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Review

Significance and Applications of the Microalgae *Chlamydomonas* and its Bacterial Consortia

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Abstract: The wide metabolic diversity of microalgae, their fast growth rates, and cost-effective production make these organisms highly promising resources for a variety of biotechnological applications, addressing critical needs in industry, agriculture, and medicine. The utilization of microalgae in consortia with bacteria is proving to be valuable in different biotechnological fields, including treating various types of wastewaters, producing biofertilizers, and extracting various products from their biomass. Monoculture of the microalgae *Chlamydomonas* has been a prominent research model for many years, extensively utilized in studying photosynthesis, sulfur and phosphorus metabolism, nitrogen metabolism, respiration, and flagella synthesis, among others. Recent research has increasingly recognized the potential of *Chlamydomonas*-bacteria consortia as a biotechnological tool for various applications. Bioremediation of wastewater using *Chlamydomonas,* and its bacterial consortia presents significant potential for the sustainable reduction of contaminants, while also facilitating resource recovery and valorization of microalgal biomass. Using *Chlamydomonas* and its bacterial consortia as biofertilizers can offers several benefits, such as enhancing crop yield, protecting crops, maintaining soil fertility and stability, aiding in CO_2 mitigation, and contributing to sustainable agriculture practices. *Chlamydomonas*-bacterial consortia play a significant role in the production of high-value products, particularly in biofuel and enhancing H_2 production. This review aims to achieve a comprehensive understanding of the potential of *Chlamydomonas* monoculture and its bacterial consortia, identifying current applications, and proposing new research and development directions to maximize its potential.

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1. Why *Chlamydomonas* and Why Its Consortia?

Microalgal cells are photosynthetic unicellular organisms that play a crucial role in global ecosystems, contributing to over half of the Earth's total photosynthetic activity and forming the backbone of the food chain [1]. Despite their prevalence in aquatic environments, microalgae thrive in various habitats, including the cold and irradiated poles, the subterranean environments of densely packed rhizospheres, and even within animal tissues in coral reefs [2]. Microalgae share a common evolutionary origin that can be traced back to a primary endosymbiotic event involving a cyanobacterium, which eventually evolved into the plastid. Subsequently, this plastid lineage expanded through secondary and tertiary endosymbiosis [3]. Microalgae play a crucial role in supporting ecosystems; however, they can also disrupt them through algal blooms, presenting substantial ecological, economic, and health risks [4]. Microalgae have demonstrate a wide range of metabolic capabilities, possessing various unique qualities that make them highly valuable for scientific studies [5]. Furthermore, the use of microalgae in diverse biotechnological applications has significantly increased in the last century. Emerging applications of microalgae include the production of biomaterials as alternatives to fossil-based materials, including biofertilizers,

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biostimulants, biopesticides, and energy sources like biodiesel, bioethanol, and biogas [6]. Microalgae are a source of bioactive compounds, including essential amino acids, polyunsaturated fatty acids, and antioxidants, which have been shown to positively impact nutrition and health [7]. Microalgae are considered a sustainable resource due to their ability to be employed in bioremediation processes, converting waste into valuable products within a circular economy model [8]. Consequently, microalgae are gaining global attention for their significant ecological and economic importance.

Chlamydomonas is a genus of single-celled biflagellated green microalgae that are commonly found in freshwater, but some species can also be found in habitats as diverse as saltwater, soil, and snow. Taxonomically, the genus *Chlamydomonas* comprises more than 500 species [9]. *Chlamydomonas reinhardtii* is undoubtedly the most studied species of *Chlamydomonas* genus. *C. reinhardtii* was isolated in 1945 from the soil of a potato field in Massachusetts, USA [10]. Since then, it has been utilized to investigate a range of research topics including photosynthesis, respiration, sulfur and phosphorus metabolism, nitrogen metabolism, amino acids and metals metabolism, biosynthetic pathways of starch, carotenoids, lipids, glycerolipids, heme groups, and chlorophyll. Additionally, it has been used to study other fundamental aspects such as the function of chaperones, proteases, flagella biogenesis, thioredoxins, and responses to various stress conditions [9]. The progress in different gene editing techniques like CRISPR-Cas9 in *Chlamydomonas* represent significant progress in addressing fundamental research inquiries and biotechnological applications [11]. All this demonstrates that *Chlamydomonas* has been intensively used in basic research; however, the practical application of *Chlamydomonas* for certain biotechnological purposes is not as numerous. Nevertheless, there has been a noticeable increasing interest for its potential biotech use in recent years [12].

Microalgae have been isolated from their natural habitats, with many proving challenging to cultivate axenically. This reliance on other microorganisms likely stems from the long-term co-evolution between microalgae and their beneficial microbe [13]. In this sense, microalgae have the capability to establish symbiotic associations with a diverse array of organisms, including bacteria, fungi, plants, and animals [14]. The term "phycosphere" refers to the area where algal exudates affect neighboring microorganisms [15]. In mutualistic relationships, microalgae typically provide their partner(s) with fixed carbon and oxygen in exchange for crucial nutrients and essential molecules like CO_2, different vitamins, and nitrogen [16]. Moreover, a wide range of molecules can be secreted, detected, and utilized by the interacting partners to stablish a complex molecular dialogue. The comprehension of the complexity and dynamics of many of these symbiotic relationships is still in its early stages [17].

Since its initial discovery, there has been a significant rise in the number of studies exploring the symbiotic relationships between *Chlamydomonas* and bacteria, mainly focusing on fundamental research [18]. However, as far as we know, a single review compiling the potential of *Chlamydomonas*-bacteria consortia in crucial biotechnological tasks such as wastewater treatment, bioremediation, biofertilizer production, biomass valorization, and bio-product production has never been undertaken. Therefore, we summarize and categorize these reports here with the aim of highlighting the potential of *Chlamydomonas*-bacteria consortia to fulfill these tasks.

2. Wastewater Types and Composition

Water can be polluted by various streams, each with varying degrees of intensity. Wastewater can originate from various sources, including households, commercial sites, residential communities, industrial areas, surface runoff, recreational sites, institutional facilities, and agricultural areas (Figure 1). The composition of wastewater varies significantly depending on the source and industrial processes involved, comprising a diverse mixture of organic and inorganic compounds, as well as synthetic substances, with carbohydrates, fats, sugars, and amino acids being among the primary contaminants [19]. Persistent organic pollutants include chlorinated and aromatic compounds, such as organochlorine pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls [20]. Inorganic constituents found in wastewater include substances such as sodium, calcium, nitrates, potassium, magnesium, sulfur, bicarbonate, arsenic, heavy metals, chlorides, phosphates, and non-metallic salts [21]. Municipal wastewater contains organic matter, nutrients, pathogens, and chemicals. Agricultural wastewater includes organic matter, pesticides, herbicides, and fertilizers. Dairy processing plant wastewater typically shows a very acidic pH, high concentrations of organic compounds, as well as high levels of organic and inorganic phosphate and nitrogen, fats, oils, grease, and detergents. Industrial wastewater may contain heavy metals, organic chemicals, and oils. Synthetic wastewater is a lab-created mimic used for research purposes [22]. Medical wastewater contains antibiotics and antibiotic-resistant genes, organic pollutants (e.g. phenol and its derivatives), refractory micropollutants (e.g., triclosan, ibuprofen, diclofenac) and toxic chemicals (e.g., cyanids, chlorinated lignin, dyes) [23]. Consequently, each type demands specific treatment due to its unique contaminants.

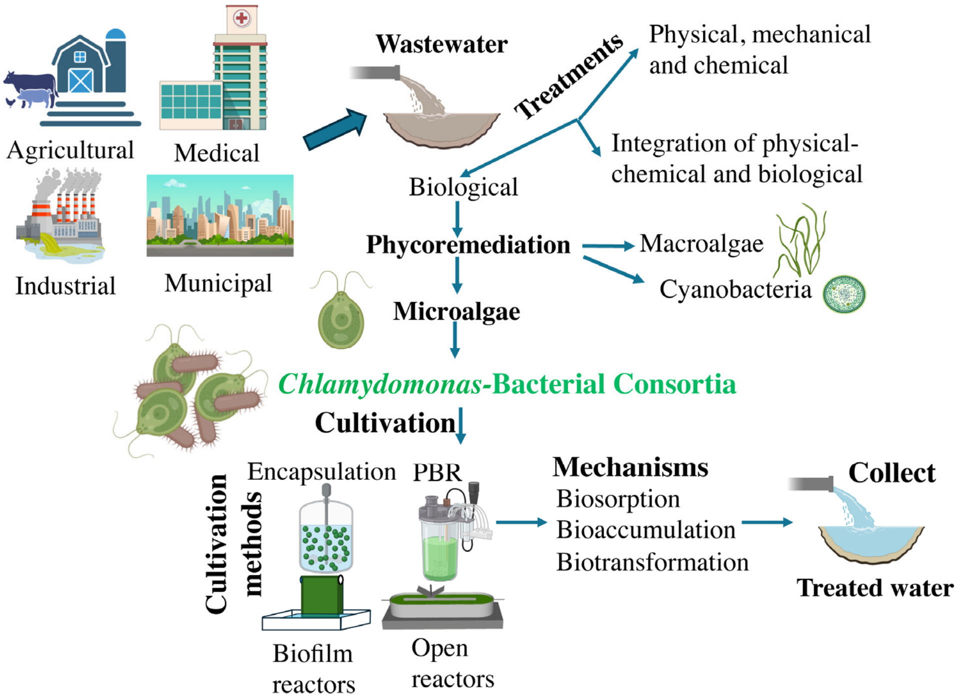

Figure 1. Schematic overview of the wastewater treatment process using *Chlamydomonas*-bacterial consortia. Wastewater treatments from various sources such as agricultural, industrial, medical, and municipal activities involve, physical, chemical, mechanical and biological processes including phytoremediation with microalgae. Consortia between *Chlamydomonas* and bacteria can be cultivated in open reactors, photobioreactors (PBR), biofilms or being encapsulated in beads. The final stage is the harvest of the biomass which results in bioremediated treated water.

3. Wastewater Treatment Methods

As anthropogenic activities increase, leading to more complex wastewater compositions, it becomes essential to develop wastewater treatment methods that are easy to deploy, effective, and eco-friendly to mitigate water pollution. Traditional wastewater treatment methods involve physical, mechanical, chemical, and biological approaches (Figure 1) [24]. Physical methods include sedimentation, screening, and skimming; mechanical methods use filtration techniques; chemical methods involve processes such as adsorption, neutralization, disinfection, precipitation, and ion exchange [25]; and biological methods use microorganisms to degrade pollutants [26]. Integrating physical-chemical and biological methods offers a sustainable solution by reducing energy and chemical usage, cutting costs, and minimizing environmental impact [27].

Among the biological methods, phycoremediation, derived from the Greek word for algae 'phyco,' is an eco-friendly approach that employs various algae varieties such as microalgae, macroalgae, or cyanobacteria, to cleanse wastewater by eliminating pollutants or extracting products from it (Figure 1). Notable applications of phycoremediation include eliminating nutrients and xenobiotic compounds, reducing excessive nutrients in organic-rich effluents, mitigating CO_2, treating effluents with heavy metal ions, and using algae as biosensors to monitor potentially harmful

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substances [28]. Phycoremediation not only helps in the removal of pollutants but also results in the production of algal biomass, which can be utilized for the production of various valuable products such as food, fertilizers, pharmaceuticals, and biofuels [29]. *Chlamydomonas* and other algae have been extensively utilized in wastewater treatment [30].

4. Microalgae Cultivation Methods

For the correct and efficient use of *Chlamydomonas*-bacteria cocultivation for any biotechnological purpose, it is essential to optimize the cultivation conditions and choose the appropriate cultivation method. A perfect microalgal cultivation system should be easy to manage, cost-effective to build, have sufficient light exposure, facilitate efficient gas-liquid transfer, and demonstrate minimal contamination risk. Photobioreactors have emerged as a potentially sustainable method for removing wastewater pollutants while producing microalgal biomass [31]. Typically, reactor configurations used in microalgal-bacteria systems include suspended systems (open reactors and closed reactors) and attached systems (such as biofilm reactors and encapsulated microalgae). Therefore, we will now present the main cultivation methods available for use with microalgae, highlighting the specific characteristics for microalgae-bacteria cocultivation and providing examples of their biotechnological potential in each case.

4.1. Open Reactors

Open reactors include natural ponds and lakes, as well as specially designed high-rate algal ponds (HRAPs) that are tanks or lagoons featuring a paddle wheel that circulates wastewater. HRAPs can be an cost-effective and environmentally friendly approach method for treating wastewater, as microalgae efficiently absorb nutrients such as nitrogen and phosphorus, as well as help remove organic and inorganic contaminants [32]. Unlike indoor laboratory-scale cultivation, outdoor algal cultivation employing HRAPs are heavily influenced by various uncontrollable environmental factors, such as seasonal changes and weather conditions [33]. The following are the generally accepted design parameters for algae-bacteria HRAPs: depths typically range from 0.2 to 1 m, depending on wastewater clarity for light penetration; horizontal water velocities between 0.09 and 0.3 m/s are recommended to ensure good mixing; hydraulic residence times (HRTs) range from 3 to 15 days; and HRAP areas vary from 1,000 to 50,000 m² [34,35]. Numerous studies have reported the use of HRAP in wastewater treatment, primarily focusing on genera such as *Scenedesmus* and *Chlorella* [36]. It has been reported that NH_4^+ was primarily removed through nitrification, followed by assimilation and denitrification, when anaerobically digested swine slurry was treated in algae-bacteria in HRAP [37]. However, very few records exist of applying HRAP with *Chlamydomonas*. In a pilot-scale experimental wastewater treatment using HRAP with *Chlamydomonas* sp., a reduction of approximately 90% in biochemical oxygen demand, 65% in chemical oxygen demand (COD), 20% in total phosphorus, and 46% in total nitrogen was reported [38]. A study on the bioremediation of piggery wastewater using HRAP with *Chlamydomonas* sp. revealed an average COD and total nitrogen removal efficiencies of 76% and 88%, respectively [39]. In another study using HRAP with *Chlamydomonas sp*. for treating municipal wastewater, the average reductions of volatile suspended solids, biochemical oxygen demand, and total nitrogen were 63%, 98%, and 76%, respectively [40].

4.2. Closed Photobioreactors

Closed photobioreactors (PBR) are enclosed systems utilized for the cultivation of microalgae and other phototrophic microorganisms. PBRs can be operated in any open space and provide excellent control over culture conditions with minimal risk of contamination. However, PBRs are costlier to build and necessitate the use of transparent materials like glass and acrylic for their construction. PBRs are characterized by a narrow light path and a large illuminated surface-to-volume ratio, which maximizes light energy capture and conversion [41]. PBR modules can be organized in various configurations, such as horizontal, inclined, vertical, or spiral arrangements. Flat plate and tubular photobioreactors are prevalent designs because of their extensive illuminated

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surfaces [42]. There are numerous studies utilizing PBR with *Chlamydomonas*, with many focusing on basic science research. Next, we will present some recent studies that have led PBR applications with significant biotechnological potential. The utilization of the phototactic response of *C. reinhardtii* to induce biomixing in PBR has been investigated. By exploiting this phototactic mechanism, *C. reinhardtii* can be stimulated to swim in opposite directions, providing mixing and ensuring access to nutrients without additional energetic costs. This approach could potentially replace mechanical agitation in certain circumstances, enhancing energy efficiency [43]. The multi-scale modular PBR, Antares I, has been tested with *C. reinhardtii* and has shown improved biomass production compared to traditional flask systems. With Antares I, the estimated doubling time for *Chlamydomonas* culture was almost half of that reported for culture using similar media and light conditions [44]. A drawback of monoculture microalgae in PBRs is that dissolved oxygen accumulation can reach up to 400% of air saturation, which is detrimental to microalgae growth [45]. However, the high oxygen consumption by bacteria in co-cultured algal-bacteria systems has been shown to reduce these negative effects by decreasing the amount of dissolved oxygen available [46]. When municipal wastewater was treated in a stirred algal-bacterial PBR, it was estimated that 40-53% and 17-20% of NH_3 removal were attributed to bacterial assimilation and nitrification, respectively [47].

4.3. Biofilm Reactors

In biofilm reactors, microalgae are immobilized on a surface that acts as a support, resulting in the formation of a continuous layer. This approach provides benefits such as increased cell concentration per volume of medium, simplified harvesting, and decreased or minimal presence of cells in the effluent [48]. Algal-bacteria biofilms can be categorized as either stationary or mobile, based on the movement of the supporting materials. One of the primary advantages of microalgal biofilm reactors compared to other techniques is that the extraction and dewatering of algae cells from biofilms are simplified. This is due to the ease with which the attached cells can be separated from their surrounding growth medium, eliminating the need for costly separation methods like filtration, centrifugation, flocculation, or settling/floating the biomass. In these systems, the biomass needs to be collected by removing it from the support medium through scraping [49]. So far, the basic principles governing the colonization of surfaces by motile, photosynthetic microorganisms remain largely unexplored. The regulation of the adhesion properties of *Chlamydomonas* could significantly enhance the efficiency of biofilm reactors by controlling surface colonization and biofilm formation. Research has shown that the surface adhesion of *C. reinhardtii* is flagella-mediated and largely substrate-independent, enabling it to adhere to any type of surface [50]. However, it has been shown that the biofilm adhesion of *C. reinhardtii* is controlled by the type of light, being activated in blue light and deactivated under red light [51]. The phosphate-hyperaccumulating strain *Chlamydomonas pulvinata* TCF-48g has been tested using the biofilm system to recover phosphate from municipal wastewater, demonstrating a high phosphorus removal rate of 70%. [52]. With *Chlamydomonas* sp. JSC4, this technique has been successfully optimized in the removal of phosphorus, nitrogen, and copper from swine wastewater [53].

In biofilm reactors, the medium passes through the bioreactor while the biomass remains attached to a stationary support medium; therefore, the residence time of the algae and bacteria is much longer. This allows algal-bacteria biofilm reactors to be operated at higher organic loading rates and shorter hydraulic retention times than suspended growth systems because communities with slow growth rates are retained in the reactor. Numerous experiments, both small-scale and large-scale, have been conducted using algal-bacteria biofilm reactors for wastewater treatment [54]. Algal-bacteria biofilm reactors can be flushed with CO_2 to enhance biomass productivity or integrated with other treatment processes to enhance the efficiency of wastewater treatment. A biomass productivity of 60 g/m^2/day has been reported in algal-bacteria biofilm reactors using synthetic wastewater and purged with 0.5% CO_2 enriched air [55]. Utilizing an algal-bacteria biofilm reactor in conjunction with additional reactors offers the potential to refine secondary wastewater effluent, resulting in a further reduction of total suspended solids concentrations to below 0.5 mg/L [56].

4.4. Encapsulation

The encapsulation of microalgae involves trapping the microalgae within coating materials, resulting in the formation of beads. This process offers various advantages, such as promoting controlled release, protecting the formation of bioactive compounds, enhancing bioavailability, and improving solubility [57]. Various materials such as alginate, chitosan, carrageenan, and polyvinyl, have been used for the immobilization of microalgae [58]. With *Chlamydomonas*, alginate has been the most extensively used for its low cost, biocompatibility, transparency, and permeability, which facilitate the diffusion of nutrients and light [59]. Additionally, the preparation of alginate beads is a rapid and straightforward process that can be easily scaled up [60]. The encapsulation of *C. reinhardtii* in alginate has been successfully carried out to remove various types of contaminants such as nitrogen, phosphorus, cadmium, lead, and mercury [61] or even phenol [62]. In *C. reinhardtii* alginate beads, pore size has been shown to be critical for contaminant removal, with the highest removal efficiency obtained with a gel bead pore size of 3.5 mm [63]. Silica hydrogels have been utilized to entrap *C. reinhardtii* cells, offering some advantages over alginate such as higher transparency and greater stability against ions and microbial attacks. Silica hydrogels encapsulating *Chlamydomonas* have demonstrated potential for applications such as hydrogen production [64]. One drawback of alginate encapsulation is its high porosity, which can lead to the release of large molecules. Nevertheless, it has been noted in *C. reinhardtii* that the combination of alginate and silica to create hybrid beads can offer enhanced properties that surpass this limitation [65]. Single-cell encapsulation involves coating individual cells with metal-phenolic networks to create a mechanical barrier. With microalgae, this method was first employed with *C. reinhardtii*, finding that this approach helped delay the proliferation of the coated cells and effectively promoted flocculation [66]. One advantage of encapsulation is that it can be performed with multiple organisms simultaneously, a process known as co-immobilization. The co-immobilization of *C. reinhardtii* with the acetate-producing cyanobacteria *Synechococcus* sp. PCC 7002 increases the biomass content [67]. Co-immobilization of *Chlamydomonas* and the cyanobacteria *Lyngbya* sp. on silica hydrogel exhibited a 92.5% removal of Pb^{2+} from wastewater [68]. Studies have also shown that in the co-immobilization of *Chlamydomonas* with the nitrogen-fixing bacterium *Azospirillum brasilense*, there is a mutualistic relationship supported by the exchange of tryptophan and indole-3-acetic acid (IAA), respectively. This relationship increases microalgal CO_2 fixation and biomass production [69].

5. Main Mechanisms and Molecules Bioremediated by *Chlamydomonas*

Chlamydomonas has demonstrated remarkable capacity and diversity in bioremediating various molecules. Next, we will outline the different mechanisms and types of molecules that can be bioremediated by microalgae, particularly *Chlamydomonas*. Microalgae have the capacity to absorb and degrade pollutants like heavy metals, hydrocarbons, and pesticides using mechanisms such as biosorption, bioaccumulation, and biotransformation [70] (Figure 1).

5.1. Biosorption

Biosorption is a passive process where microalgae act as a biological sorbent to bind and concentrate pollutants. Microalgae utilize their cell wall and various chemical groups to attract and retain metallic and organic contaminants. The pollutants adhere to the algal membrane and are separated due to the presence of receptors that can bind and attract them [71]. Biosorption is indeed a well-studied mechanism for removing heavy metals from wastewater. *C. reinhardtii* has demonstrated a high capacity to remove through biosorption, arsenic [72], copper, boron and manganese [73], nickel [74], uranium [75], zinc and cadmium [76].

5.2. Bioaccumulation

Microalgae can eliminate contaminants through the process known as bioaccumulation. The difference between biosorption and bioaccumulation processes lies in the fact that biosorption is a passive process in which microorganisms utilize their cellular structure to trap pollutants, while

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bioaccumulation is an active process characterized by the buildup of pollutants in the biomass of microalgae, achieved through either accumulation or uptake into intracellular spaces [77]. *C. reinhardtii* has been shown to bioaccumulated several compounds as o-nitrophenol [78], Prometryne a herbicide [79], and *Chlamydomonas mexicana* carbamazepine, an antiepileptic agent [80].

5.3. Biotransformation

Biotransformation involves the degradation of pollutants, either within or outside cells, aided by enzymes [81]. While biosorption and bioaccumulation do not raise significant concerns, biotransformation presents greater challenges due to the potential for its byproducts to be more toxic than the original compounds. Some of the pollutants removed by biotransformation by *C. reinhardtii* include polystyrene [82], polycyclic aromatic hydrocarbons such as benz(a)anthracene [83], organophosphorus pesticide such as trichlorfon [84], microplastics as bisphenol A [85], anti-inflammatory as ibuprofen [86], and antibiotics as sulfadiazine [87]. Genetically modified *C. reinhardtii* expressing the cyanase gene from *Synechococcus elongatus* showed the ability to remediate high levels of potassium cyanide, up to 150 mg/L [88]. These findings underscore the ability of *Chlamydomonas* to eliminate different substances, highlighting their role in environmental processes and potential applications in bioremediation.

6. ***Chlamydomonas*-Bacterial Consortia for Bioremediation**

The use of microalgae-bacteria consortia in bioremediation is known as a promising approach for wastewater treatment [89]. These consortia utilize the synergistic relationship between microalgae and bacteria to efficiently degrade organic matter, remove inorganic compounds, enhance biomass production, or improve influent quality, among other benefits, [90]. In fact, the use of microalgae-bacteria consortia can provide several advantages over the use of just microalgae monocultures for the bioremediation treatment of different wastewater such as improve the nutrient or antibiotics removal as well as to reduce the contamination risk. The combined metabolic activities of microalgae and bacteria reduce the risk of contamination compared to microalgae monocultures, as the diverse microbial community is more resilient to environmental changes and potential invaders [91]. A key interaction between microalgae and bacteria involves the exchange of CO_2 and O_2. Aerobic bacteria consume the oxygen generated by algal photosynthesis and, in turn, produce CO_2 which supports algal growth. The bacteria can highly contribute to break down complex organic matter, making it more readily available for microalgal uptake [92]. Microalgae-bacteria consortia have shown superior performance in removing veterinary antibiotics from synthetic wastewater and swine wastewater in pilot-scale photobioreactors [93].

While there are numerous studies supporting the use of microalgae-bacteria consortia in different wastewater bioremediation [94], the use of *Chlamydomonas*-bacteria based consortia is quite unexplored topic with promising results. Different types of bacteria have shown the ability to enhance the potential of *Chlamydomonas* to phycoremediate. The potential of *C. reinhardtii* in consortia with three different bacterial strains (*Stenotrophomonas maltophilia*, *Microbacterium paraoxydans*, and *Paenibacillus lactis*) for bioremediating wastewater contaminated with phenol has been investigated. The results demonstrate that the consortium of *M. paraoxydans* and *C. reinhardtii* was very effective in phenol removal due to the synergistic interactions between the microalgae and bacteria, which enhance algal growth [95]. Furthermore, a cooperative consortium between *C. reinhardtii* and the bacterium *Methylobacterium oryzae* was found to lead an increased biomass generation and inorganic nitrogen removal when grown in urban wastewater [96]. The collaboration between microalgae and bacteria in wastewater treatment significantly enhances the efficiency of nitrogen removal. Bacteria play crucial roles in the nitrification and denitrification processes essential for completely eliminating nitrogen as nitrogen gas, and both algae and bacteria also contribute to nitrogen removal through assimilation [97]. As mentioned earlier, under illumination, microalgae produce oxygen, which aids in bacterial nitrification while reducing the energy required for culture aeration. *C. reinhardtii* in consortia with bacteria has been shown to remove nitrogen species from municipal wastewater, achieving up to 80% removal of dissolved organic nitrogen (DON) [98]. In a study using animal

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wastewater collected from two different sources, an animal feedlot wastewater storage tank and a sheep wastewater storage lagoon, the results showed that between 36-79% of initial DON was eliminated by the *Chlamydomonas*-bacteria consortia [99]. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* in consortia with *Chlamydomonas* have been shown to reduce the contamination of sediments containing heavy metals (Cu, Pb, Zn, Mn, Cd, As) [100]. The co-culture of *C. reinhardtii* with different bacterial strains has allowed the identification of bacterial species such as *Sphingobium yanoikuyae*, capable of degrading hydrocarbons and aromatic compounds [101], *Acidovorax sp. A16OP12*, which can utilize common environmental pollutants as a carbon source [102], and *Microbacterium sp.*, capable of removing heavy metals and antibiotics [103].

7. *Chlamydomonas*-Bacterial Consortia for Biomass and Bio-Product Generation

7.1. Biomass

Microalgae biomass is highly valued in a bio-based economy, being utilized for the sustainable production of various products such as fuel, food, energy, pharmaceuticals, among others [104]. Microalgae have various advantages in biomass production; they are more productive per unit of land area than any plant system, do not compete for arable lands, and can be cultivated throughout the year [105]. Microalgae biomass is a versatile resource that offers a wide range of applications through the extraction of specific compounds for various purposes. The composition of biomass, including proteins, lipids, and carbohydrates, as well as active compounds, is significantly influenced by the strains of microalgae and the cultivation conditions they are exposed to [106]. Various approaches have been explored to obtain microalgae biomass enriched in specific biomolecules. Studies on increasing biomass production through the co-cultivation of *Chlamydomonas* and bacteria have shown promising results. Next, we will present studies in which an increase in biomass production is achieved through the interaction of bacteria and *Chlamydomonas*. The *Chlamydomonas* vitamin B12 auxotroph strain was able to increase its biomass when co-cultured with B12-producing bacteria, including the rhizobium *Mesorhizobium loti* or even an *E. coli* strain engineered to produce and release B12 [107]. *Methylobacterium* spp. have excellent biotechnological potential in agriculture because they produce phytohormones, promote plant growth through N_2 fixation, and provide protection against pathogens and pollutants [108]. The cocultivation of *Mesorhizobium oryzae* and *C. reinhardtii* in ethanol-containing media significantly increases biomass production, with a potential increase of up to 700%. The crucial metabolic aspect of this association depended on the bacterial conversion of ethanol into acetate, which supported *C. reinhardtii* heterotrophic growth [96]. *Methylobacterium aquaticum* has been shown to enhance the *C. reinhardtii* biomass production in a proline-rich medium by transferring a portion of the NH_4^+ obtained through its metabolization to the alga. In turn, *C. reinhardtii* donates part of its fixed carbon in the form of glycerol to *M. aquaticum*, which uses it as its carbon source [109].

Diazotrophs are microorganisms that have the capability to convert atmospheric nitrogen into bioavailable forms like ammonia. The microalgae, including *Chlamydomonas*, have shown notable interactions with various types of nitrogen-fixing organisms [110]. *Azotobacter* spp., which are free-living symbiotic diazotrophs, have been widely employed as biofertilizers, effectively boosting the yields of various crop plants [111]. Different *Azotobacter* species, including *A. chroococcum, A. beljerinckii, A. agilis*, and *A. vinelandii*, have been demonstrated to enhance *C. reinhardtii* biomass production through the transfer of a portion of the fixed nitrogen to *Chlamydomonas* [112]. On the other hand, researchers have also observed that *C. reinhardtii* can support *Azotobacter* growth by transferring some of the carbon fixed through photosynthesis; however, the specific compounds involved in this process remain unidentified [113]. This *Chlamydomonas-Azotobacter* mutualism could have great biotechnological importance, as it would allow for the production of a large amount of biomass using only N_2 and CO_2 from the air, without the need to add organic nitrogen and carbon sources, which would decrease the economic profitability of the process.

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7.2. Biofuels

Biofuels originate from renewable biological sources such as plants or plant-derived materials and include biodiesel, bioethanol, biogas, and biohydrogen. First-generation biofuels are made from food crops, second-generation biofuels come from non-food sources like waste and third-generation biofuels are produced from sources that do not compete with arable land. Biofuels produced from algae are third-generation biofuels [114]. These biofuels can be produced through transesterification, photosynthesis-mediated microbial fuel production, and other thermochemical and biochemical conversions [115]. Several approaches have been undertaken to increase biofuel production through the co-cultivation of microalgae and bacteria [116], with studies mainly focusing on biohydrogen production in *Chlamydomonas*.

7.2.1. Biohydrogen

Biohydrogen is the term used to refer to hydrogen produced by living organisms such as bacteria, cyanobacteria, and algae [117]. The first indications suggesting that *Chlamydomonas* had the capability to produce H_2 were noted in experiments involving *C. moewusii* under anaerobic conditions [118]. In biological processes, hydrogenases are the enzymes responsible for hydrogen production [119]. *Chlamydomonas* has two distinct hydrogenases that have been the subject of extensive research, with the primary goal of exploring ways to enhance the efficiency of hydrogen production [120]. Hydrogenases can utilize two processes to obtain the energy for the reduction of protons into H_2: they can either use the energy from light, a process known as biophotolysis, or oxidize organic compounds, such as starch, a process known as dark fermentation. The primary challenge in utilizing *C. reinhardtii* for hydrogen production is the rapid inactivation of both hydrogenases by oxygen, especially given that oxygen is produced during photosynthesis. Therefore, with *C. reinhardtii*, the first evidence of its capacity to produce H_2 was reported under anaerobic conditions [121]. The use of sulfur-deprived *C. reinhardtii* was the first successful strategy demonstrating significant and consistent hydrogen production under aerobic conditions [122]. This occurs because the lack of sulfur inhibits protein synthesis, consequently disrupting photosynthesis and the generation of oxygen.

Chlamydomonas-bacteria consortia have opened a new window to improve hydrogen production. Several studies have successfully employed different consortia between *C. reinhardtii* and various bacterial partners, and analyzed the factors behind the improved hydrogen production [123]. One of the main reasons why the co-cultivation of *Chlamydomonas* and bacteria enhances hydrogen production is because the bacteria consume oxygen, thus preventing the inhibition of hydrogenase. This has been demonstrated in the co-cultivation of *Chlamydomonas* with *A. chroococcum* [124] or with *Bradyrhizobium japonicum* [125]. In the co-culture of *Chlamydomonas* with *P. putida, P. stutzeri, Rhizobium etli,* and *E. coli*, an increase in *Chlamydomonas* hydrogen production was also observed. This enhancement was attributed not only to the bacterial consumption of oxygen but also to the bacterial production of acetic acid from sugars, which *Chlamydomonas* utilizes as a carbon source [126,127]. In the coculture of *C. reinhardtii* and *Methylobacterium oryzae*, the improved hydrogen capacity of *Chlamydomonas* was due to the ability of *M. oryzae* to oxidize ethanol to acetate , which supports the heterotrophic growth of *Chlamydomonas* [96]. In the co-cultivation of *C. reinhardtii* and *Mesorhizobium sangaii*, the addition of various nitrogen compounds, such as $NaNO_2$, $NaNO_3$, and NH_4Cl, led to enhanced photobiological hydrogen generation compared to monocultures. Specifically, the addition of 3 g/L $NaNO_2$ resulted in the maximum H_2 production of 226.98 µmol/mg chlorophyll, which was 5.2 times higher than that of the pure algal culture [128].

A very promising approach of *Chlamydomonas*-bacterial consortia is to achieve hydrogen production from wastewater (Figure 2). Although producing hydrogen from wastewater is very challenging, research on hydrogen production from waste degradation presents an intriguing avenue with significant scale-up potential. Considering that *Chlamydomonas* serves as a model microalga for biohydrogen production, and bacteria contribute to enhancing hydrogen generation, it is logical to explore the capacity of *Chlamydomonas*-bacteria consortia for biohydrogen production alongside wastewater bioremediation [129]. In fact, the biohydrogen production by three microalgae, including *C. reinhardtii*, has led to a novel approach for autotrophic denitrification using hydrogen-consuming

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denitrifiers [130]. Nitrate contamination in drinking water is a significant environmental concern. Autotrophic denitrification, which converts nitrate into harmless gases using microorganisms, is a promising alternative but an electron donor is needed to sustain this process. In this sense, the hydrogen produced by *Chlamydomonas*, in co-culture with hydrogen-consuming denitrifiers, serves as an electron donor, providing a sustainable, innovative, and eco-friendly solution for addressing nitrate contamination [130]. Mixed culture of *Chlamydomonas-Rhizobium* consortia has also been shown for coupled hydrogen and biogas production [131]. After the hydrogen production stage, the *Chlamydomonas-Rhizobium* biomass was used for biogas generation in the second stage of the process. This study demonstrates that the *Chlamydomonas-Rhizobium* consortium can produce hydrogen without the need for sulphur deprivation, which is typically required for biohydrogen production by *Chlamydomonas* monoculture.

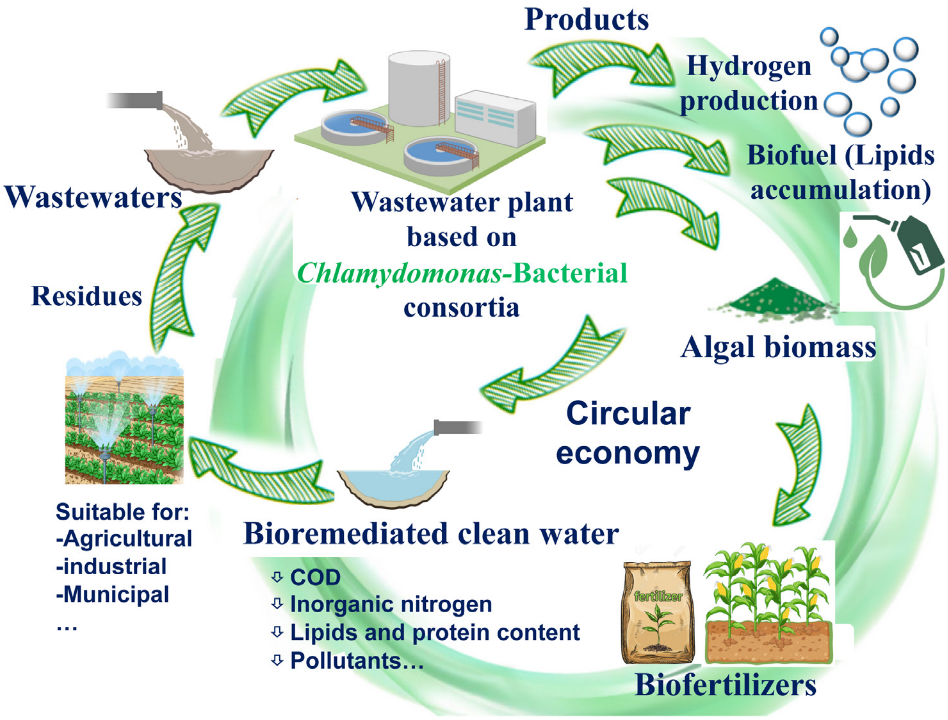

Figure 2. Schematic overview of a hypothetical model based on the concept of a circular economy using *Chlamydomonas*-bacteria consortia in wastewater treatment plants. Different wastewater sources are treated in wastewater treatment plants utilizing *Chlamydomonas*-bacteria consortia. This treatment results in algal biomass, which can be converted into bio-hydrogen production, biofuel through lipid accumulation and biofertilizers. The clean, bioremediated water produced exhibits reduced COD (Chemical Oxygen Demand), low inorganic nitrogen levels, and low lipid and protein content, rendering it suitable for reuse in various applications, including the irrigation of agricultural crops, among others.

7.2.2. Lipids

Triacylglycerols (TAG) are crucial lipids in microalgae for biofuel production. Oleaginous microalgae, rich in TAG, can be converted into biodiesel through transesterification, a process that transforms TAG into fatty acid methyl esters (FAME), the key components of biodiesel [105]. Given that biodiesel production is closely linked to the quantity of lipids and TAGs, various strategies have been explored to enhance their production in *Chlamydomonas-bacterial* consortia. High lipid content was found when a consortium of *Chlamydomonas* and cyanobacteria was cultured in wastewater from a dairy farm. The consortium was found to be capable of removing more than 98% of nutrients from wastewater, reaching a high biomass production and algal lipid content which could be converted into biodiesel [132]. It has been observed that immobilizing a co-culture of *C. reinhardtii* with the acetate-producing cyanobacteria *Synechococcus* sp. PCC 7002 increases the microalgal lipid yield [67].

The addition of *A. chroococcum* to *C. reinhardtii* culture significantly boosted lipid accumulation and productivity compared to the traditional nitrogen deprivation condition for increasing lipid accumulation, making it an efficient and economical strategy for enhancing lipid production [133]. Co-culturing *Chlamydomonas* sp. in the presence of the floc-forming bacterium *Bacillus infantis* showed that the co-cultures had higher microalgae biomass and lipid content compared to the axenic culture [134]. The co-culture of *C. reinhardtii* in municipal and swine wastewater effluents exhibited enhanced algal growth, biomass production, and lipid accumulation when indigenous bacteria were present [135].

7.3. Biofertilizers

Microalgae serve as biofertilizers and biostimulants, enhancing crop growth and enriching soil nutrients, which in turn reduces the need for chemical fertilizers and supports sustainable agriculture [136]. However, despite their abundance in natural soil ecosystems, *Chlamydomonas* species have been largely overlooked and underutilized in agricultural practices. In this context, lyophilized powders obtained from *C. reinhardtii* have been found to have a beneficial impact on maize plant growth, producing bioactive substances that act as biostimulants, improving plant growth, yield, quality, and crop performance [137]. It has been shown that *Chlamydomonas sajao* can enhance soil physical characteristics, such as aggregation and stability, thereby aiding in the improvement of soil structure and the retention of nutrients [138]. Research investigating the impact of *Chlamydomonas applanata* M9V as a biofertilizer on wheat growth revealed that both live and dead forms of the microalgae performed even better than a specific quantity of chemical fertilizer in terms of fresh weight, plant height, carotenoid and chlorophyll content [139]. Applying live *Chlamydomonas* cells through the soil drench method significantly enhanced leaf size, fresh weight, shoot length, pigment content, and the number of flowers in *Medicago truncatula* [140]. The acid-hydrolyzed dry biomass of *C. reinhardtii* enhanced the nitrogen, phosphorus, and carotenoid levels in *Solanum lycopersicum* [141]. Extracts from the biomass of *Chlamydomonas* sp. demonstrated auxin-like activity, leading to an increased number of roots in cucumber plants [142].

The significance of microalgae in the plant microbiome has only begun to be recognized recently, and their application for enhancing soil fertility, conserving water, and promoting plant growth is now emerging as a promising strategy for sustainable agriculture [143]. The plant hormone IAA, known as auxin, is a key signaling molecule involved in regulating plant growth and physiology [144]. Microalgae can release L-tryptophan, which can be converted by bacteria into IAA to stimulate algal growth [145]. In this regard, *Chlamydomonas* has the ability to release L-tryptophan, which serves as a suitable substrate for symbiotic microbes to produce IAA [146]. Moreover, it has been shown that *Chlamydomonas* itself can also synthesize IAA from L-tryptophan, an activity mediated by the enzyme L-amino acid oxidase (LAO1) [147]. Interestingly, elevated levels of IAA inhibit *Chlamydomonas* cell proliferation and chlorophyll degradation. However, in a consortium of *C. reinhardtii* with the plant-growth-promoting bacterium *Methylobacterium aquaticum*, these inhibitory effects were alleviated. These results may have significant agricultural implications, as *Chlamydomonas* and Methylobacterium spp. coexist in the plant rhizosphere [148]. Their ability to regulate IAA levels could influence plant health and may be utilized for enhancing crops in sustainable agriculture practices.

8. Future Perspective

This review aims to highlight the great potential of *Chlamydomonas* in monoculture and in co-culture with bacterial partners across various aspects, including the bioremediation of wastewater, biomass production, biofuels, and biofertilizers. As mentioned, one of the main products derived from the cultivation of microalgae-bacteria is their biomass, which is used as a raw material for obtaining other derived bioproducts. It would be highly beneficial economically to use the biomass resulting from the bioremediation process for bioproduct purification (Figure 2). However, the utilization of biomass and the water derived from wastewater treatment faces several inherent challenges. These challenges include the presence of xenobiotic residues and heavy metals within the biomass, the scalability of biomass production, as well as contamination with bacteria, fungi, and viruses, all of which limit their extensive application. Achieving future improvement and optimization of these processes to avoid these drawbacks is one of the most promising future perspectives, which would allow establishing a relationship with the circular economy by reducing waste and reusing materials, thus minimizing environmental and economic impact.

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