

Article

The Role of Microalgae in Improving The Quality of Industrial Wastewater and Reducing Environmental Pollution

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Abstract: Industrial water pollution results in severe health hazard to the people besides enhancing the level of environmental pollution. Given its capabilities to successfully filter pollutants and generate useful biomass, microalgae have become a suitable and efficient manner of wastewater treatment. This work investigates the efficiency of *Chlorella saccharophila* (CSA) and *Chlorella vulgaris* (CVS) for wastewater treatment. The outcomes suggested reliable reductions in some of the chemical parameters including chemical oxygen demand, total nitrogen, total phosphorus, electrical conductivity and total suspended solids when *Chlorella* species were applied on the wastewater. Decrease in the quantity of total ammoniacal nitrogen (TAN) also clearly indicates that how effectively *Chlorella* species remove hazardous materials from wastewater. Another important effect was the decrease of the pH after treatment by *Chlorella* species, which affects pathogen inactivation as well as the further treatment steps. The differences in the growth rates as well as the capacities for nutrient removal between CVS and CSA might have been responsible for any differences in the efficiency of pollutant removal. CSA offered superiority over CVS for the removal of TDS; TAN and COD were better removed by CVS. Overall, the study indicated that *Chlorella* species may prove to be a cheap and effective bio-remediation method of wastewater. Heavy metals, organic pollutants and nutrients, amongst other, could be removed by the microalgae. The treated water met the discharge standards for some parameters because the treated wastewater could be released into the environment. Overall, the results point to the possibility of using *Chlorella* species as an affordable and environmentally friendly wastewater treatment technique.

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1. Introduction

The economy, the environment, and human health are all severely harmed when untreated industrial wastewater is released (Pratap et al., 2023). Pollutant releases into water bodies, such as those involving metals, chemicals, and sewage, lead to significant air, soil, and water pollution, which deteriorates aquatic ecosystems and reduces biodiversity (Malik et al., 2020). The process of eutrophication occurs when an excessive amount of nutrients in the water promotes the growth of harmful algae and other plants. These plants then cause hypoxia, block out sunlight, and raise carbon dioxide levels to dangerous levels for aquatic life, which in turn causes ocean acidification (Findlay & Turley, 2021). In addition, industrial waste pollution of drinking water poses a serious risk to human and wildlife health. Industrial pollution has an influence on the environment as well as the economy (Garg et al., 2022). For example, the textile industry uses a lot of water for finishing and dyeing, which increases competition for this resource

and likely drives up water prices. This industry can obtain its resource supply locally during low rainfall conditions or typically during low government water availability (Mishra, 2023). Once more, the pulping and bleaching processes used in the paper-making industries can lead to pollution. These industries also use a lot of water. The massive release of untreated wastewater into the Vistula River in Warsaw had a negative impact on the environment (Szalkowska & Zubrowska-Sudol, 2023). This is one example of how inadequate wastewater treatment and management practices can lead to catastrophic infrastructure failure. This would require the setting up of sustainable wastewater management practices, reusing treated wastewater for agriculture and industry, and putting in place strict regulations and policies to bind industries in their environmental impact.

Microalgae are a broad class of unicellular photosynthetic organisms with many benefits for a range of industries and uses (Elshafey et al., 2023). With respect to cost, renewability, and environmental concerns, they are an ideal alternative to liquid fossil fuels as a source of biofuels, bioactive pharmaceuticals, and food ingredients. Microalgae are useful for the pharmaceutical industry and the development of functional foods because they are high in nutrients and active compounds and exhibit a variety of biological activities, including antibacterial, anti-inflammatory, antioxidant, and antitumor effects (Saeed et al., 2022). They can be produced sustainably, so they are some of the lowest greenhouse gas emitters and best carbon caches. Hence, they can be exploited for the bioremediation of polluted waters. The microalgae, in addition to its easy form of cultivation and rapid production rates, Microalgae's are a successful source of protein as well as other useful commercially exploitable compounds with nutraceutical significance or medicinal properties like lipids polysaccharides etc (Orejuela-Escobar et al., 2021). They have also been tested for use as biomaterials in cosmetics and medicine, alongside biofuels, bioplastics, and even biofertilizers. Not only, industrial wastewater are a nutrient rich source to cultivate microalgae but provide an excellent opportunity for improving algal productivity and concomitant domestic sewage treatment in the process which is low cost energy efficient friendly technology (Ezzat et al., 2024). Overall, microalgae are widespread and favorable source in many fields benefitting environment and human health also have the potential to play good role for environment preservation (Dolganyuk et al., 2020) ; Ezzat et al., 2024).

Microalgae is a sustainable and efficient wastewater treatment more so due to the following (Morillas-España et al., 2022). A major advantage is that they can remove microorganisms and chemicals from the wastewater like nitrogen, phosphorus, and heavy metals that when untreated cause eutrophication and other issues (Tiwari & Pal, 2022). Also, the parameters of the treated wastewater are improved in which the biological oxygen demand (BOD) and chemical oxygen demand (COD) are significantly reduced, hence making it safe to discharge in water sources (Song et al., 2022). Moreover, microalgae organisms can grow well in almost all the types of wastewater like municipal, agricultural, or industrial effluents (La Bella et al., 2022). It can remove the bacteria from water sources that have been polluted such as mine water. Microalgae are known to create biomass that can be collected for further use in different processes including: production of important chemicals, animal feed, and energy source in the form of biofuels (Elshafey et al., 2022). This feedback is an efficient and sustainable process since it deals with wastewater and at the same time is able to generate useful products. Microalgae-dependant wastewater treatment is the key inside for combating climate change due to its efficiency, which does not require large amounts of energy and even minimizes emissions of carbon dioxide compared to conventional methods (Sadvakasova et al., 2023). In mild of the above discussions, it's miles consequently very clear that the use of microalgae inside the treatment of wastewater is therefore an effective, sustainable technique for the rising task of water pollution. In preferred, it can be said that the primary purpose of this painting was to observe the opportunity of using *Chlorella* species (*Chlorella vulgaris* and *Chlorella saccharophila*) for the elimination of water contaminants. The examination aimed to evaluate the capacity of those algae to put off diverse pollutants from wastewater, consisting of nutrients, organic dependents, and heavy metals. They look at also aim to

evaluate the effect of *Chlorella* treatment on the overall high quality of the wastewater, which includes the pH and overall ammoniacal nitrogen (TAN) tiers. The aim of the take a look at turned into to decide the feasibility of the usage of *Chlorella* species as a fee-effective and sustainable technique for wastewater remedy.

2. Materials and Methods

2.1 Isolation of Microalgae and Preparation

Ten milliliters of pure isolates composed of the microalgae species *Chlorella vulgaris* and *Chlorella saccharophila* are taken from the Canadian Center for Environmental Studies. Then different algae isolates were transferred to suitable media for algae cultivation in glass flasks of (250 ml), each containing a liquid (100 ml) of Bold's Basal Medium (BBM) added to it (10 ml) of algal isolate and left to grow at a temperature of (25 °C) (Sadvakasova et al., 2023).

2.2 Experimental design and sampling

The study used nine glass aquaria (20 cm x 40 cm x 30 cm) representing three treatments in three replicates this treatment is control "wastewater alone" (WWA), wastewater treated with *Chlorella vulgaris* (CVS), and wastewater treated with *Chlorella saccharophila* (CSA) as represented in (Table 1). All aquaria were filled with 10 L of water from Industrial wastewater. Microalgae were batch-added in the treatment aquaria whereas no microalgae were added to the control. The control treatment consisted solely of wastewater alone in the test water. The study used an inoculum of microalgae at a concentration of approximately 8×10^4 cells/ml. Samples of test water were taken at different periods throughout the duration of the experiment for water quality analysis.

2.3 Analysis of water quality

The parameters collected for the water quality analysis were as follows: pH, water temperature, electrical conductivity (EC), and total suspended solids (TSS) for in situ analysis; and chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), total dissolved solids (TDS), biological oxygen demand (BOD), total solids (TS), and ammoniacal nitrogen (TAN) for ex situ analysis. A portable gadget was used to gather the in-situ parameters. Water samples were taken from each of the three sites and submitted for laboratory analysis in accordance with standard protocols in order to determine ex situ parameters (Table 2) according to (Maiolo & Pantusa, 2021).

2.4 Statistical analysis

The distribution's normality was examined using the Shapiro-Wilk normality test. Before processing the percentage data, an arcsine transformation was performed. Data analysis was conducted using GraphPad Prism 9 (GraphPad Prism v9.0, San Diego, CA, USA). As of right now, all results are given as means with a standard error of the mean. A one-way ANOVA was used to compare various treatments. Tukey's multiple comparisons were appropriately employed as a post hoc test in these situations.

3. Results

Table 1. Treatments and Experimental design.

NO.	Treatments	Description	Abbreviation	Number of aquariums
1	Control	wastewater alone	WWA	3 Glass aquarium
2	<i>Chlorella vulgaris</i>	Wastewater treated with <i>Chlorella vulgaris</i>	CVS	3 Glass aquarium
3	<i>Chlorella saccharophila</i>	Wastewater treated with <i>Chlorella saccharophila</i>	CSA	3 Glass aquarium

Table 2. Parameters of water quality and the kinds of analysis techniques used.

Abbreviation	Parameters	The Analysis instrument Used
pH	pH	pH meter
Temp. (°C)	Temperature (°C)	EC meter
EC (µS/cm)	electrical conductivity (µS/cm)	EC meter
BOD (mg/L)	Biological oxygen demand (mg/L)	Standard method
COD (mg/L)	chemical oxygen demand (mg/L)	HACH-DR6000 UV VIS— Spectrophotometer
TN (mg/L)	Total nitrogen (mg/L)	HACH-DR6000 UV VIS— Spectrophotometer
TS (mg/L)	Total solids (mg/L)	HACH-DR6000 UV VIS— Spectrophotometer
TP (mg/L)	Total phosphorus (mg/L)	HACH-DR3900 UV Spectrophotometer
TAN (mg/L)	Ammoniacal nitrogen (mg/L)	HACH-DR6000 UV VIS— Spectrophotometer
TSS (mg/L)	total suspended solids (mg/L)	Gravimetric method
TDS (mg/L)	Total dissolved solids (mg/L)	EC meter

3.1 Comparison of Physicochemical Characteristics of *Chlorella* Species-Treated Wastewater

Chlorella vulgaris, *Chlorella saccharophila*, and wastewater alone were the three treatments that were found to differ greatly from one another in the physico-chemical analysis of the water samples. The treated samples had reduced electrical conductivity, total nitrogen, total solids, total phosphorus, and total suspended solids values when compared to the untreated wastewater. There was no significant difference found between CVS and CSA, but the results did show a significantly lower biochemical oxygen demand in CVS and CSA when compared with WWA. Conversely, when compared to both WWA and CSA, chemical oxygen demand demonstrated notable variations in CVS. When comparing total dissolved solids to WWA and CVS, the value in CSA was noticeably lower. The statistical analysis showed that treatment with *Chlorella* species significantly reduced different physicochemical parameters of wastewater. This difference was statistically significant at $p < 0.05$, indicating that there is no chance involved with respect to the reduction of said parameters. Overall, the results obtained in this study indicated that the species of *Chlorella* may be further explored for the treatment of waste water due to their capability to clear pollutants in the water that show in (Table 3).

Table 3. Analysis of the physicochemical parameters (mean \pm SEM) in the water sample results.

Water Quality Parameters	Treatments			P-values
	WWA	CVS	CSA	
Temp. (°C)	27.98 \pm 0.3435	27.98 \pm 0.3544	28.20 \pm 0.3682	0.8804
EC (µS/cm)	618.1 \pm 14.08 ^a	384.3 \pm 6.204 ^b	325.0 \pm 10.10 ^c	<0.0001
BOD (mg/L)	27.88 \pm 0.1808 ^a	21.02 \pm 0.6245 ^b	19.81 \pm 0.2524 ^b	<0.0001
COD (mg/L)	143.1 \pm 1.435 ^a	132.8 \pm 0.8947 ^b	127.4 \pm 1.979 ^b	0.0001

TN (mg/L)	32.78±0.6134 ^a	18.43±1.089 ^b	21.83±0.7254 ^c	<0.0001
TS (mg/L)	925.1±16.93 ^a	643.1±7.514 ^b	585.4±13.26 ^c	<0.0001
TP (mg/L)	5.145±0.1252 ^a	4.008±0.1357 ^b	4.183±0.04922 ^b	<0.0001
TSS (mg/L)	152.6±8.929 ^a	45.80±1.142 ^b	67.54±8.067 ^b	<0.0001
TDS (mg/L)	384.9± 2.324 ^a	220.7±7.863 ^b	196.9± 4.638 ^c	<0.0001

Where "WWA" is wastewater alone, "CVS" is Wastewater treated with *Chlorella vulgaris* and "CSA" is Wastewater treated with *Chlorella saccharophila*. Means within each raw that lack common superscripts differ significantly at $p < 0.05$.

3.2 Impact of Wastewater Treatment with *Chlorella* Species on pH

Figure.1 show the wastewater samples treated with *Chlorella saccharophila* (CSA), wastewater treated with *Chlorella vulgaris* (CVS), and wastewater alone (WWA) all had significantly different pH levels. In comparison to WWA, the pH values in CVS and CSA were considerably lower. The results of the statistical analysis demonstrated that the *Chlorella* species treatment was successful in lowering the wastewater's pH levels. The statistical significance of the differences between the treatments ($p < 0.05$) suggests that the observed pH reductions were not the result of random variation. Overall, the findings point to the possibility of using *Chlorella* species in wastewater treatment due to their ability to lower pH levels and enhance water quality.

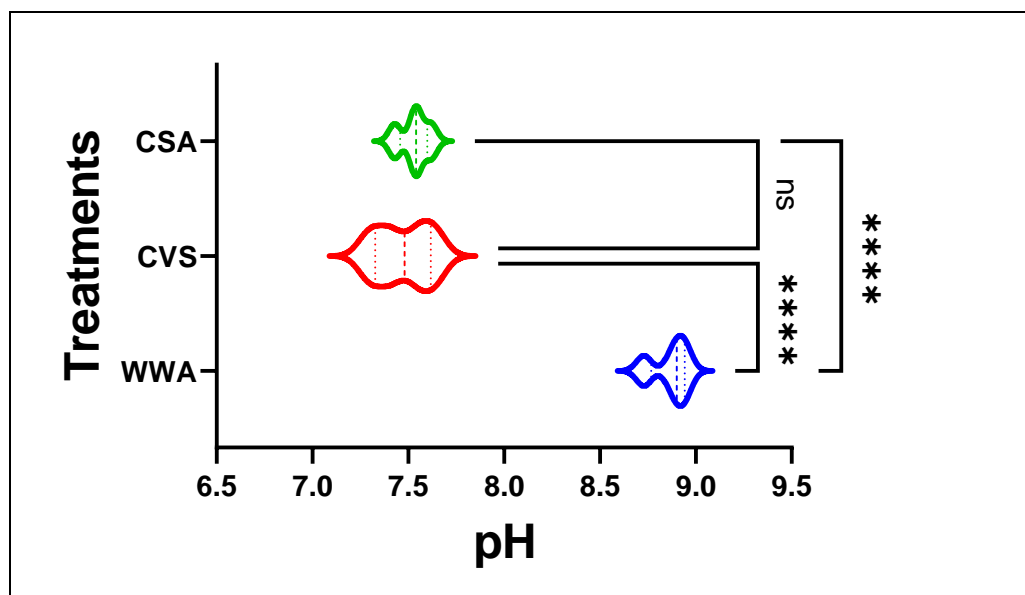


Figure1. The values of pH that recorded across the experimental.

Values are expressed as mean \pm SEM from triplicate groups. Asterisks on the data bars indicate significant differences between the experimental groups to their control when $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

3.3 Removal of Total Ammoniacal Nitrogen (TAN) from Wastewater Using *Chlorella* Species

Three treatments were found to have significantly different mean levels of TAN in wastewater samples: wastewater alone, wastewater treated with *Chlorella vulgaris* and *Chlorella saccharophila*, and WWA combined with wastewater treated with these two species. In comparison to WWA, the TAN level was discovered to be extremely low

under both CVS and CSA. As a result, the analysis did show that treating the wastewater with *Chlorella* species was successful in lowering its TAN level. The statistically significant difference between the treatments (p -value < 0.05) indicates that the observed reductions in TAN were not coincidental. In conclusion, because *Chlorella* spp. efficiently removes TAN and enhance water quality, they may be used to treat wastewater see Figure2.

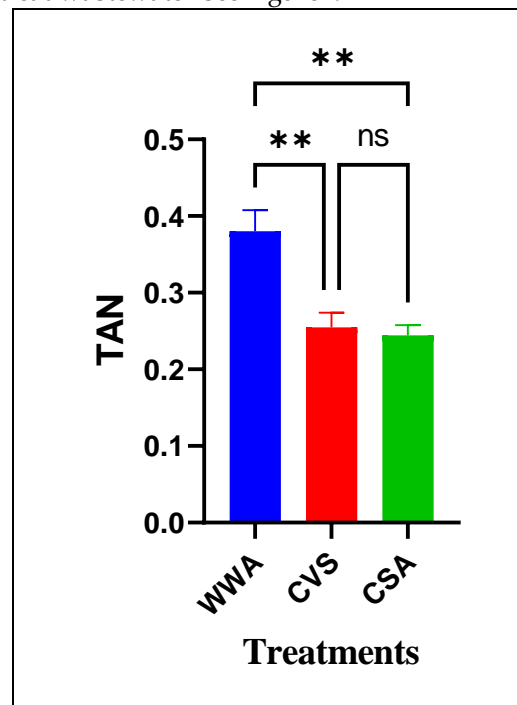


Figure2. the values of Total ammoniacal nitrogen (TAN) that recorded across the experimental.

Values are expressed as mean \pm SEM from triplicate groups. Asterisks on the data bars indicate significant differences between the experimental groups to their control when $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

4. Discussion

Chemical analysis showed that treating wastewater with *Chlorella vulgaris* (CVS) and *Chlorella saccharophila* (CSA) resulted in a notable improvement in wastewater quality. When compared to untreated wastewater, treated samples showed a significant decrease in electrical conductivity (EC), a measure of dissolved salts. In a similar manner, both microalgae species exhibited the desirable ability to decrease the total solids (TS) defined as all suspended and dissolved inorganic and organic materials as well as the total nitrogen (TN) which is one of the nutrients affecting eutrophication. Also, total suspended solids (TSS), that pollute water and pose threat to aquatic life forms, and total phosphorus (TP), another nutrient of interest were lowered in both CVS, CSA. Despite the superiority of the pseudonymised contaminated water treatment group, both treatment groups were able to reduced the biochemical oxygen demand (BOD), which means that there is a decrease of organic matter that may lead to the depletion of dissolved oxygen in water that is vital for the inhabitants of water bodies. Compared with both negative control, CVS had a more pronounced efficiency in the removal of COD, which indicated that it was better at absorbing and removing easily oxidizable organic matter than that of the levels in untreated wastewater and CSA samples (Oruganti et al., 2022). Interestingly, CSA was found to be better than both, untreated wastewater and CVS-treated samples in the efficiency of removing dissolved solids – TDS and hence it seems to have a positive application in certain areas. *Chlorella* species prove to be very effective in wastewater treatment in concerning the eradication of several pollutants and enhancing the overall quality of the water. The variation in the approach utilized by *Chlorella* species in cleaning

water is most likely leads to the recorded declines in pollutants. The absorption of nitrogen and phosphorus by these microalgae serves the purpose of these nutrition-rich substances for their own growth, thus acting as nutrient sinks at the micro scale, removing these two nutrients from the water (Jacob-Lopes et al., 2020). Additionally, *Chlorella* species use a two-pronged strategy to break down organic matter: physisorption and chemisorption along with the process of biodegradation (Bharti et al., 2022). They produce enzymes that secrete substances that destroy large molecules, found in natural compounds, into smaller and more easily absorbed molecules. By way of notably lowering the natural load, this –pronged attack improves water pleasant. The observed variations in pollutant removal efficiency among CVS and CSA may be because of intrinsic organic variations. CVS's stepped-forward removal of general dissolved solids (TDS) and effortlessly oxidizable natural be counted (COD) might be attributed to better metabolic activity. CVS can also have a bigger urge for food than CSA, allowing it to ingest those additives greater speedy, resulting in an extra fall in awareness. Extra observation into each species' specific metabolic techniques and dietary options may shed mild on those capacity blessings, bearing in mind the choice of the most effective *Chlorella* species for positive wastewater treatment programs (Mu et al., 2021). The cutting-edge observation provides solid proof for the efficacy of *Chlorella* species as an efficient and ecologically friendly wastewater treatment approach. The microalgae displayed achievement in handling an extensive form of contaminants through considerably lowering nutrients which includes nitrogen and phosphorus, natural remember as seen with the aid of a drop in BOD and COD, and perhaps even heavy metals. This multi-pronged method resulted in handled wastewater that met discharge criteria across numerous metrics, suggesting that it was secure to launch into the surroundings. *Chlorella* species additionally have a protracted-term benefit for the reason that they could thrive within the equal contaminants they cast off, and they may even create treasured biomass as a byproduct. All matters taken into consideration, those outcomes reveal the potential of *chlorella*-based totally bioremediation as an economical and sustainable method of coping with wastewater.

When both CVS and CSA were incorporated in to the wastewater treatment both the flask containing the CVS and CSA treated wastewater showed comparatively lower pH value than the untreated wastewater (WWA). This shift in favor of acidity is probably due to the combined effect of two prongs of the algae. The first is that to feed *Chlorella* species, they require nitrogen and phosphorus; hence reduce the pH of the water solution since these are line alkaline nutrients (Tan et al., 2021). In addition, due to their own metabolic processes, algae may produce organic acids that will make the solution get even more acidic (Cheng et al., 2022). Due to the fact that some of the industrial wastewaters may require a change in their pH before they can be discharged safely, this observed change in pH is yet again, an indication of another strength of using *Chlorella*-based treatment. The fact that the pH of the water reduces after the application of *chlorella* in the treatment of wastewater holds a lot of interesting applications. First of all, procedures such as flocculation and coagulation, which are the downstream treatment procedures, will be enhanced by acid conditions (Fu et al., 2021). These methods are more effective at lower pH levels and are critical in the reduction of suspended solids. Second, the acidity may hinder the growth of some pathogenic bacteria, which would essentially increase the overall quality and safety of the treated wastewater (Hashem & Qi, 2021). "This twofold memorialisation hits the better downstream processing and possible pathogen control making *Chlorella* based bioremediation an even more valuable and adaptable instrument in the WWTP portfolio." The difference in the biological make up of CVS and CSA might explain the observed differences in the extent of decrease in pH between the two. Workers have stated that *Chlorella vulgaris* multiplies faster and absorbs more nutrients compared to *Chlorella saccharophila* (Piasecka & Baier, 2022). As to why CVS resulted in a greater extent of pH reduction in wastewater, it could have been due to this enhanced biochemical activity (Zhao et al., 2022). Because CVS has a stronger hunger for nutrients, particularly nitrogen and phosphorus, it would remove these alkaline substances faster than CSA, resulting in a greater pH drop. Greater research into every species' precise metabolic

pathways and nutritional choices could help determine the quality Chlorella stress for optimum pH control in wastewater remedy packages (Taghavijeloudar et al., 2021). The cutting-edge have a look at provides strong evidence for the efficacy of Chlorella species as an efficient and ecologically pleasant wastewater treatment technique. Microalgae considerably reduced nutrients, organic debris, and probably even heavy metals, effectively fighting a selection of contaminants (Singh et al., 2021). This multi-pronged method ended in treated wastewater that met discharge criteria throughout several metrics, suggesting that it changed into secure to release into the environment. Chlorella species additionally have a protracted-term benefit considering the fact that they are able to thrive in the identical contaminants they cast off, and they may even create usable biomass as a byproduct (Deviram et al., 2020). Past pollutant removal, the observed pH drop improves downstream treatment performance and inhibits pathogenic bacteria improvement (Oruganti et al., 2022). However, further research is wanted to enhance factors together with developing conditions and algae pressure selection in an effort to fully recognize the promise of Chlorella-primarily based bioremediation. Furthermore, in order to guarantee the system's sustainability and efficacy in actual wastewater treatment applications, long-term performance assessments are essential. All things considered, this study opens the door to a viable and sustainable method of managing wastewater.

Regarding the TAN content in the water samples, the results also demonstrated that Chlorella species were effective in reducing the content in the treated WW (WWB) compared to the untreated one (WWA). This drastic decrease in ammonia could be attributed to the combined assault of the algae which is seen as two blades. First are the growth needs of the nutrients such as ammonia since the Chlorella species are understood to be repulsive eaters of used up nutrients (Ahmad et al., 2022). Second, microalgae employed in this research possess the metabolic equipment required for nitrification that is, the conversion of ammonia to nitrate (Salbitani & Carfagna, 2021). This dangerous component in wastewaters, ammonia is directly uptaken and converted in the course of this two-step process for utilization, for instance, as nitrate by even other organisms in wastewaters' ecosystem (Shu et al., 2024).

There are significant implications for the subsequent treatment processes of wastewater and for the environment as a whole based on the lower levels of TAN observed when chlorella is used to treat wastewater. Ammonia is well known toxic substance that inhibits in growth and respiration of the aquatic life (Jahanbani et al., 2023). Chlorella treatment also serves to shield the receiving environments as it hastily detoxifies ammonia, thereby making sure that the water discharged out is far less hazardous (Baldisserotto et al., 2020). Second, during disinfection this decrease minimizes formation of chloramines (Baldisserotto et al., 2020). Indeed when chlorine comes in contact with ammonia it forms chloramines which are far more dangerous to the aquatic life than chlorine itself (Wang et al., 2022). Thus, chlorella-based treatment provides a twofold advantage: It dislodges one toxin outright to prevent the formation of a potent toxin during disinfection, which leads to the release of wastewater that are less toxic (Chai et al., 2021). CSV might outcompete CSA for substrates in the wastewater and thus lead to different removal efficiencies of TAN. In previous research, Chlorella vulgaris has been identified to grow much faster and assimilate nutrients at a faster rate compared to *Chlorella saccharophila*; considering that increased metabolic activity is usually associated with higher detoxification abilities, this might have been the reason why CVS was able to achieve a greater decrease in TAN levels compared to the other algal treatments. Ammonia is a nutrient that would to some extent be incorporated in the nitrogen CV S would more easily take up ammonia if it had a high nutrient. Taking into account all presented aspects, it can be concluded that current research offers significant evidence of positive impact of Chlorella species as efficient, economic and ecological wastewater treatment possibility. (Dragone, 2022). As it can be observed, the results showed low TAN content after the process, as well as considerable reduction of other impurities, thus contributing to the increased quality of the water.

5. Conclusion

The study confirms the potential of *Chlorella* species for low-cost, sustainable wastewater treatment. The algae effectively removed various pollutants, including nutrients, organic matter, and heavy metals. The treated wastewater met all discharge standards, indicating its safety for environmental discharge. *Chlorella* species positively impacted pH and TAN levels in the wastewater, potentially improving the quality and safety of discharge. The study suggests the potential application of *Chlorella* species in low-cost and sustainable wastewater treatment, but further research is needed to optimize the treatment process and assess the long-term performance of *Chlorella*-based systems.

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