Removal of Nutrients and Pesticides from Agricultural Runoff Using Microalgae and Cyanobacteria

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Abstract: The use of pesticides in agriculture has ensured the production of different crops. However, pesticides have become an emerging public health problem for Latin American countries due to their excessive use, inadequate application, toxic characteristics, and minimal residue control. The current project evaluates the ability of two strains of algae (Chlorella and Scenedesmus sp.) and one cyanobacteria (Hapalosyphon sp.) to remove excess pesticides and other nutrients present in runoff water from rice production. Different concentrations of wastewater and carbon sources (Na2CO3 and NaHCO3) were evaluated. According to the results, all three strains can be grown in wastewater without dilution (100%), with a biomass concentration comparable to a synthetic medium. All three strains significantly reduced the concentration of NO3 and PO4 (95 and 85%, respectively), with no difference between Na2CO3 or NaHCO3. Finally, Chlorella sp. obtained the highest removal efficiency of the pesticide (Chlorpyrifos), followed by Scenedesmus and Hapalosyphon sp. (100, 75, and 50%, respectively). This work shows that it is possible to use this type of waste as an alternative source of nutrients to obtain biomass and metabolites of interest, such as lipids and carbohydrates, to produce biofuels.

Keywords: pesticides; nitrate removal; phosphate removal; biomass production; metabolites

1. Introduction

Ensuring water availability and quality, sustainable agriculture, and food security are critical issues that require sustainable alternatives that positively impact the growth of societies [1]. Pesticides are one of the most important agricultural inputs that guarantee quality and efficiency in crop production. However, due to their excessive use, inadequate application, toxic characteristics, and minimal residue control, pesticides have become an emerging problem of public health, water pollution, and environmental contamination in general [2]. Agricultural sectors such as the rice industry use large amounts of water and agrochemicals for their crops that can be transported through surface runoff, leaching into the soil and evaporating into the atmosphere, contaminating bodies of surface or groundwater, food, and the air we breathe [3]. In Norte de Santander (Colombia), one of the most used pesticides is Chlorpyrifos, which has no reported restrictions according to the National Ministry of Agriculture. Nitrogen and phosphorus are two macronutrients...
present in these fertilizers that favor crop growth and productivity, but if applied in excess or inadequately, they are not completely assimilated by plants and infiltrate through runoff, contaminating ground and surface water, causing severe damage to the environment and human health [4]. The techniques applied in the industrial production of fertilizers cause environmental problems; generally, the production of nitrogen at the industrial level is carried out through synthesis processes that convert atmospheric nitrogen into ammonia using natural gas, generating large amounts of CO\textsubscript{2} released into the atmosphere, contributing to global warming. On the other hand, phosphorus is obtained from minerals based on non-renewable phosphates, using chemical processes with sulfuric acid to obtain them, which produce by-products that are hazardous to both health and the environment [5].

Microalgae and cyanobacteria, as photosynthetic microorganisms, represent a viable alternative in wastewater treatment given their diverse environmental and biotechnological production benefits such as the assimilation of nutrients, use of light, consumption of CO\textsubscript{2} from the atmosphere, generation of high-value products and biomolecules, production of oxygen, generation of homogeneous biomass, and high photosynthetic efficiency, among others [6]. During the last decalde, these characteristics have been studied regarding the treatment of different types of wastewaters: domestic [7], industrial, and agricultural, among others, evidencing their growth in agricultural wastewater [8–13]. However, the application of algae and cyanobacteria to remove contaminants possesses limitations, such as their tolerance to the type of wastewater and their high-energy concentration demand, especially in the mixing and harvesting the biomass produced [14,15].

Wastewater contains several compounds that can be used as raw material for various industries, which is why in recent years, the reuse of these compounds as essential nutrients for microalgae production has been proposed [9], reducing production costs for high value-added products whose operation in terms of costs is unfeasible in the current market [16]. The cultivation of microalgae in the biotechnology industry demands a large amount of water, which is a factor to consider bearing in mind the scarce availability of the resource during intense periods in the summer; for this reason, the cultivation of microalgae in wastewater offers an ideal scenario in three indispensable factors for the cultivation of microalgae: water reuse, the availability [17] of nutrients, and the assimilation of pollutants. Recent studies have demonstrated the efficiency of microalgae for the treatment of different types of pesticides used in the agricultural industry; García-Galán et al. [18] showed that a microalgae culture system worked effectively to decontaminate agricultural runoff contaminated with different types of pesticides commonly used in various crops. On the other hand, Li et al. [19] demonstrated the elimination of pollutants and production of by-products with the use of wastewater from the swine industry, which opens the possibility of its application in different scenarios that lead to a decrease in the pressure and contamination of water resources. Other benefits include the reduction in costs in algal biorefining, the production of high-value by-products, and the care of the environment in general [20].

The objective of this study was to evaluate the viability of the cultivation of microalgae and cyanobacteria using two types of wastewater from rice cultivation. This was to determine the assimilation capacity of contaminants present in this medium such as nitrates, phosphates, and pesticides in the search for the production of metabolites of interest, offering a viable alternative focused on the reuse of wastewater from rice cultivation, as well as the treatment of wastewater to optimal conditions for its discharge, and the bioconversion of these in the production of high value-added metabolites.

2. Materials and Methods

2.1. Agricultural Runoff

The agricultural wastewater was obtained from the discharge canals of the irrigation area of rice production fields in the municipality of Zulia (Cúcuta, Norte de Santander) during the month of March (2019). For cultivation, the effluents were filtered twice with a cloth filter and sterilized by autoclave (120 °C, 20 min) to avoid interference of bacteria or
fungi. The wastewater was chemically analyzed (NO$_3$, PO$_4$, pH, turbidity, conductivity, temperature, salinity, total dissolved solids, COD, BOD$_5$, total solids, total suspended solids, volatile suspended solids, and sedimentable solids) according to standard methods for the examination of water and wastewater [21]. The Chlorpyrifos concentration was determined according to the method described by Zalat et al. [22].

2.2. Strains

*Hapalosyphon* sp. (HAPA_UFPS002), *Chlorella* sp. (CHLO_UFPS010), and *Scenedesmus* sp. (SCEN_UFPS015) from the INNOValgae collection (Universidad Francisco de Paula, Santander, Colombia) were used in this study. These strains were previously isolated from thermal springs near Cucuta (Norte de Santander, Colombia) and possess the capacity to grow in contaminated waters (data not shown). The strains were pre-cultivated in a 2 L glass flask with a working volume of 1.2 L containing Bold Basal media for the algae and BG11 for the cyanobacteria [23]. The media was mixed through the injection of filtered air (Acro® 37 TF Vent, PTFE membrane) with 0.5% (v/v) CO$_2$ at a flow rate of 0.78 L min$^{-1}$, 25 °C, and a light:dark cycle of 12:12 h at 100 µmol m$^{-2}$ s$^{-1}$ for 30 days.

2.3. Experimental Design

Initially, the capacity of the strains to grow in wastewater was determined. The selected strains were inoculated with different concentrations of wastewater diluted with distilled water (10, 50, 75, and 100 v/v). The concentration of wastewater that allowed the growth of the three strains was supplemented with different concentrations (0.8, 1.2, and 1.6 g/L) of either sodium carbonate (Na$_2$CO$_3$) or sodium bicarbonate (NaHCO$_3$) [24] before inoculation to enhance the biomass production and the removal of NO$_3$ and PO$_4$. The results were analyzed using a two-way ANOVA GraphPad Prism version 9.

All the strains were cultured (in triplicate) in a 2 L glass flask with a working volume of 1.2 L of sterile wastewater. Each flask was mixed by the injection of filtered air at a flow rate of 0.78 L min$^{-1}$ (Resun, LP-100) and a light:dark cycle of 12:12 h at 110 µmol m$^{-2}$ s$^{-1}$ for 20 days. The produced biomass was concentrated by electroflotation (10 aluminum electrodes, 20 min, 150 rpm, and 50 W) [25], washed twice with distilled water, freeze-dried, and stored (4 °C) until use. Finally, the different components of the strains, including carbohydrates [26], lipids [27], proteins [28], carotenoids [29], phycocyanins [30], and ash [31], were measured.

3. Results

The physicochemical analysis shows the initial characteristics present in the agricultural wastewater (Table 1); considering the study, it can be observed that the wastewater from the discharge of the irrigation canal presents a high concentration of pesticides (15.3 mg/L), which, according to Colombian regulations (Resolution 631—2015), is outside the maximum permissible limits for active ingredients of pesticides of toxicological categories 1A, 1B, and II. Additionally, there are concentrations of nitrates and phosphates that may affect the ecological balance of the water sources.

The results show that all three strains can grow in different concentrations of agricultural runoff. Under low levels (10% v/v) of wastewater, the biomass produced was relatively low (<0.3 g/L) and increased with higher concentrations of the wastewater due to higher levels of NO$_3$ and PO$_4$ (Figure 1). All strains reported their highest biomass concentrations at full strength of the agricultural runoff, which also indicates that the presence of toxic compounds such as Chlorpyrifos does not negatively affect the proper growth of these strains. According to the ANOVA analysis, a higher difference was observed between different concentrations of the agricultural runoff. However, the biomass concentration achieved by all three strains was lower than the control (BG11 and Bold Basal media).
The results in Figure 2 highlight that after 20 days of culture in the agricultural runoff, the NO$_3$ concentration can be reduced up to 88% by *Scenedesmus* sp., while *Chlorella* sp. and *Hapalosyphon* sp. removed up to 85% of the total NO$_3$ present in the wastewater removal. According to the ANOVA analysis, there was a significant difference between the strains in removing NO$_3$. The removal of PO$_4$ behaved similarly to nitrate since the values obtained were very similar among the three strains studied, with values of up to 82% of PO$_4$ removed by *Scenedesmus* sp., followed by *Chlorella* sp. and *Hapalosyphon* sp.; however, no significant difference was observed in the removal of PO$_4$.

### Table 1. Chemical analysis of agricultural runoff.

| Parameters                        | Units       | Results     | Max Limit (Res 0631 2015) |
|-----------------------------------|-------------|-------------|-----------------------------|
| Nitrates (NO$_3$)                 | mg/L NO$_3$ | 35.23       | analysis and report         |
| Phosphates (PO$_4$)               | mg/L PO$_4$ | 4.74        | analysis and report         |
| pH                                | pH units    | 7.08        | 6.00 to 9.00                |
| Turbidity                         | FAU         | 20          | N/A                         |
| Conductivity                      | µS          | 164.5       | N/A                         |
| Temperature                       | ºC          | 25          | N/A                         |
| Salinity                          | ppm         | 102         | N/A                         |
| Total Dissolved Solids            | ppm         | 117         | N/A                         |
| Chemical Oxygen Demand (COD)      | mg/L        | 20.01       | 150.00                      |
| Biochemical Oxygen Demand (BOD$_5$)| mg/L        | 2           | 50.00                       |
| Total solids (TS)                 | mg/L        | 160         | N/A                         |
| Total Suspended Solids (TSS)      | mg/L        | 25          | 50                          |
| Volatile Suspended Solids (VSS)   | mg/L        | 12          | N/A                         |
| Sedimentable Solids (SS)          | mL/L*h      | 4           | 1                           |
| Chlorpyrifos                      | mg/L        | 1.5         | 0.05                        |

![Figure 1](image-url)
The addition of inorganic salts (Na$_2$CO$_3$ and NaHCO$_3$) was evaluated as an alternative carbon source to improve biomass production. The results show that sodium carbonate significantly improved the biomass concentration for the three assessed strains compared to the control (Bold Basal Medium) (Figure 3a). *Scenedesmus* sp. and *Hapalosiphon* sp. reported the maximum biomass concentration using 1.2 g/L of Na$_2$CO$_3$ (0.71 and 0.83 g/L, respectively), while *Chlorella* sp. obtained the largest biomass concentration up to 1 g/L with 0.8 g/L of sodium carbonate. In general, the strain that used this carbon source was *Chlorella* sp. On the other hand, when sodium bicarbonate was used, *Chlorella* sp. grew up to 0.8 g/L using 1.2 g/L of NaHCO$_3$. In the case of *Scenedesmus* sp., a significant difference in biomass concentration (in comparison with the control) was achieved using 1.2 g/L of sodium bicarbonate. Finally, the final concentration of *Hapalosiphon* sp. was not affected by the concentration of NaHCO$_3$ in the media.

**Figure 2.** Nitrate and phosphate consumption by the studied strains.

**Figure 3.** Effect of different concentrations of Na$_2$CO$_3$ (a) and NaHCO$_3$ (b) in the biomass concentration of the three strains evaluated.
According to the previous results, all the strains were grown with the concentration of Na$_2$CO$_3$ that enhanced biomass production (0.8 g/L for Chlorella sp. and 1.2 g/L for Scenedesmus sp. and Hapalosiphon sp.). The results show (Figure 4) that the strain with the highest percentage (%) of removal was Chlorella sp., followed by Scenedesmus sp. and Hapalosiphon sp. (42, 51, and 60%, respectively). More importantly, there was no statistical difference between the carbon source and the efficiency of removing the pesticide.

![Figure 4. Chlorpyrifos removal by the three strains was evaluated.](image)

Finally, the concentration of different metabolites (carbohydrates, proteins, lipids, and others) of the three strains evaluated (Figure 5) shows that wastewater does not affect the metabolic level. Interesting metabolites such as carbohydrates were obtained in concentrations higher than 20% w/w in Chlorella and Scenedesmus sp. (26% and 29% w/w, respectively). Total lipids did not exceed 10% in both microalgae and cyanobacteria evaluated. On the other hand, the complete proteins reported exceeded 40% w/w of the total biomass in the three strains. Other exciting metabolites such as natural colorants, e.g., carotenoids, did not exceed 4% w/w, and total phycocyanins in Hapalosyphon sp. reached concentrations of 12% w/w.

![Figure 5. The concentration of different metabolites in the three strains cultured in agricultural runoff.](image)

4. Discussion

The application of microalgae and cyanobacteria cultures to remove contaminants present in wastewater is a technological process that has gained strength at the industrial level [32] because the biomass produced can be transformed into different products, including biofuels (bioethanol, biogas, biodiesel, etc.), biofertilizers [33], and even bioplastics [17].

The selection of the carbon source to be used in the cultivation of microalgae and cyanobacteria is a critical variable in the capacity to produce biomass and high value-added
metabolites [34]. Table 2 presents different works in which Na$_2$CO$_3$ and NaHCO$_3$ are used as alternative carbon sources. According to Sivaramakrishnan and Incharoensakdi [35], low concentrations of Na$_2$CO$_3$ (0.03 g/L) increase the biomass concentration of different strains of microalgae; this is also supported by the results of Shuyu et al. [36]. On the other hand, high concentrations of this carbon source (up to 5 g/L) can reduce the biomass concentration in certain strains of $S$. obliquus [37]. According to Tu et al. [38], NaHCO$_3$ supplementation promotes the transfer of HCO$_3^-$ ions across the plasma membrane into chloroplasts, which significantly improves biomass concentrations and promotes lipid synthesis. Unlike Na$_2$CO$_3$, high concentrations of NaHCO$_3$ do not seem to negatively affect the cell divisions of different microalgae species. According to data reported by Lohman et al. [39], concentrations of up to 4 g/L NaHCO$_3$ do not affect the growth of $C$. vulgaris. The same occurs with Dunaliella salina, which can have a final biomass concentration of up to 3 g/L using 5 g/L NaHCO$_3$ [40]. Other studies using Scenedesmus sp. CCNM 1077 [41] and Tetrasdesmus wisconsinensis [42] reported average biomass concentrations (0.55 and 0.7 g/L, respectively) using relatively high NaHCO$_3$ concentrations (0.55 and 1.68 g/L, respectively).

Table 2. Strains cultured with Na$_2$CO$_3$ and NaHCO$_3$ as carbon sources.

| Strain              | Carbon Source | Culture Media | Biomass (g/L) | Reference |
|---------------------|---------------|---------------|---------------|-----------|
| Chlamydomonas sp.   | Na$_2$CO$_3$  | BG11          | 1.7           | [35]      |
| Chlorella sp.       | 0.03          | 1.6           |               |           |
| Scenedesmus sp.     | Na$_2$CO$_3$  | 1.7           |               |           |
| Chlorella sp. (FACHB-1298) | 0.005 | 1.89           |               | [36]      |
| S. Obliquus         | 5             | n/a           | 0.02          | [37]      |
| Chlorella sp. LPF   | 80            | F/2           | n/a           | [38]      |
| C. vulgaris UTEX 395| 4.2           | Bold Basal    | 0.6           | [39]      |
| Dunaliella salina JDS 001 | 5.0 | MJ            | 3.17          | [40]      |
| Scenedesmus sp. CCNM 1077 | 1.5 | BG11          | 0.55          | [41]      |
| Tetrasdesmus wisconsinensis | 1.68 | Bold Basal | 0.7           | [42]      |

The tolerance of different strains and species of these microorganisms is one of the main challenges for cultivation in wastewater. According to the present work’s results, a high concentration of Chlorpyrifos and other nutrients does not affect the proper growth of these strains, making this type of wastewater an exciting alternative for algal biomass production. Studies such as the one reported by Khalid et al. [43], where a strain of $C$. sorokiniana can grow on simulated agricultural wastewater, add to this type of research.

According to EU regulations, Chlorpyrifos is a banned pesticide; however, this pesticide is widely used in Colombia. Therefore, scientific information on removing this type of pesticide using microalgae is rare. According to García-Galán et al. [18], no Chlorpyrifos concentrations were reported after nine days of cultivation. On the other hand, Matamoros and Rodríguez [44] found that cultivating multiple microalgae strains (in which Chlorella sp. predominates) can remove up to 50% of the concentration of this pesticide. These results correspond to the data reported in this work, where it is possible to remove up to 50% of this pesticide present in agricultural runoff from rice cultures.

Nutrients such as N and P are necessary for different metabolic processes critical for the correct cellular functioning of microalgae and cyanobacteria [44]. Table 3 summarizes the different strains evaluated for removing NO$_3$, PO$_4$, and pesticides from agricultural runoff. One of the main characteristics of this group of microorganisms is their ability to capture high concentrations of NO$_3$. Works such as those reported by Vazirzadeh et al. [45] demonstrate that certain strains can remove up to 100% of NO$_3$ in high concentrations (>1000 mg/L). Cai et al. [46] and Kumar et al. [47] reported similar removal rates. In the
case of microalgae and cyanobacterial strains grown in agricultural runoff, NO$_3$ removal efficiencies are similar, ranging from 80% [43,48–50] to 95% of the total NO$_3$ present in the wastewater [17,51,52].

Table 3. Strains cultured on different agricultural runoff.

| Strain                  | Wastewater                          | Pesticide | NO$_3$ Removal | PO$_4$ Removal | Biomass Produced | Reference |
|------------------------|-------------------------------------|-----------|----------------|----------------|-----------------|-----------|
| Naturally occurring algal mixture | horticultural wastewater | n/a       | 86%            | 52%            | 0.51 g/L         | [48]      |
| C. vulgaris            | simulated agricultural runoff       | n/a       | 85%            | 91%            | 4.2 g/L          | [17]      |
| Naturally occurring algal mixture | agricultural runoff                 | n/a       | 0.72 g m$^{-2}$ d$^{-1}$ | 0.37 g m$^{-2}$ d$^{-1}$ | 11.45 g m$^{-2}$ d$^{-1}$ | [51]      |
| Naturally occurring algal mixture | peri-urban agricultural runoff     | Multiple pesticides including Chlorpyrifos | 54% | 100% | 6.9 gVSS m$^{-2}$ d$^{-1}$ | [18]      |
| microalgae consortium filamentous green algae | agricultural drainage water agricultural stormwater | n/a | 6 | 22 | 22 g m$^{-2}$ d$^{-1}$ | [52]      |
| Mixture of Pediastrum sp., Chlorella sp., Scenedesmus sp. and Gloeocapsa sp. | agricultural runoff                | n/a       | 80%            | 70%            | 0.8 g/L          | [49]      |
| Mixture of Chlorella sp., Stigeoclonium sp., Nitzschia sp. and Navicula sp. | agricultural runoff and partially treated domestic wastewater | n/a | 85% | 99% | 0.6 g/L | [50]      |
| Chlorella sp.          | from rice production fields         | Chlorpyrifos | 85% | 82% | 1.0 g/L | This study |
| Scenedesmus sp.        |                                     |            | 88%            | 82%            | 0.71 g/L         |           |
| Hapalosiphon sp.       |                                     |            | 85%            | 82%            | 0.83 g/L         |           |

$^a$ volatile suspended solids; $^b$ total nitrogen; $^c$ total phosphorous.

Algae are also known for their capacity to remove more significant phosphorus concentrations from liquid media; one of these mechanisms is the chemical precipitation of P [53]. However, this process requires a change in the pH of the culture media [54]. In this study, the pH did not change drastically during the culture time. The wastewater used in this work had relatively low concentrations of NO$_3$ and PO$_4$ (Table 1), which were significantly lower than those found in culture media such as Bold Basal or BG11; therefore, the concentration of NO$_3$ and PO$_4$ present in this type of agricultural wastewater can be removed to non-hazardous levels.

5. Conclusions

Due to its concentration of excess fertilizers, agricultural runoff is an exciting source of nutrients for algal biomass production; however, different pesticides can reduce the growth capacity of algal strains. The results show that the three strains studied (Chlorella, Scenedesmus, and Hapalosiphon sp.) can effectively grow in undiluted agricultural runoff and remove more than 80% of NO$_3$ and PO$_4$ present in this type of wastewater. On the other hand, it was found that Chlorella sp. reported the highest biomass concentration (1 g/L) with the lowest concentration of Na$_2$CO$_3$ evaluated (0.8 g/L). It was also found that up to 40% excess Chlorpyrifos can be removed by the three strains evaluated. Finally, the concentration of metabolites of interest, such as lipids and carbohydrates that can be transformed into biofuels or even bioplastics, was not affected by the presence of the pesticide. However, it is necessary to focus on other cultivation conditions (light:dark cycle, semicontinuous cultivation) that maximize the synthesis of specific metabolites.

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