lamella separators and sedimentation tanks. Therefore, the lamella separators offer a greater area of sedimentation in comparison with the conventional thickeners owing to the plates' orientation. Microorganism suspension is continuously pumped, while the slurry is continuously eliminated. The energy required in this process is for pumping the slurry. Flocculants can be added to increase the separation of biomass and its sedimentation rate. These flocculants can also be increased to intensify the separation of microorganisms and the sedimentation rate. The separation of microorganisms using sedimentation tanks is an economic process. Density of microorganisms plays an essential role in the removal of solids [91].

3.3.4. Flocculation

Flocculation is a separation process using chemicals (flocculants) that allows the cells suspended in a cell culture to adhere to each other and form a floccule so that they sediment more easily. The flocculants used are divided into long-chain organic and inorganic coagulant categories. The microbial cells have a negative superficial charge, and the addition of metal salts allows the aggregation of them. This process is widely used in the industry to eliminate suspended solids [7,86,89,90].

Flocculation is more convenient when there are large quantities of medium to be treated through a biomass-harvesting process and can be applied to many species [17,91], although sometimes this method alone can be inefficient [91]. There are a variety of chemicals studied for flocculation, and among the most effective are ferric chloride, aluminum sulfate, ferric sulfate, some cationic polyacrylamide polymers (praestol) (zetag 63 and zetag 92), and cationic polyamines that produce complexes at optimum pH [88,91]. However, the disadvantage in the use of inorganic flocculants is that high concentrations are required, and this results in a biomass with aluminum or iron in its concentration [86]. Despite this, it has become the first choice in the wastewater treatment industries, where it is achieved by adding salts such as aluminum sulfate [17].

Chitosan has recently been studied as an organic flocculant that allows greater flocculation, permitting faster sedimentation and a purer supernatant. It is also non-toxic and biodegradable, which allows a reuse of the medium [86].

Furthermore, the biomass is sensitive to certain pH values of the culture medium, and some studies show how increasing the pH improves the efficiency of flocculation [86].

Sometimes, when there is a limitation of carbon or nitrogen or other environmental factors such as pH, dissolved oxygen, and the presence of some metal ions, a spontaneous aggregation of particles is generated, and this phenomenon is called auto-flocculation [7,88,91].

However, one disadvantage of the flocculation method is that after the biomass has been collected, it cannot be separated again into individual cells [90].

3.3.5. Flotation

Flotation can be considered as a more effective and advantageous process for sedimentation, and it is promising in algae with small-diameter cells at laboratory scale. Flotation is a physicochemical method, where charged bubbles (air or gas) are used by ozonation, interacting with the hydrophilic surfaces that are negatively charged in algae. At the end of the process, a flotation is created and eliminated. The stability of the flotation created depends on the contact area of the air or gas particles. There are three main floating techniques: air flotation, flotation by dissolved air, and electrolytic flotation. However, it can be an expensive process [7,91].

4. Benefits from the Cultivation of Photosynthetic Microorganisms

Photosynthetic microorganisms biotechnology, in addition to the above-mentioned advantages in the tertiary treatment, may also provide other benefits: (i) its metabolism allows the bio-fixation of carbon dioxide, helping to mitigate the greenhouse effect; and (ii) microbial biomass, obtained by different harvesting processes, may be employed for several purposes, as presented below.

4.1. CO_2 as Greenhouse Gas and Bio-Fixation

Anthropic activities may also provoke other environmental problems. It is well known that industrialization and other human activities release greenhouse gases (GHG) in the air, mainly CO_2, which substantially threatens environmental sustainability. For this reason, several strategies have been investigated for CO_2 fixation, especially employing biological processes. Photosynthetic microorganisms are the main candidates for performing CO_2 bio-fixation, and some species have a relative high range of CO_2 tolerance [11,92,93].

Bio-fixation of this gas represents a process with efficiency and economy mostly because of the photosynthetic capacity of the microorganism [94]. In addition, these microorganisms can convert CO_2, derived from the burning of organic material or metabolic pathways of industrial microorganisms, into biomass or a variety of compounds derived from it, such as fatty acids, pigments, vitamins, minerals, antioxidants, polysaccharides, and other interesting compounds, due to their bioactive activity. These may be applied in the production of cosmetics, food, and pharmaceutical products [95,96].

In this sense, Matsudo et al. [97] and Matsudo et al. [98] indicate that the cyanobacterium *Arthrospira platensis* can be continuously cultivated in a tubular photobioreactor for bio-fixation of CO_2 (including that released from alcoholic fermentation) to mitigate the greenhouse effect and, at the same time, remove urea from the medium and produce single-cell protein.

In such an application, the CO_2 is almost pure, and no treatment is necessary to purify it. However, there are cases in which this gas is accompanied by a substantial quantity of another gas, as occurs in biodigestion [99], where there is the methane, and methods for separation of the gases can be applied. The isolated CO_2 can be introduced in the culture medium in order to supply a carbon source as well as to maintain the pH of the medium of photosynthetic microorganisms. The separation by polymeric membranes has been used for this purpose. There are different shapes of membranes such as asymmetric (fibers wound in spiral) and hollow (although maintaining their efficiency of separation) [100]. The decarboxylation process allows the promoted thermal crosslinking to be effective for the membrane stabilization against plasticization. Therefore, a hollow membrane free of defects is essential for performance. In the study by Chen et al. [101], the resistance to plasticization was demonstrated by applying CO_2 pressures of 400 psi, which allowed us to conclude that these membranes are promising for the purification of natural gas. Another example of membranes is observed in the study by Wind et al. [102], where membrane-derived copolymers (6FDA-DAM: DABA and 6FDA-6FpDA: DABA) were used for separation of CO_2/CH_4 gas mixtures and mixtures of synthetic natural gas at 35 °C with feeding pressures up to 55 atm.

4.2. Biomass Uses

After microorganism growth with removal of the nutrients in wastewater, collected biomass can be used in value-added products. Depending on the microorganism species, whole or modified biomass can be used for animal feed, organic fertilizer, extraction of value-added products (carotenoids, pigments, vitamins, amino acids, polysaccharides, lipids), or production of biofuels [7,35,85].

Food products can be improved by adding the biomass of microorganisms, due to the biochemical composition. The carbohydrates present in microorganisms are represented by starch, glucose, sugar, and other polysaccharides, and the digestibility of these compounds is high, which is why the biomass can be used in integral powder in concentrates. Regarding total content of lipids present in the biomass, it varies in the range of 1–70% of dry weight, depending on the species. Lipids composition includes glycerol, sugars, esterified bases, and fatty acids [103–105]. They can be polar or neutral and are present in cell membrane components as phospholipids, glycolipids, tri-, di-, and monoacylglycerol, and carotenoids [93].

In the production of biofuel, several investigations have been carried out to produce biodiesel (transesterification of lipids), biomethane, bioethanol (fermentation of carbohydrates), biohydrogen, and biobutanol [7]. Among the advantages found in the production of biodiesel from photosynthetic microorganisms are the following: higher growth rates, short production times, smaller cultivation area in comparison with plants, high concentration and variety of lipids produced, and use of wastewater as culture medium. However, methodologies must be improved for the extraction of lipids, since they are complex and possess lower yield [7].

In the study performed by Neveux et al. [12], the biomass produced from *Oedogonium* sp. to generate biocrude oil, via reactions of hydrothermal liquefaction (HTL), showed a composition with carbon content of 38.5% of the dry weight in the eighth week. The study estimated that the biomass would produce between 26–27% of biocrude in dry weight [12].

However, the composition of the biomass can differ significantly in growth phases (logarithmic or stationary), in addition to variations in the concentration of the nutrient, salinity, temperature, pH, and light intensity [106]. Currently, some studies use methods such as genomics, lipidomics, proteomics, and metabolomics for the development of new strains with higher growth and lipid biosynthesis rates or the ability to produce and accumulate several high-value byproducts [7].

The emphasis on biotechnology for the production of microbial biomass is characterized by technology, product properties, yields, nutrition, toxicology, and economics [85]. Considering this, there are multiple purposes to wastewater treatment: recovering water for reuse (irrigation), mitigation of greenhouse gases (CO_2), and production of byproducts [85,92].

4.3. Benefit Analysis

Harvesting of biomass contributes 20 to 30% of total production costs. For this reason, an efficient harvesting of biomass indicates a high-quality treatment and cost-effective production [17,87]. Likewise, another key aspect is selecting the cultivation system, since it greatly affects the efficiency and cost–benefit ratio of a production process [82].

Ji et al. [36] showed that tertiary treatment of wastewater with microalgae and addition of CO_2 could significantly improve microorganism growth, resulting in higher biomass yield and larger productivity of lipids of *Ourococcus multisporus*, *Chlorella vulgaris* and *Scenedesmus obliquus*. Likewise, a decrease in the total concentrations of nitrogen and phosphorus (below their detection limits) was demonstrated after four days of culture, demonstrating that it is an environmentally sustainable method with a good cost–benefit ratio.

Moreover, cultivation of microalgae with artificial medium generates more residues, and additional costs for components can be eliminated with the use of wastewater [35].

Another benefit of tertiary wastewater treatment is the possibility of water reuse. Around the world, 181 km^3 of municipal wastewater must be treated per year, but only 13% of this treated effluent is reused [29]. Therefore, the volume of discarded water is very large.

Water reuse is important mainly when available water supply is overcommitted and it is not possible to supply increasing water demands in growing community. The reclaimed water may be appropriate for many non-potable applications, such as industrial cooling, cleaning water, and irrigation. The reuse of water also helps to protect the environment by reducing the quantity of treated effluent released to water bodies [107].

5. Conclusions

In this review, it was possible to observe how important performing tertiary treatment in wastewater is for removing nutrients coming from secondary treatment and, consequently, to mitigate eutrophication in waterbodies. This treatment may represent a crucial strategy in water management and reuse, mitigation of the greenhouse effect with the concomitant production of microbial biomass with potential application in fertilizer, and biofuels.

Taking into account that, in secondary wastewater, organic compounds are present in small amounts, the best strategy is to apply photosynthetic microorganisms, because they have the ability to use inorganic nitrogen and phosphorus for their growth, particularly those species already present in wastewater treatment plants.

Another important piece of information is that, in sewage treatment plants, it is possible to employ an anaerobic biodigester that produces methane, besides carbon dioxide. Burning of methane, after its separation from CO_2, which can occur due to using membrane separation, produces even more carbon dioxide that may be bio-fixed by photosynthetic microorganisms in tertiary treatment.

Several laboratory-scale experiments have been performed, although more effort needs to be addressed towards pilot or, mainly, full-scale projects.

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