Chapter

Potential of Native Microalgae from the Peruvian Amazon on the Removal of Pollutants

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Abstract

Environmental pollution is a severe and common problem in all the countries worldwide. Various physicochemical technologies and organisms (e.g., plants, microorganisms, etc.) are used to address these environmental issues, but low-cost, practical, efficient, and effective approaches have not been available yet. Microalgae offer an attractive, novel, and little-explored bioremediation alternative because these photosynthetic organisms can eliminate pathogenic microorganisms and remove heavy metals and toxic organic compounds through processes still under study. Our research team has conducted some experiments to determine the bioremediation potential of native microalgae on some pollutant sources (i.e., leachate and wastewater) and its ability to remove hazardous chemical compounds. Therefore, in this chapter, we provide the results of our research and updated information about this exciting topic. Experiments were conducted under controlled culture conditions using several native microalgae species, variable time periods, different pollutant sources, and hazardous chemicals such as ethidium bromide. The results indicated that native microalgae can remove pollutants (i.e., phosphorus, ammonia, etc.) of wastewater, leachate, and some hazardous chemical compounds such as ethidium bromide. In conclusion, native microalgae have an excellent potential for removing several pollutants and, consequently, could be used to develop bioremediation technologies based on native microalgae from the Peruvian Amazon.

Keywords: bioremediation, native microalgae, leachate, pollutants, wastewater

1. Introduction

Microalgae have aroused the scientific community's interest by their biotechnological potential and increased commercial demand because these microorganisms

are an excellent source of a wide range of chemicals with biomedical interest (e.g., carotenoids, essential fatty acids, polyphenols, polysaccharides, etc.) [1–3]. In addition, they are helpful for bioremediation applications in wastewater treatment and other decontamination applications [4, 5]. Some advantages of this biological system are that bioremediation reinforces biogeochemical processes, toxic chemicals are degraded and not simply physically separated from the environment, and the process requires less energy than other technologies and uses less manual supervision. Furthermore, the bioaccumulation of heavy metals by microalgae cells may represent a feasible method for the treatment of leachates and wastewater containing bioavailable heavy metals [6–10].

Additionally, microalgae could be cultivated in wastewater lagoons with small nutrient requirements for their maintenance and development. This component usually constitutes the final step to completing the decontamination process in many wastewater treatment systems [11–13]. Therefore, massive cultivation of microalgae using wastewater as a source of nutrients is a cost-effective approach due to the simplicity of the technology allowing both pollutants (i.e., biological and chemical) remotion and the obtention of a valuable microalgae biomass rich in proteins, lipids, pigments, bioactive chemicals, etc. [14–17].

In this context, this chapter aims to provide updated information based on the results of investigations conducted by our research team using some strains from the freshwater microalgae collection culture native from the Peruvian Amazon.

2. Use of native microalgae for pollutants removal

2.1 Leachate treatment from an open-air garbage dump

Solid waste production in Iquitos city and other cities worldwide has been increased in direct relation to the demographic explosion. Commonly, cities such as Iquitos and other main cities of the Peruvian Amazon have an inefficient garbage collection system, and their main streets and popular markets are often full of garbage (**Figure 1**). In addition, these cities do not have a proper garbage disposal approach, and landfill sites are missing; consequently, the solid wastes are directly deposited in open-air garbage dumps (**Figure 1**).

In these open-air garbage dumps, the solid wastes can be dispersed and degraded by abiotic and biotic factors, producing a gamma of solid, gaseous, and liquid products; the latter is known as leachate. This wastewater flows out from a landfill or an open-air garbage dump sites due to precipitation, ground-water intrusion, moisture content of waste, and rate of evaporation [18]. The volume and pollutant composition of this leachate wastewater fluctuate over time; therefore, in the early acid phase, there exists a high concentration of the four groups of pollutants (dissolved organic matter, heavy metals, inorganic macrocomponents, and xenobiotic organic compounds); finally, in the long methanogenic phase, the leachate liquid has a lower concentration of the four groups of pollutants and is characterized by its very low concentration of heavy metals and biochemical oxygen demand/chemical oxygen demand (BOD/COD) ratio [19–21]. In addition, leachate liquid has a great diversity and composition of bacterial and archaeal populations of the members *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Epsilonproteobacteria*, among others [22–24]. For these reasons, leachate liquid should be appropriately disposed and treated to keep away ecotoxicological and environmental damage [25].

Figure 1.
Solid waste accumulation in the main streets and popular markets of Iquitos city (A, B, and C) and its final disposal in an open-air garbage dump (D).

According to these necessities, our research team evaluated the potential use of native microalgae of the Peruvian Amazon for leachate treatment generated in an open-air garbage dump. To do these experiments, leachate liquid samples (3 L) were collected from leachate pools generated from an open-air garbage dump of Nauta city, Loreto, Peru. After, leachate liquid samples were subsequently filtered through 0.45- and 0.25-μm filter membranes to remove particulate matter and microorganisms.

The experiments were conducted for 5 days with three native microalgae strains (*Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp.) of the freshwater microalgae collection culture native from the Peruvian Amazon. Each experiment included a control group (microalgae strain cultured with Chu-10 medium) and two treatments. The first treatment contained 100% leachate and the second 50% leachate and 50% Chu-10 culture medium. Assays were conducted by triplicate in 500-mL Erlenmeyer flasks and started using in each one a 200-mL final culture volume, 3×10^8 microalgae cells, a light intensity of 100 $\mu E \cdot m^2 \cdot s^{-1}$, a photoperiod regime of 12–12 h (light-dark), ambient temperature at 25°C ± 2°C, and constant homogenization at 200 rpm.

We evaluated the microalgae capabilities for chemical pollutant removal in leachates by quantifying these pollutants in the culture medium at the beginning and on the 5th day of the experiments, using standardized methods with the multiparameter LaMotte 3633-04 Fresh Water Aquaculture Test Kit. In addition, phosphate was quantified using a spectrophotometric method [26].

The results showed that the three microalgae strains were able to eliminate chemical pollutants in leachate (**Table 1**). Ammonium was efficiently removed from 90%

Pollutant compound/ chemical parameter	Microalgae strain	Concentration at the beginning of the experiments (mg/L)	Concentration on the 5th day of the experiments (mg/L)	Percentage decrease
Ammonium	*Ankistrodesmus* sp.	0.20 ± 0.01	0.00 ± 0.000	100 ± 0.00
	Chlorella sp.	0.20 ± 0.03	0.02 ± 0.001	90 ± 0.95
	Scenedesmus sp.	0.20 ± 0.01	0.01 ± 0.000	95 ± 0.22
Nitrite	*Ankistrodesmus* sp.	0.50 ± 0.05	0.05 ± 0.002	90 ± 0.72
	Chlorella sp.	0.50 ± 0.01	0.04 ± 0.001	92 ± 0.16
	Scenedesmus sp.	0.50 ± 0.06	0.03 ± 0.002	94 ± 0.33
Chloride	*Ankistrodesmus* sp.	24 ± 1.00	2 ± 0.10	91.7 ± 0.68
	Chlorella sp.	24 ± 1.15	2 ± 0.21	91.7 ± 0.49
	Scenedesmus sp.	24 ± 0.58	2 ± 0.10	91.7 ± 0.33
Phosphate	*Ankistrodesmus* sp.	100 ± 5.03	10 ± 0.26	90 ± 0.39
	Chlorella sp.	100 ± 3.61	10 ± 0.17	90 ± 0.21
	Scenedesmus sp.	100 ± 1.00	10 ± 0.20	90 ± 0.30
Carbon dioxide	*Ankistrodesmus* sp.	37 ± 1.00	0.0 ± 0.0	100 ± 0.0
	Chlorella sp.	37 ± 1.73	0.0 ± 0.0	100 ± 0.0
	Scenedesmus sp.	37 ± 0.58	0.0 ± 0.0	100 ± 0.0
Calcium and magnesium salts (hardness)	*Ankistrodesmus* sp.	160 ± 1.53	28 ± 0.50	82.5 ± 0.24
	Chlorella sp.	160 ± 0.58	28 ± 0.92	82.5 ± 0.59
	Scenedesmus sp.	160 ± 1.53	48 ± 1.00	70.0 ± 0.90
Carbonate and bicarbonate salts (alkalinity)	*Ankistrodesmus* sp.	180 ± 0.58	76 ± 1.00	57.8 ± 0.44
	Chlorella sp.	180 ± 1.00	96 ± 0.50	46.7 ± 0.29
	Scenedesmus sp.	180 ± 1.15	96 ± 1.00	46.7 ± 0.31
pH	*Ankistrodesmus* sp.	9 ± 0.50	9 ± 0.50	0.0 ± 0.0
	Chlorella sp.	9 ± 0.51	9 ± 0.50	0.0 ± 0.0
	Scenedesmus sp.	9 ± 0.50	9 ± 0.00	0.0 ± 0.0

Table 1.
Decrease in pollutant compound concentration and some chemical parameter values in landfill leachate cultures (100% leachate) of three native microalgae strains from the Peruvian Amazon.

(*Chlorella* sp.) to 100% (*Ankistrodesmus* sp.). These microalgae strains displayed similar pollutant elimination capabilities for nitrite, chloride, phosphate, carbon dioxide, and other chemical pollutants. CO_2, carbonate, and bicarbonate decrease can be related to its consumption by the microalgae cells in the photosynthetic process.

2.2 Wastewater treatment

The generation of great volumes of wastewater in the main cities of the Peruvian Amazon is increasing notably in the past 20 years. This environmental issue is associated with the intense migration of people from rural to urban areas with the hope to get better opportunities to improve their life qualities. This unplanned migration is generating unorganized human settlements in the marginal areas of the big cities, which lack basic services, such as electric fluid, potable water, and sewage system (**Figure 2**). In addition, none of these cities have wastewater treatment plants; then, wastewater is directly disposed into the main rivers of the Amazon basin, causing significant pollution of the aquatic ecosystems and affecting the aquatic flora, fauna, microbiota, and, of course, the human settlements located along the main rivers.

In this context, with a view to alleviate this pollution problem, we need to investigate eco-friendly, efficient, and low-cost options to treat wastewater. In this sense, we did experiments to determine whether native microalgae are useful to decontaminate wastewater generated in Iquitos city because there are several successful experiences around the world using these microorganisms [4, 5, 11, 27].

Therefore, to do the experiments, wastewater samples (5 L) were collected from the two main wastewater drainage systems of Iquitos city (Moronacocha and Huequito), Loreto, Peru. Furthermore, particulate matter and microorganisms were removed from the wastewater samples using the same previously described filtration approach (item 2.1) and were sterilized by autoclaving at 121°C for 30 min.

The experiments were conducted for 15 days with two native microalgae strains (*Ankistrodesmus* sp. and *Chlorella* sp.) of the freshwater microalgae collection culture native from the Peruvian Amazon. Each experiment included a control group (microalgae strain cultured with Chu-10 medium) and three treatments. The first treatment contained 100% wastewater from the Moronacocha wastewater drainage system and the second one contained 100% wastewater from the Huequito wastewater drainage system. Assays were conducted by triplicate in 250-mL Erlenmeyer flasks and started using in each one a 100-mL final culture volume, 4×10^{10} microalgae cells, a light

Figure 2.
Typical open-air sewage systems in Iquitos city and other main cities of the Peruvian Amazon.

intensity of 150 $\mu E \cdot m^2 \cdot s^{-1}$, a photoperiod regime of 12–12 h (light-dark), an ambient temperature at 27°C ± 2°C, a relative humidity at 83%, and constant aeration with an air pump system.

We evaluated the microalgae capabilities for chemical pollutants removal in wastewater by quantifying these pollutants in the culture medium at the beginning and on the 15th day of the experiments, using standardized methods with the multiparameter LaMotte 3633-04 Fresh Water Aquaculture Test Kit. In addition, phosphate was quantified using a spectrophotometric method [26].

The results showed that the two microalgae strains were capable to remove chemical pollutants from the two wastewater samples (**Tables 2** and **3**). However, there are marker differences; for example, ammonium was efficiently removed from wastewater of the Huequito wastewater drainage system (from 97.2% to 100%); in contrast, this pollutant was poorly removed from wastewater of the Moronacocha wastewater drainage system (only 20% with both microalgae strains).

2.3 Ammonium removal using an immobilized microalgae

Ornamental fish export is an important economic activity in Iquitos city, they provide benefits to several families dedicated to this area. A frequent problem during the process of ornamental fish transportation is high mortality rate, which could be attributable to decrease in water quality during transportation. These changes are due to the accumulation of toxic and metabolites of the fish catabolic process, such as ammonium [28], which, in turn, alkalinizes the pH and decreases the dissolved oxygen concentration in the aqueous medium [29]. Oxygen deficiency, toxin

Pollutant compound/ chemical parameter	Microalgae strain	Concentration at the beginning of the experiments (mg/L)	Concentration on the 15th day of the experiments (mg/L)	Percentage decrease
Ammonium	*Ankistrodesmus* sp.	50 ± 1.00	40 ± 1.00	20 ± 0.40
	Chlorella sp.	50 ± 1.50	40 ± 2.00	20 ± 3.47
Chloride	*Ankistrodesmus* sp.	24 ± 0.76	2 ± 0.10	91.7 ± 0.57
	Chlorella sp.	24 ± 0.76	2 ± 0.26	91.7 ± 1.29
Phosphate	*Ankistrodesmus* sp.	2 ± 0.10	1.10 ± 0.20	45.0 ± 0.83
	Chlorella sp.	2 ± 015	1.05 ± 0.07	47.5 ± 0.72
Carbon dioxide	*Ankistrodesmus* sp.	40 ± 1.53	8.5 ± 0.57	78.8 ± 2.08
	Chlorella sp.	40 ± 1.53	5.0 ± 0.55	87.5 ± 1.33
Calcium and magnesium salts (hardness)	*Ankistrodesmus* sp.	65 ± 0.70	60 ± 1.73	7.7 ± 1.86
	Chlorella sp.	65 ± 1.76	52 ± 2.29	20.0 ± 4.63

Table 2.
Decrease in pollutant compound concentration in wastewater cultures obtained from the Moronacocha wastewater drainage system using two native microalgae strains from the Peruvian Amazon.

Pollutant compound/ chemical parameter	Microalgae strain	Concentration at the beginning of the experiments (mg/L)	Concentration on the 15th day of the experiments (mg/L)	Percentage decrease
Ammonium	*Ankistrodesmus* sp.	36 ± 1.00	0.0 ± 0.00	100 ± 0.00
	Chlorella sp.	36 ± 1.73	1.0 ± 0.10	97.2 ± 0.39
Chloride	*Ankistrodesmus* sp.	38 ± 1.00	36 ± 2.00	5.3 ± 0.06
	Chlorella sp.	38 ± 2.00	28 ± 1.05	26.3 ± 3.28
Phosphate	*Ankistrodesmus* sp.	1.9 ± 0.10	1.5 ± 0.10	21.1 ± 4.94
	Chlorella sp.	1.9 ± 0.20	1.2 ± 0.10	36.8 ± 1.25
Carbon dioxide	*Ankistrodesmus* sp.	50 ± 3.46	14 ± 1.59	72 ± 1.30
	Chlorella sp.	50 ± 1.73	4 ± 0.15	92 ± 0.54
Calcium and magnesium salts (hardness)	*Ankistrodesmus* sp.	60 ± 1.73	38 ± 1.01	36.7 ± 3.28
	Chlorella sp.	60 ± 2.00	32 ± 2.00	46.7 ± 1.56

Table 3.
Decrease in pollutant compound concentration in wastewater cultures obtained from the Huequito wastewater drainage system using two native microalgae strains from the Peruvian Amazon.

accumulation, and an increase in total ammonium concentration in the water are believed to be the main cause of fish mortality during transportation [30].

To help solve this problem, our research team evaluated the hypothesis that by using immobilized microalgae, the ammonium concentration decreased significantly. To test the formulated hypothesis, the experiments were conducted by triplicate for 2 weeks with one native microalgae strain (*Chlorella* sp.) of the freshwater microalgae collection culture native from the Peruvian Amazon. To do the experiment, first, *Chlorella* sp. (**Figure 3**) was cultured in increasing volumes of BG-11 medium (100, 250, and 500 mL) to obtain sufficient microalgal biomass to begin the experiments. Once sufficient microalgal biomass was generated, the microalgae cells were harvested by centrifugation. The culture conditions were a light intensity of 100 $\mu E \cdot m^{2} \cdot s^{-1}$, a photoperiod regime of 12–12 h (light-dark), an ambient temperature at 27°C ± 2°C, a relative humidity at 83%, and constant aeration with an air pump system, after microalgae cells were immobilized (**Figure 3**) according to Zamani et al. [31]. Finally, 2×10^{3} alginate beads with trapped microalgae cells were transferred to polypropylene boxes of 40 × 50 × 40 cm (W × H × L) with 5 L of distilled water and ammonium chloride (NH_4Cl) at 800 μM. These immobilized cells were cultured for 2 weeks under the conditions described earlier, monitoring in the culture supernatant each 24 h the ammonium levels according to Solórzano [32, 33].

The results showed that immobilized *Chlorella* sp. can efficiently remove the toxic ammonium from the aqueous medium. Thus, 33.07% and 76.10% of ammonium were removed from the culture system on the 7th and 14th days of culture, respectively (**Figure 4**). These results are similar to previously reported studies that showed that microalgae of the genus *Chlamydomonas*, *Chlorella*, *Scenedesmus*, *Picochlorum,* and others can efficiently remove ammonium ions from several kinds of wastewater [34–39].

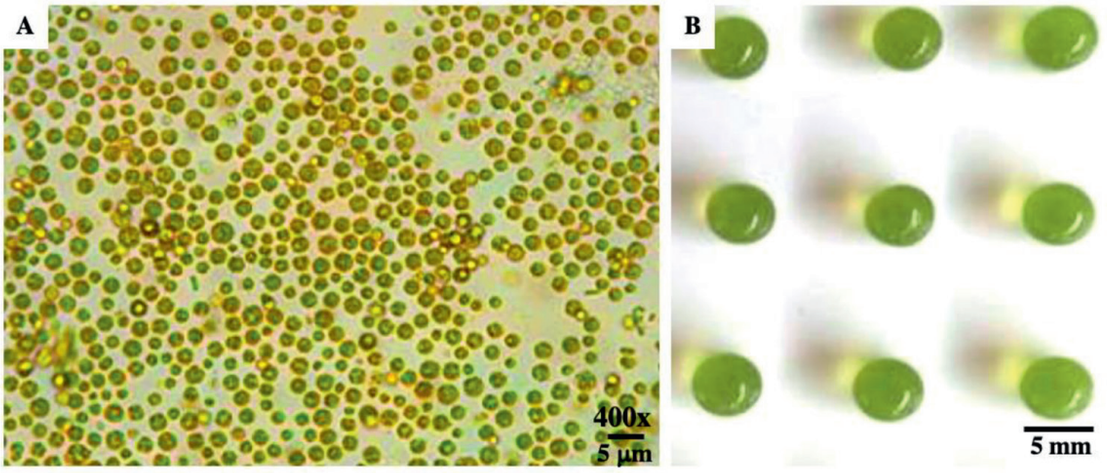

Figure 3.
Microphotography of Chlorella sp. cells (A) and immobilized microalgae cells in alginate beads (B).

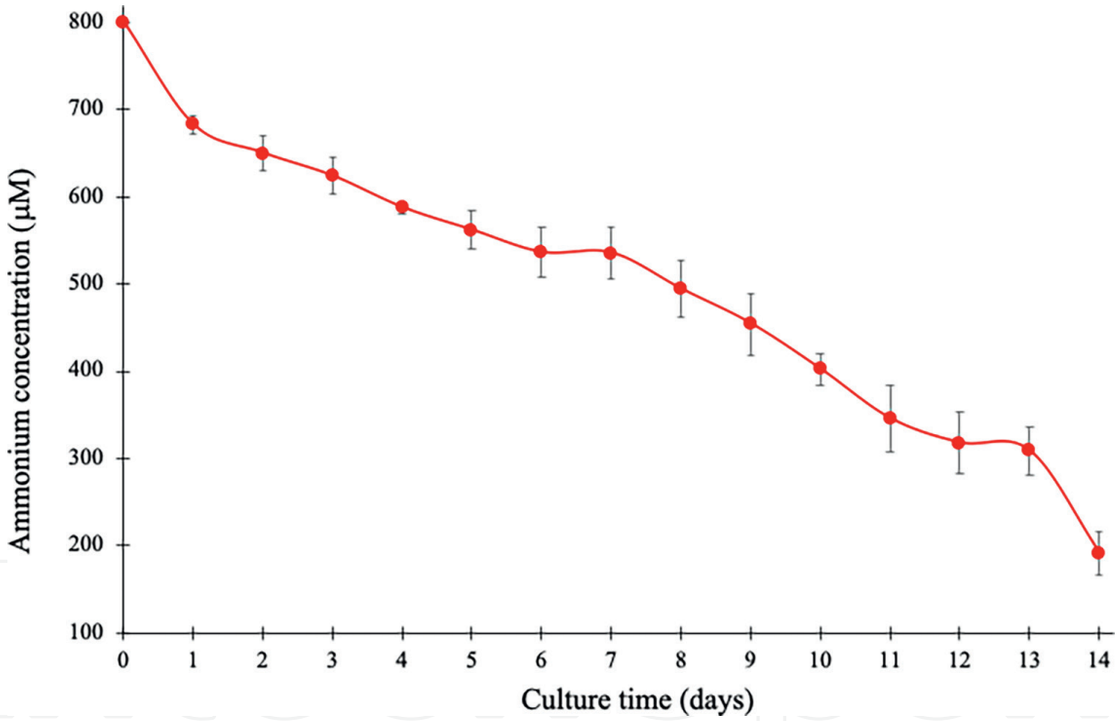

Figure 4.
Ammonium removal from the aqueous medium by Chlorella sp. immobilized in alginate beads.

Ammonium ions enter microalgae cells through ammonium transporters/ammonia permeases (AMTPs) embedded into the plasmatic membrane. These membrane-spanning proteins be made of 11 highly conserved transmembrane domains that fold into a channel across ammonia or ammonium translocates [40, 41]. According to X-ray crystallographic studies of some prokaryotic partners of these protein transporters, these are characterized as a compact trimer with 11 transmembrane helices per monomer and a narrow, mainly hydrophobic, channel for substrate conduction, located at the center of each monomer of the trimeric molecule. In addition, at the

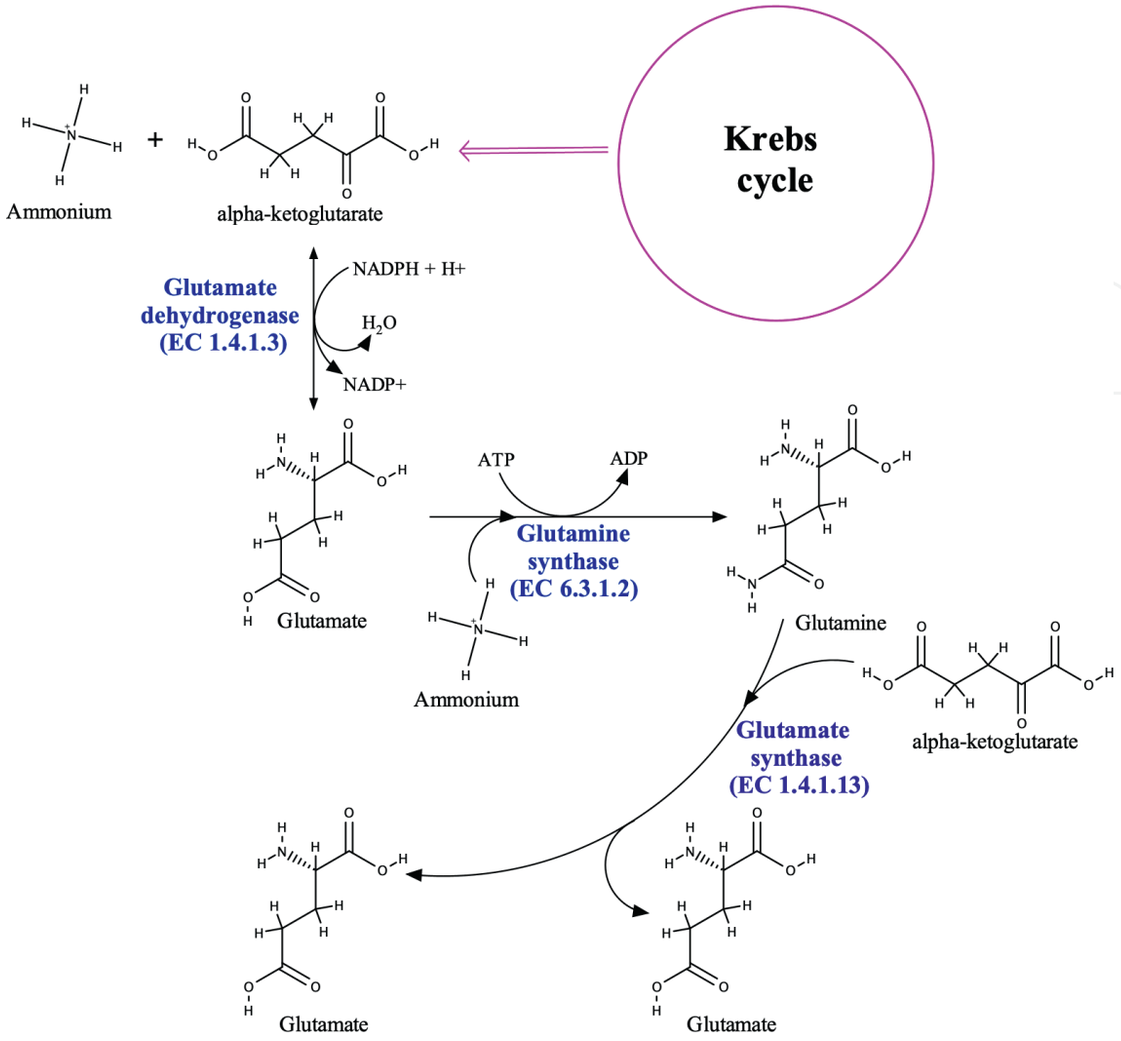

Figure 5.
Three key enzymes of microalgae involved in the incorporation of ammonium into amino acids and proteins.

periplasmic side of the transporter protein, a binding site for NH_4^+ is observed [42, 43]. In the particular case of *Chlamydomonas reinhardtii*, this microalga possesses the largest family of ammonium transporters consisting of eight members, which have complexly and finely regulation mechanisms at transcriptional and post-translational levels (**Figure 5**) [44–46].

According to Ahmad and Hellebust [47], the microalga *Chlorella autotrophica* can use two mechanisms to incorporate inorganic nitrogen sources into amino acids and proteins, which are related to the levels of the enzymes glutamate dehydrogenase (GDH) and glutamine synthetase (GS). Thus, GS levels are high in microalgae cells grown in nitrate and under nitrogen-starved conditions. However, in cells growing on ammonium, the GDH catalytic activity is increased. Both the enzymes require ammonium as a secondary substrate (**Figure 6**). Frequently, plant and microalgae cells prefer ammonium (NH_4^+) since it has the lowest metabolic energy cost than other inorganic nitrogen forms [36] because it can be directly ligated to amino acids by the action of three enzymes: glutamate dehydrogenase, glutamine synthetase, and glutamate synthase.

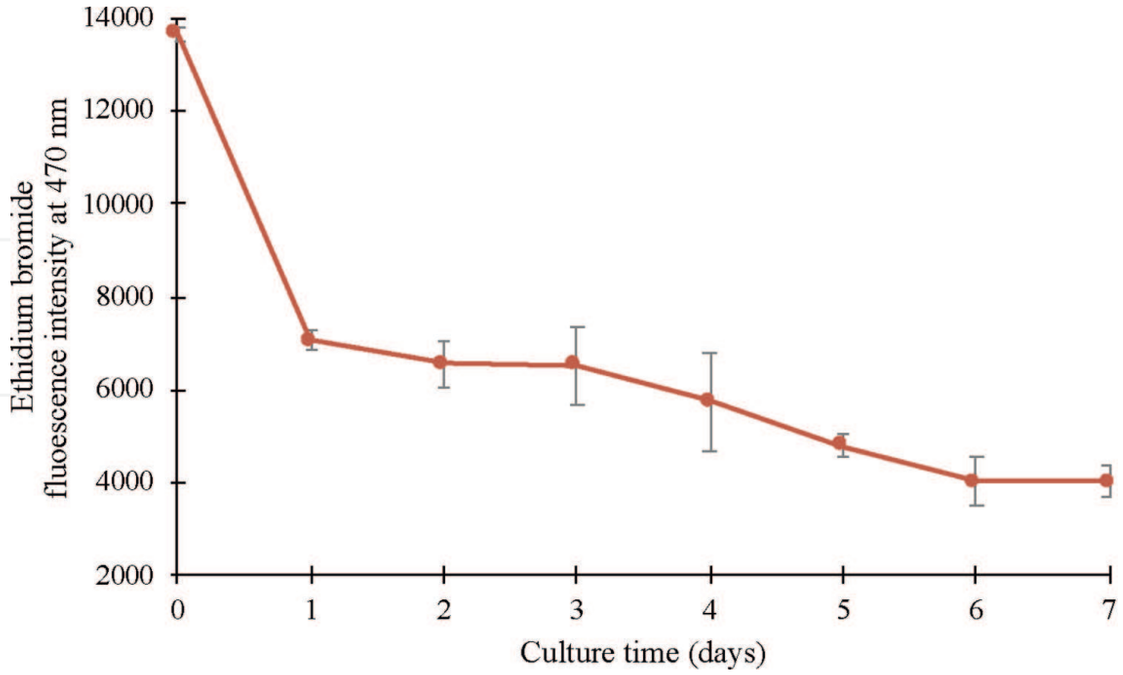

Figure 6.
Ethidium bromide removal kinetics by a microalgae consortium from the Peruvian Amazon.

2.4 Ethidium bromide removal using microalgae

The release of untreated effluent from research laboratories in our country and worldwide into water bodies is a major threat to the environment and human health. Commonly, effluent from laboratories and other research facilities is rich in toxic organic compounds, such as dyes used in the nucleic acid analysis, especially ethidium bromide, which is considered a serious biohazard due to its mutagenic, carcinogenic, teratogenic, and very toxic potentials when inhaled, ingested, or absorbed through the skin, and can irritate the eyes, mouth, and upper respiratory tract [48, 49]. To overcome these pollution problems, ethidium bromide and other toxic compounds could be partially or completely degraded to nontoxic forms before disposal. Consequently, some research laboratories worldwide are testing the biodegradation of ethidium bromide using plants and various kinds of microorganisms, including bacteria and microalgae [50–53], to develop, in the next future, modern, cost-effective, and eco-friendly bioremediation approaches.

In this context, our research team has evaluated the ability of a microalgae consortium for the removal of ethidium bromide from aqueous medium. For this experiment, three previously cultured native microalgae strains *Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp. were proportionally mixed (10^6 microalgae cells per milliliter of each strain) and transferred by triplicate into 250-mL Erlenmeyer flasks until a 100-mL final culture volume of BG-11 medium containing ethidium bromide at 1 mg/mL. In the experiments, a control group containing the same quantity of heat-inactivated microalgae cells and ethidium bromide at equal concentrations was included. Then, the assays were conducted for 7 days with a light intensity of 150 $\mu E \cdot m^2 \cdot s^{-1}$, a photoperiod regime of 12–12 h (light-dark), an ambient temperature at 27°C ± 2°C, a relative humidity at 83%, and constant aeration with an air pump system. Ethidium bromide concentrations were monitored every day measuring the

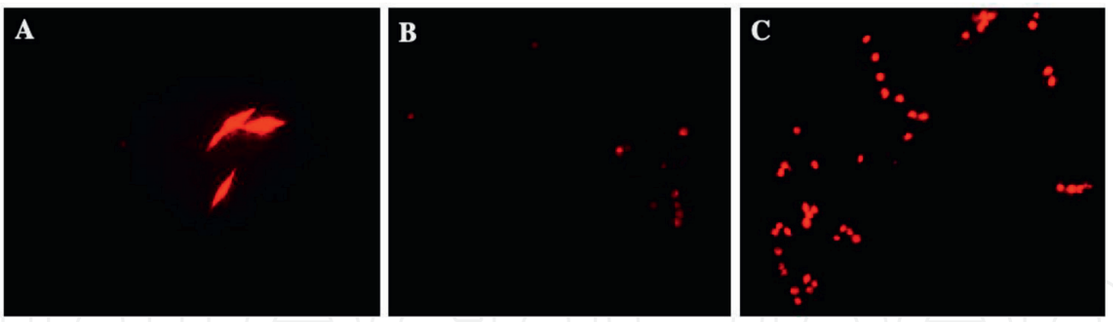

Figure 7.
Fluorescence microphotography of three native microalgae cells exposed to ethidium bromide. Ankistrodesmus sp. (A), Chlorella sp. (B), and Scenedesmus sp. (C).

intensity of fluorescence emission at 470 nm with a Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

The results showed that on the 7th day of starting the experiments, it was evidenced that the microalgal consortium was able to decrease the ethidium bromide concentration (directly related to fluorescence intensity) in the culture supernatant until 70.5% (**Figure 6**). These results corroborate the previous report of Cavalcante de Almeida et al. [52]. These authors also evaluated the capability of the microalgae *Chlorella vulgaris*, *Desmodesmus subspicatus,* and *Raphidocelis subcapitata* separately and in a consortium for ethidium bromide removal from an aqueous medium [52]. Their results strongly suggest the great potential of these microalgae species for phycoremediation application for ethidium bromide removal, which was directly dependent on the microalgae biomass.

Probably, the phycoremediation process used for microalgae to remove ethidium bromide is similar to several detoxification strategies used against aromatic organic pollutants (e.g., polycyclic aromatic hydrocarbons, phenolic compounds, dyes, etc.), including biosorption, bioaccumulation, biotransformation, and biodegradation [54, 55]. The first one is a metabolically independent process, which is a physicochemical phenomenon, supported by a gamma of mechanisms comprising absorption, adsorption, surface complexation, ion exchange, and precipitation [56]. The second one consists in the selective transportation by the monovalent cation uptake transport system [57] and other unidentified transporters, followed for its accumulation into some organelles such as nucleus, mitochondria, and chloroplast, which can be intercalated with DNA molecules (**Figure 7**). Finally, biotransformation and biodegradation are dependent on the metabolic capabilities of the microalgae cells, which are determined for their genomic background that codes a repertory of required enzymes [54]. To date, however, none of the metabolic pathways for ethidium bromide biodegradation has been described.

3. Conclusions

Native microalgae isolated from the Peruvian Amazon have a potential biotechnological application in the remotion of diverse chemical pollutants. These microorganisms showed abilities to remove pollutants contained into leachate generated in an open-air garbage dump and from two wastewaters from Iquitos city. In addition, an immobilized version of the microalgae *Chlorella* sp. was capable to remove ammonium

efficiently. Finally, a microalgae consortium composed of three microalgae from the genus *Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp. was competent to remove the toxic compound ethidium bromide. Together, these experimental pieces of evidence indicate that native microalgae have an excellent potential for removing several pollutants and, consequently, could be used to develop bioremediation technologies based on native microalgae from the Peruvian Amazon.

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Conflict of interest

The authors declare no conflict of interest.

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