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RESEARCH ARTICLE

Phycoremediation Potential of *Nostoc ellipsosporum* and *Spirulina subsalsa* for Pollutant Removal from Textile Wastewater with Integrated Biodiesel Production

S.M.D.C. Bandara, Yuansong Wei, C.L. Abayasekara and R.R. Ratnayake

Highlights

- First use of *Nostoc ellipsosporum* and *Spirulina subsalsa* for textile wastewater treatment, integrating biodiesel production
- Achieved >90% pollutant removal efficiency, showing strong potential for eco-friendly remediation
- Treated textile wastewater enhanced green gram germination and growth, reducing phytotoxicity
- Wastewater-grown biomass yielded 21–25% (w/w) lipids with suitable fatty acids for biodiesel production

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Phycoremediation Potential of *Nostoc ellipsosporum* and *Spirulina subsalsa* for Pollutant Removal from Textile Wastewater with Integrated Biodiesel Production

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Abstract

The present study investigated the potential of two cyanobacterial species, namely *Nostoc ellipsosporum* (NE) and *Spirulina subsalsa* (SS), to remediate textile wastewater (TWW) while obtaining wastewater-grown biomass for biodiesel production. This study reports their multi-faceted benefits using unsterilized and undiluted (100%) TWW for the first time. Both cyanobacterial species were cultivated in TWW under greenhouse conditions, focusing on their growth, TWW decolorization, pollutant removal, phytotoxicity, lipid content, and fatty acid profile. Results demonstrated significant growth and decolorization of TWW by both species, highlighting their potential for sustainable treatment of TWW. NE-treated TWW (NE-TWW) achieved a chemical oxygen demand (COD) removal efficiency of 96.48%, while SS-treated TWW (SS-TWW) reached 93.11%. Ammonia removal efficiencies were recorded as 76.28% for NE-TWW and 96.86% for SS-TWW. NE-TWW and SS-TWW achieved nitrate removal of 69% and 73%, respectively. Phosphate removal was 81.14% for NE-TWW and 33.33% for SS-TWW. Both species exhibited more than 75% removal of heavy metals, particularly Cr, Fe, Zn, Cu, and Ni. Seed germination studies showed enhanced germination and seedling growth of green gram *(Vigna radiata)* when irrigated with NE-TWW and SS-TWW, indicating phytotoxicity reduction in treated TWW. Lipid yields (w/w) of 21.5% and 25% were recorded from TWW-grown biomass of NE and SS, respectively. Both species exhibited favorable fatty acid methyl ester (biodiesel) profiles, dominated by palmitic acid (C16:0), oleic acid (C18:1), stearic acid (C18:0), lauric acid (C12:0), and myristic acid (C14:0), indicating their suitability as biodiesel feedstocks. Overall, this integrated approach not only provides an eco-friendly and cost-effective strategy for TWW treatment but also offers a sustainable feedstock for biodiesel production.

Keywords: Biodiesel; Bioremediation; Cyanobacteria; Phytotoxicity; Textile wastewater

INTRODUCTION

The textile industry is one of the most water-intensive industries, consuming large volumes of water for different manufacturing processes like sizing, de-sizing, scouring, bleaching, mercerizing, dyeing, and printing (Brar et al., 2019; Khan & Malik, 2014), in turn generating significant amounts of wastewater. These wastewaters are typically highly colored due to the high load of toxic dyes, and contain heavy metals, recalcitrant organic compounds, and excessive amounts of inorganic ions. Textile wastewater (TWW) is toxic, carcinogenic, and mutagenic due to the presence of synthetic dyes such as azo, anthraquinone, and phthalocyanine dyes (Khan & Malik, 2014; Khatri et al., 2018). They reduce light penetration and photosynthetic activity in water bodies, consequently limiting oxygen supply to aquatic organisms and inhibiting their growth (Kishor et al., 2020). The discharge of nutrient-rich (nitrogen and phosphorus) wastewater is also a major source of water pollution owing to eutrophication. Thus, nutrient removal has become crucial alongside organic pollutant removal (Hasan et al., 2021).

Conventional wastewater treatment methods, such as chemical coagulation, electro-flocculation, adsorption, advanced oxidation processes, and membrane technology, often present challenges related to high costs, secondary pollution, sludge management, and inefficiency in

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removing complex contaminants (Bharagava & Bhimrao, 2018; Mona et al., 2020). As a sustainable and cost-effective alternative, bioremediation of TWW using cyanobacteria has gained significant attention due to their ability to metabolize pollutants while simultaneously consuming them to produce valuable biomass. This biomass could be employed in the generation of biofuels, biopolymers, and biofertilizers, etc (Sadvakasova et al., 2021).

Cyanobacteria, also known as blue-green algae, are prokaryotic photosynthetic microorganisms known for their rapid growth and high tolerance to extreme environmental conditions. They have demonstrated remarkable potential in the bioremediation of textile effluents (Mathimani et al., 2024; Selvaraj & Arivazhagan, 2024). As photosynthetic organisms, cyanobacteria can release O_2 while assimilating CO_2, unlike bacteria-based wastewater treatment, where a significant amount of CO_2 is emitted. Cyanobacteria employ several mechanisms in TWW bioremediation (Tsolcha et al., 2017). These mechanisms include dye decolorization, biosorption, biomineralization, biotransformation, bioaccumulation, and enzymatic biodegradation, while sequestering heavy metals and assimilating inorganic and organic compounds, leading to improved water quality (Mona et al., 2020; Touliabah et al., 2022). Furthermore, cyanobacteria accumulate high lipid contents under nutrient-stress conditions, making them a promising feedstock for biodiesel production (El-Sheekh et al., 2023). Hence, integrating TWW bioremediation with successive biodiesel production not only addresses environmental concerns but also enhances the economic feasibility of the process. However, the complexity of wastewater and algal ecological characteristics induces techno-economic challenges for its industrial implementation. These challenges include large-scale cultivation, downstream processing, strain robustness, biomass yield, and quality for biodiesel production (Brar et al., 2019; Nguyen et al., 2022).

This study explored the dual role of two filamentous cyanobacterial species, namely *Nostoc ellipsosporum* (NE) and *Spirulina subsalsa* (SS), in TWW remediation and their potential for biodiesel generation. Limited studies have been conducted to explore the potential of cyanobacteria in real TWW bioremediation, compared to other wastewater types (Cuellar-Bermudez et al., 2017; El-Sheekh et al., 2022). Many studies use synthetic dye solutions rather than real TWW, which has complex mixtures of dyes, salts, surfactants, heavy metals, and organic and inorganic compounds. To the best of our knowledge, this could be the first report of utilizing the above-mentioned cyanobacterial species to treat unsterilized and undiluted (100%) real TWW under greenhouse conditions without the addition of any extra nutrients, with a key focus on investigating the practicability of this strategy, saving energy and freshwater usage, in terms of sterilization and dilution, respectively.

The study aimed to evaluate the growth performance of NE and SS in TWW, while focusing on their ability to decolorize TWW. The study assessed their effectiveness in removing COD, heavy metals, and inorganic nutrients (ammoniacal nitrogen, nitrate, phosphate) from TWW. Moreover, this study investigated the impact of treated TWW on seed germination, particularly focusing on shoot and root development. Furthermore, the research objectives were to explore the total lipid content of TWW-grown biomass while assessing their fatty acid profile for biodiesel production. Thus, this research aimed to establish an eco-friendly and cost-effective approach to TWW treatment while employing TWW-grown biomass for biodiesel production. The findings of this study could pave the way for a circular bioeconomy, where wastewater treatment and energy generation are seamlessly integrated for environmental and industrial sustainability.

MATERIALS AND METHODS

Cyanobacterial species and collection of textile wastewater

The cyanobacterial cultures (NE and SS) were obtained from the NIFS-Sri Lanka culture collection (NIFS-SLCC), registered in the World Data Centre for Microorganisms (WDCM) (Reg. No: 1245), and maintained in BG-11 (Stanier et al., 1971) medium in 250 ml conical flasks at room temperature under 2500 lux of white fluorescent light till the exponentially growing phase is attained. TWW was collected from a leading textile company in the Biyagama Export Processing Zone (6.9775° N, 79.9800° E), Sri Lanka, and its initial physicochemical parameters were assessed in accordance with the established guidelines provided by the American Public Health Association (APHA, 2012). The pH, electric conductivity (EC), salinity, and total dissolved solids (TDS) were measured onsite using a portable multiparameter (Oyster™, NH, USA) (Table 1).

Evaluation of cyanobacterial growth and TWW decolorization

A 10% (v/v) homogenous algal suspension was inoculated into transparent glass tanks containing a total volume of 20 L of unsterilized and undiluted (100%) TWW. Each treatment was triplicated, and they were maintained under greenhouse conditions with a photoperiod of 12:12 h. Continuous aeration was provided using conventional aerator pumps, ensuring uniform bubbling across all tanks. Biotic control (10% algal suspension inoculated in BG-11 medium) and abiotic control (uninoculated 100% TWW) were maintained along with the treatments. The growth of NE and SS was monitored during three-day intervals by measuring optical density at 680 nm using a UV–visible spectrophotometer (Agilent Carry 60, CA, USA) over a period of 28 days to monitor their growth. To assess the decolorization of the TWW by NE and SS, a UV-spectral scan was done initially within the visible range (400 nm to 800 nm) to find the maximum absorbance wavelength (λ_{max}) of the untreated TWW, and it was found as 775 nm. Samples were withdrawn at three-day intervals, centrifuged at 3500 rpm for 10 mins to pellet the cyanobacterial cells, and the absorbance of the supernatant was measured at the pre-

determined λ_{max}. The percentage of TWW decolorization was calculated using the following equation (Javed et al., 2022).

$$\text{Decolorization}\left(\%\right) = \frac{\text{Abs (I)} - \text{Abs(F)}}{\text{Abs (I)}} \text{ x 100}$$

Where Abs (I) and Abs (F) are the initial and final absorbance values of the sample, respectively.

Analysis of pollutant removal efficiency

Ammoniacal nitrogen (NH_4^+) was analyzed by the salicylate method (Method 10023) using HACH powder pillows. Ammonia salicylate reagent powder pillows and ammonia cyanurate reagent powder pillows were used for the analysis. Nitrate (NO_3^-) was measured by the cadmium reduction method (Method 8039) using HACH powder pillows. NitraVer® 5 Nitrate reagent powder pillows were used for analysis. COD was determined by the reactor digestion method (Method 8000) using HACH COD digestion vials (High range). Samples were analyzed in a DR900 colorimeter (HACH, CO, USA). The phosphate (PO_4^{3-}) removal efficiency was determined by the ascorbic acid method with potassium hydrogen phosphate (dibasic) as the standard. The above procedures followed the stipulated protocols in standard methods for wastewater analysis (APHA, 2012). Heavy metals were determined in micro-filtered TWW samples using an ICP-OES (Thermo Scientific, iCAP 7000, US) with an argon plasma. The removal efficiencies were calculated according to the following equation.

$$\text{Removal efficiency}\ (\%) = \frac{\text{Conc (I)} - \text{Conc (F)}}{\text{Conc (I)}} \text{ X 100}$$

Where Conc (I) and Conc (F) are initial and final concentration values (mg/L) of the sample, respectively.

Phytotoxicity assay

A phytotoxicity assay was performed to evaluate the toxicity of untreated and treated TWW. The test was carried out using green gram (*Vigna radiata)* under laboratory conditions. The undamaged seeds of almost identical size were surface sterilized using 2% sodium hypochlorite, washed thoroughly, and soaked overnight in sterile distilled water. Four seeds were placed in sterilized petri plates layered with two Whatman qualitative (Grade 01) filter papers. Two milliliters of TWW treated with NE and SS were watered into each plate daily up to 5 days. Positive control was run by watering the seeds with distilled water, while the negative control was run by watering with untreated TWW. Each treatment was triplicated. Germination (%) was calculated according to the following equation, while the growth of seedlings was observed by measuring the lengths of plumule/shoot, and radicle/root compared to the negative control using the ImageJ (Image processing and analysis in Java) (version 1.53t) software.

$$\text{Germination}\ (\%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Harvesting of TWW-grown cyanobacterial biomass for lipid analysis

Fresh biomass of NE and SS grown in 100% TWW was harvested after 28 days of initial culturing by the filtration method using a cell strainer (pore size of 5 µm). The harvested biomass was washed thrice with distilled water to remove any residual particles attached to the biomass and transferred into aluminum trays. They were oven-dried at 55°C until a constant weight was achieved. The dried biomass was ground into a fine powder, weighed, and stored in the freezer (-4˚C) for lipid extraction and analysis.

Lipid extraction and quantification

Lipid extraction was done in a Soxhlet apparatus (Biobase®, China) using hexane as the extraction solvent. Two grams of dried TWW-grown biomass of NE and SS were taken and wrapped in a Whatman No. 1 qualitative filter paper. It was refluxed with 50 ml of hexane for six hours with a water condenser. The hexane layer containing the extracted lipids was collected and vacuum-dried in a rotary evaporator (Heidolph™, Germany) at 37 ˚C to evaporate the solvent. The dried lipid was weighed, and the total lipid content was calculated gravimetrically on w/w % of dry biomass by taking the difference in the pre- and final weights of the vial. For each treatment, three replicates were maintained.

$$\textbf{Total lipid content (\%)} = \frac{\text{Final weight of the vial} - \text{Initial weight of the vial}}{\text{Weight of the initial dry algal sample}} \times 100$$

Fatty acid profile analysis

Fatty acid methyl esters (FAMEs)/ biodiesel were prepared from extracted lipids following an acid-base transesterification reaction as follows: 200 µl of lipids were reacted with 2 ml of methanol containing 2% (w/v) NaOH for 15 min at 65 ºC in a water bath. Then, 2 ml of methanol containing 5% (v/v) H_2SO_4 was added to the screw-capped glass tube and heated for another 15 min at 65 ºC. Esterified fatty acids were extracted into 1 ml of hexane by centrifugation (1500 rpm for 10 mins) (Javed et al., 2022). FAMEs were analyzed using a gas chromatograph (Agilent 7890B, CA, USA) equipped with a flame ionization detector (FID) in a capillary column (100 m×0.25 mm×0.2 µm) (Agilent J & W CP-Sil 88 for FAME, CA, USA). Ultra-high purity nitrogen gas (99.9995%) was used as the carrier gas. Initial column temperature was set at 100 °C, which was progressively raised to 180 °C at 8 °C/min, and then to 230 °C at 1 °C/min. The injector was retained at 260 °C with an injection volume of 1 µL in a splitless mode. The peaks were quantitatively identified by comparing the retention times with those of a mixture of external standard methyl esters (Supelco® 37 FAME Mix, Sigma Aldrich, St. Louis, MO, USA). The results were expressed as the relative percentage of the total peak area of FAMEs considered.

Statistical analysis

All experiments were replicated three times. Data were presented as mean ± standard deviation. The statistical analysis of the data was performed using analysis of variance (ANOVA), and when significant effects were detected, the groups were compared using a post-hoc Tukey's HSD test. The level of significance used for all statistical tests is 5% ($p < 0.05$). The statistical program used was Minitab version 19.2.

RESULTS & DISCUSSION

Characterization of textile wastewater

The pH, electric conductivity (EC), salinity, and total dissolved solids (TDS) were recorded as 9.93, 704 µS, 350 mg/L, and 470 mg/L, respectively. The initial characteristics of the TWW revealed a high COD value of 3046 mg/L, along with higher concentrations of phosphates (168 mg/L), ammoniacal nitrogen (70 mg/L), and nitrates (200 mg/L) (Table 1). Initial heavy metal concentrations were observed as Cr (36.91 µg/L), Fe (0.7 µg/L), Zn (7.79 µg/L), Cu (2.34 µg/L) and Ni (0.78 µg/L). These findings highlighted the necessity for adequate treatment of TWW before discharge into the environment. The changes of physicochemical parameters after TWW treatment by NE and SS are also shown in Table 1.

Cyanobacterial growth in textile wastewater

The growth patterns of NE and SS in TWW were assessed by measuring optical density (OD) at 680 nm (Figure 1), a standard wavelength for monitoring cyanobacterial growth and chlorophyll content. Both strains exhibited a gradual increase in OD values over the cultivation period, indicating successful adaptation and proliferation in the wastewater environment. During the study, average daytime temperatures ranged from 24 ± 2 °C to 30 ± 2.0 °C, and light intensity varied between 18,000 and 55,000 lux depending on seasonal fluctuations. The pH of the TWW gradually declined over time, from an initial value of 9.93 to 8.54 (NE-treated TWW) and 8.62 (SS-treated TWW), providing an optimum condition for cyanobacterial growth (Table 1). Among the two, NE showed the most promising growth, achieving a growth rate of 90.82% ± 0.09, consistently displaying a higher OD increase compared to SS, which recorded a growth rate of 83.57% ± 0.06.

The observed growth trends highlighted the adaptive capabilities of cyanobacteria in TWW, demonstrating their ability to utilize TWW as a nutrient source while tolerating its toxic components. Cyanobacteria are known for their resilience to environmental stressors and efficient uptake of inorganic nutrients (e.g., ammoniacal nitrogen, nitrates, and phosphates) from industrial effluents, promoting biomass accumulation (Cai et al., 2013). The higher growth rate of NE (a heterocystous filamentous cyanobacterium) may be attributed to its nitrogen-fixing ability, allowing it to thrive even in nitrogen-limited conditions commonly found in TWW. Conversely, SS is a non-heterocystous cyanobacterium that relies on available nitrogen sources for growth, which may explain its relatively lower but still significant growth rate. Inorganic phosphates presented in the TWW also could have contributed to the notable algal growth, playing a key role in their metabolism (Mouga et al., 2024). These findings suggest that TWW could serve as a low-cost, sustainable cultivation medium for cyanobacterial biomass production, reducing the need for synthetic growth media while simultaneously supporting bioremediation applications. Further optimization of cultivation parameters, such as light intensity, aeration, and supplementation with additional nutrients, could enhance the growth efficiency of these strains in industrial-scale applications.

Decolorization of textile wastewater

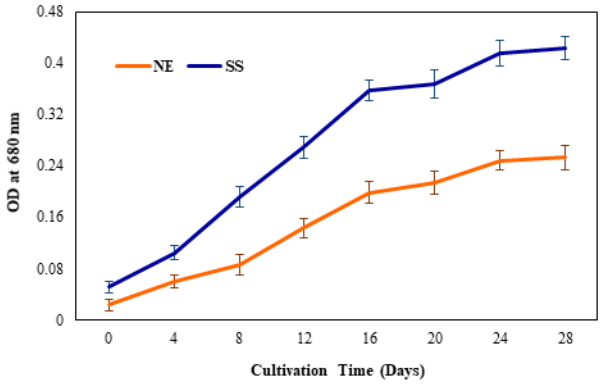


Figure 1: Growth performance of *Nostoc ellipsosporum* (NE) and *Spirulina subsalsa* (SS), expressed as optical density at 680 nm (OD680) over cultivation time

Decolorization of TWW by NE and SS was evaluated by measuring OD at 775 nm. Significant color removal of TWW was observed from NE and SS with 79.03% ± 0.09 and 82.67% ± 0.09, respectively (Figure 2). These results indicated that both strains possess strong biosorption and biodegradation capabilities, contributing to dye removal from TWW. The observed decolorization can be attributed to several mechanisms, including biosorption (cell surface adsorption), bioaccumulation (active transport of dye molecules into the cell), extracellular polymeric substance (EPS) secretion, and enzymatic degradation (biodegradation). The negatively charged cell walls of cyanobacteria facilitate electrostatic interactions with dye molecules, allowing passive dye adsorption, while intracellular metabolic processes and oxidative enzymes (e.g., azoreductases, laccases, peroxidases) further contribute to biodegradation (Dudeja et al., 2025; Mishra and Maiti, 2019; Sudarshan et al., 2023).

Efficiency of Chemical Oxygen Demand (COD) removal

The NE and SS significantly reduced the initial COD value of 3042 mg/L to 107 mg/L and 210 mg/L, achieving COD removal efficiencies of 96.49% and 93.11%, respectively.

This directly implied their ability to successfully consume organic matter present in TWW suggesting their capability to bioremediate TWW with high COD levels. A high COD level indicates a complex mixture of organic pollutants, which may require extended treatment time to remediate (Nguyen et al., 2022). Since COD is an indirect measure of organic compound concentration, assessing its reduction is crucial before TWW is discharged into the environment. According to Selvaraj and Arivazhagan, (2024), there is a strong correlation between high biomass productivity and COD removal efficiency since cyanobacteria utilize organic carbon as an energy source and a nutrient substrate for their growth. This ultimately improves the wastewater quality to permissible levels, and it was evident in this study.

The effective COD utilization by NE and SS can be attributed to factors such as species-specific metabolic activity, growth conditions, and nutrient availability in TWW. Additionally, oxygen generation by these photosynthetic prokaryotes enhances the biological degradation of organic matter, further contributing to COD reduction in TWW (Selvaraj and Arivazhagan, 2024). In a similar study, Javed et al., (2022) observed a COD removal of 59% by *Chlorella vulgaris* on unsterilized, 100% TWW, which was lower than the COD removal efficiencies observed in this study using *Nostoc* sp. and *Spirulina* sp. Brar et al., (2019) observed a 50% reduction of COD by *Anaebaena ambigua* in sterilized, 75% TWW. Raza et al., (2024) observed a COD reduction of 91.5% by a consortium of *Chlorella vulgaris* and *Staphylococcus* sp.

Nutrient removal efficiency

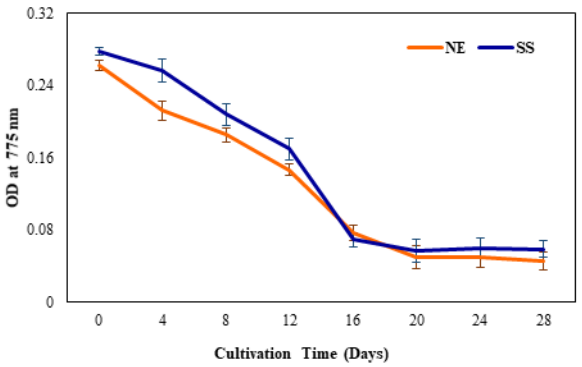


Figure 2: Textile wastewater decolorization by *Nostoc ellipsosporum* (NE) and *Spirulina subsalsa* (SS); expressed as optical density at 775 nm (OD775) over cultivation time

There was a significant reduction in the concentration of ammoniacal nitrogen, nitrate, and phosphate present in the TWW after the phycoremediation experiment. NE achieved a 76.28% ammoniacal nitrogen removal, while SS exhibited a higher removal of 96.86%. The nitrate reduction was recorded as 69% and 73% with NE and SS, respectively. Meanwhile, the phosphate removal was detected as 81.14% with NE and 33.33% with SS. Overall, SS demonstrated a superior nitrogen removal efficiency, whereas NE was more efficient in phosphate removal. All these findings highlighted the potential of selected cyanobacterial species for nutrient removal from TWW. These reductions in nutrient levels corresponded with an increase in algal biomass, demonstrating a direct relationship between nutrient uptake and growth. However, some studies indicated that phosphorus uptake by cyanobacteria may not always be efficient, depending on species and environmental conditions (Tsolcha et al., 2017), which is also evident by SS in this study.

Phosphorus (P) and Nitrogen (N) are key pollutants present in wastewater, posing significant threats to aquatic ecosystems, leading to eutrophication (Preisner et al., 2021). Cyanobacteria play a crucial role in nutrient removal from wastewater through N assimilation and phosphorylation. These two processes facilitate the uptake of N and P, which are vital for algal metabolism, growth, and biomass production. They assimilate N by converting inorganic N sources: nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) into organic forms required for the synthesis of peptides, proteins, enzymes, chlorophylls, and genetic materials (RNA and DNA) via enzymatic reduction (Inabe et al., 2021; Raza et al., 2024). Among the different N sources, ammonium (NH_4^+) is preferred by cyanobacteria due to its lower energy requirement for assimilation. Wastewater rich in ammonium, therefore, serves as a promising medium for cyanobacteria cultivation (Nguyen et al., 2022; Znad et al., 2018). Phosphorus is another essential macronutrient required for the production of lipids, proteins, nucleic acids, and ATP (adenosine triphosphate). Phosphate assimilation by cyanobacteria through phosphorylation is the primary mechanism for phosphate removal in wastewater (Znad et al., 2018). Inorganic phosphorus exists primarily in the forms of $H_2PO_4^-$ (phosphoric acid) and HPO_4^{2-} (hydrogen phosphate). These forms are absorbed by cyanobacteria through phosphorylation, where phosphate is incorporated into intracellular organic compounds via specialized phosphate transporters in the plasma membrane (Burford et al., 2023; Markou et al., 2014)

Various environmental factors affect the nutrient uptake rates in different cyanobacterial species, including initial nutrient concentration, light intensity, airflow rate, extracellular pH, temperature, and inoculum size (Tsolcha et al., 2017). Due to their diverse physiological and morphological traits, different species of cyanobacteria demonstrate varied effectiveness in removing N and P from wastewater effluents. Hence, the selection of an appropriate algal strain is crucial for optimizing nutrient removal efficiency. Based on a study by Brar et al., (2019), a nitrate removal of 52.95% and phosphate removal of 63.05% were achieved on sterilized, 75% TWW by *Anaebaena ambigua.* Raza et al., (2024) observed increased removal efficiencies by a *Chlorella vulgaris-Staphylococcus* consortium, achieving NO_3^- (58.57%), and PO_4^{3-} (89.19%), compared to algae-only treatment.

Table 1: Changes in physicochemical characteristics of textile wastewater before and after treatment with *Nostoc ellipsosporum* and *Spirulina subsalsa*

Initial assessment of textile wastewater (TWW)			TWW treated with *Nostoc ellipsosporum*		TWW treated with *Spirulina subsalsa*	
Characteristics of TWW	**Unit**	**Before treatment**	**After treatment**	**Removal efficiency (%)**	**After treatment**	**Removal efficiency (%)**
pH	N/A	9.93	8.54	-	8.62	-
Electric conductivity (EC)	µS	704	906	-	1677	-
Salinity	mg/L	350	457	-	839	-
Total dissolved solids (TDS)	mg/L	470	375	20.2	390	17.02
Chemical oxygen demand (COD)	mg/L	3046	107	96.49	210	93.11
Total phosphate (PO_4^{3-})	mg/L	168	31.68	81.14	112	33.33
Ammoniacal nitrogen (NH_4^+)	mg/L	70	16.6	76.28	2.2	96.86
Nitrate (NO_3^-)	mg/L	200	62	69	146	73
Chromium (Cr)	µg/L	36.91	7.31	80.20	9.14	75.24
Iron (Fe)	µg/L	0.7	0.07	90	0.09	85.71
Zinc (Zn)	µg/L	7.79	1.25	83.95	1.51	80.61
Copper (Cu)	µg/L	2.34	0.17	92.74	0.55	76.50
Nickel (Ni)	µg/L	0.78	0.17	78.21	0.05	93.59

Heavy metal removal efficiency

The data in Table 1 showed that heavy metal concentrations in untreated TWW were significantly reduced after biotreatment by NE and SS. NE exhibited higher removal efficiencies for Fe (90%), Zn (83.95%), Cu (92.74%), and Cr (80.20%) compared to SS, which achieved removal efficiencies of 85.71%, 80.61%, 76.50%, and 75.24%, respectively. However, SS showed superior Ni removal (93.59%) relative to NE (78.21%). Other heavy metals were not detected in the untreated TWW, suggesting that their concentrations were below the detection limit.

Algae exhibit a high potential for heavy metal removal due to their large surface area and strong binding affinity. Metal uptake mainly occurs through surface/extracellular adsorption (biosorption) as well as intracellular accumulation (bioaccumulation), where metals bind to compounds such as phytochelatins, metallothioneins, polyphosphates, and other intracellular ligands. These metal-binding ligands result in a change of the oxidation state of heavy metals and accumulation in the vacuole, where conversion of toxic form into a non/less toxic form occurs (Dudeja et al., 2025; Selvaraj et al., 2023).

On the other hand, the algal cell wall contains various functional groups, including sulphydryl, carboxyl, amino, phosphoryl, and hydroxyl groups, which impart a net negative charge to the cell surface. Since most heavy metals in wastewater occur as positively charged ions, they are readily attracted and adsorbed onto algal biomass. Cellular metabolic activities can transform absorbed metals into non/less toxic forms (Minhas et al., 2026). Furthermore, cyanobacteria can uptake certain heavy metals as essential micronutrients required for their growth and metabolism (Fe, Cu, Zn, Ni). However, when these detoxification mechanisms fail, excessive metal accumulation can lead to toxic effects on algal cells, particularly when heavy metal concentrations in the effluent exceed the permissible limits (Dudeja et al., 2025; Minhas et al., 2026). Although concentrations of heavy metals present even in the untreated TWW used in this study were much lower than the permissible discharge limits, heavy metal analysis of dried cyanobacterial biomass after TWW treatment is recommended before downstream applications.

Effect of cyanobacteria-treated textile wastewater on seed germination and seedling growth

The effects of tap water (positive control), untreated TWW (negative control), and treated TWW by NE (NE-TWW) and SS (SS-TWW) on seed germination and seedling growth are depicted in Table 2. Seeds in the positive control, NE-TWW, and SS-TWW treatments exhibited a 100% germination rate, whereas the negative control had a significantly lower germination rate of 25%. The initial physicochemical analysis revealed that TWW was toxic in nature due to the excess pollutant concentrations, as shown in Table 1. It was further confirmed by having an inhibitory effect on seed germination and seedling growth. After phycoremediation, the toxicity of TWW was significantly reduced and showed improved seed germination, indicating their pollutant removal capability.

A seed was deemed to have successfully germinated if its root system was visible and measurable, with a minimum length of 1 mm. Seeds that did not germinate were assigned a root length of zero (Rekik et al., 2017). In this study, shoot length was highest in the positive control (8.12 cm), followed by NE-TWW (6.88 cm) and SS-TWW (6.54 cm), while the negative control exhibited the shortest shoot length (1.99 cm). Similarly, root length followed the same trend, with the positive control (6.44 cm) having the longest roots, followed by NE-TWW (4.97 cm) and SS-TWW (4.24 cm), whereas the negative control had the shortest root length (1.02 cm). These findings are in accordance with a similar study conducted by Selvaraj and Arivazhagan (2024), which had maximum shoot and root lengths of approximately 8.5 cm and 5 cm, respectively, after 05 days irrigated with TWW treated with *Spirulina platensis*. Elakbawy et al. (2022) reported a shoot length of 5.69 cm on tomato seedlings when testing the effect of *Nostoc muscorum* crude extracts on treated sewage wastewater.

Cyanobacteria release secondary metabolites to the medium during their growth, including plant growth hormones, micronutrients, macronutrients, and vitamins (Elakbawy et al., 2022). The release of these beneficial compounds by cyanobacteria could also be one of the reasons for the enhanced shoot and root development observed in the present study. These results suggest that the phycoremediated TWW enhances the germination process by providing additional nutrients to the medium, highlighting the potential use of phycoremediated TWW for irrigation. The reason for reduced seed growth in untreated TWW could be due to the excess nutrient concentrations, high levels of COD, and heavy metals, which retard seedling growth by affecting water absorption and other metabolic activities (Dhaouefi et al., 2019). Different crop plant species also differ widely in response to different concentrations of wastewater with respect to seed germination, seedling growth, and productivity (Fendri et al., 2013).

Total lipid content of textile wastewater-grown cyanobacterial biomass

Lipid content and lipid composition in cyanobacterial cells are key factors determining their potential for biodiesel production (Hawrot-Paw et al., 2021; Nagappan et al., 2020). The total lipid contents of the TWW-grown cyanobacterial strains are shown in Figure 3. It is worth mentioning that NE and SS demonstrated significant lipid yields of 21.5% (w/w) and 25% (w/w), respectively, compared to several cyanobacterial strains used in TWW bioremediation (Mathimani et al., 2024). To the best of our knowledge, this is the first report that the lipid content and lipid profile of NE and SS grown in unsterilized and undiluted TWW have been analysed. These results suggest that both strains can thrive in unsterilized-raw TWW, utilizing it as a favorable growth medium while achieving enhanced lipid production. The variation in lipid content among the two species could be a result of differences in their morphology, biochemical composition, and native environmental adaptations. Moreover, lipid profile and productivity are also influenced by multiple factors, including cultivation conditions, biomass harvesting, cell disruption methods, strain selection, solvent polarity, and extraction techniques (Fazal et al., 2018; Javed et al., 2022). Importantly, harvested biomass could be contaminated by residual toxic compounds such as dye intermediates and heavy metals. Therefore, further investigations are required to quantify contaminant accumulation in cyanobacterial biomass and evaluate effective biomass purification strategies (e.g., repeated washing, solvent-based purification, and monitoring of residual contaminants) to improve biomass safety before downstream applications.

Higher lipid accumulation in algae under wastewater conditions is attributed to nutrient limitation, which enhance lipid synthesis (Feng et al., 2011; Mahmood et al., 2023). Specifically, the nitrogen (N) and phosphorus (P) levels in wastewater may be lower than those in standard growth media (i.e., BG-11, Zarrouk), inducing nutrient starvation that triggers lipid accumulation (Purba et al., 2022). Mostafa et al., (2012) reported lipid contents of 15.5% and 7.3% for *Nostoc humifusum* and *Spirulina platensis*,

Table 2: Phytotoxicity of treated and untreated textile wastewater, showing germination (%), shoot length, and root length

Treatment	Tap water	TWW	NE-TWW	SS-TWW
Germination (%)	100.00[a]	25.00[b]	100.00[a]	100.00[a]
Shoot length (cm)	8.12[a]	1.99[c]	6.88[b]	6.54[b]
Root length (cm)	6.44[a]	1.02[c]	4.97[b]	4.24[b]

respectively, when cultivated in domestic wastewater for biodiesel production. Hossain et al., (2018) reported a lipid content of 15.43% in *Nostoc* sp. cultured in half-strength BG-11 medium, while Nagappan et al., (2020) observed a lipid content of 15.7% in *Nostoc* sp. MCC41, when grown in BG-11 medium. Ambrozova et al., (2014)reported a lipid content of 13.41% for *Spirulina platensis* cultivated in BG-11, whereas Haberle et al., (2020) obtained a lipid content of 15.3% for *Spirulina subsalsa* grown in oil refinery wastewater. These findings highlighted the notable lipid yields achieved in TWW-grown NS and SS in this study, reinforcing their potential as viable feedstocks for biodiesel production.

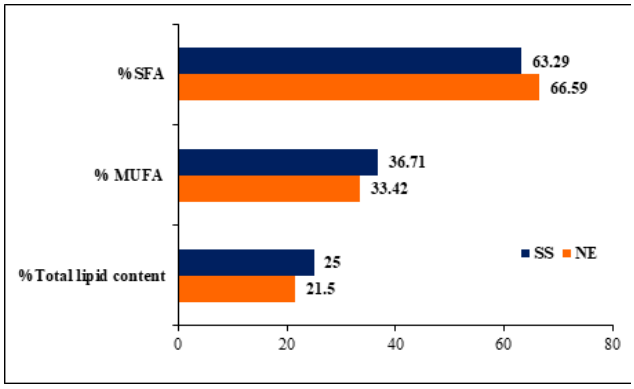


Figure 3: Total lipid content (%), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA) in textile wastewater-grown *Nostoc ellipsosporum* (NE) and *Spirulina subsalsa* (SS)

Fatty acid methyl ester (FAME) analysis for biodiesel production

Characterization of the FAME profile is crucial to determine the biodiesel quality of cyanobacteria (Mathimani et al., 2024). Therefore, the fatty acid composition of TWW-grown NE and SS was analyzed using GC-FID. In algal FAME profiles, fatty acids with carbon chain lengths of C16–C18 are the most commonly identified, as also confirmed by the results of the present study. The key FAMEs that determine biodiesel quality include palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and low concentrations of linoleic acid (C18:2) and linolenic acid (C18:3) (Hawrot-Paw et al., 2021). According to Knothe, (2005), two critical factors influencing biodiesel quality are oxidative stability and low-temperature performance.

In the present study, both cyanobacterial strains exhibited a similar fatty acid composition, though slight variations were observed in the percentages of each fatty acid. The key FAMEs identified in both cyanobacterial strains are palmitic acid (C16:0), oleic acid (C18:1), stearic acid (C18:0), lauric acid (C12:0), and myristic acid (C14:0) (Table 3). Notably, palmitic acid and oleic acid together accounted for more than 70% of the dominant FAMEs recorded in both species. Similarly, *Phormidium lucidum* grown in oil refinery wastewater was investigated for biodiesel production and was found to have palmitic and oleic acids as the predominant fatty acids, comprising 64% of the total FAMEs (Haberle et al., 2020), which is consistent with our findings.

Cyanobacterial lipids are rich in both saturated and unsaturated fatty acids. Unsaturated fatty acids (UFAs) enhance biodiesel's cold flow and cloud point, preventing fuel solidification in cold weather. However, UFAs are more prone to oxidation and rancidification unlike saturated fatty acids (SFAs). In contrast, SFAs contribute to crystal formation at low temperatures, leading to poor cold-flow properties (Sadvakasova et al., 2021; Sinha et al., 2016). Since UFAs improve low-temperature performance, while SFAs enhance oxidative stability, a well-balanced FAME composition is essential for optimal biodiesel quality, specifically with low levels of UFAs. Among UFAs, monounsaturated fatty acids (MUFAs) are ideal for biodiesel production, whereas polyunsaturated fatty acids (PUFAs) are less favorable. Oleic acid (C18:1), a MUFA, is considered optimal due to its balanced oxidative stability and low-temperature performance (Feng et al., 2011; Knothe, 2005). High PUFA content can increase nitrogen oxide emissions, but a low PUFA concentration positively affects biodiesel's flow properties, particularly at low temperatures (Hawrot-Paw et al., 2021). In this study, palmitic acid (C16:0) was the most abundant fatty

Table 3: Major fatty acid methyl esters (FAMEs) present in cyanobacterial biomass cultivated in textile wastewater

Name of fatty acid methyl ester	Carbon number	Fatty acid percentage (%) in NE biomass	Fatty acid percentage (%) in SS biomass
Palmitic acid	C16:0	45.92	45.88
Oleic acid	C18:1 (cis (n9)	24.77	28.13
Stearic acid	C18:0	8.82	5.63
Cis-10-pentadecanoic acid	C15:1	8.65	-
Cis-10-heptadecanoic acid	C17:1	-	8.58
Lauric acid	C12:0	6.66	6.64
Myristic acid	C14:0	5.19	5.14

acid, followed by oleic acid (C18:1) in both strains. NE contained 45.92% (C16:0) and 24.77% (C18:1), while SS had 45.88% (C16:0) and 28.13% (C18:1). As shown in Figure 3, the SFA and MUFA percentages in both strains were optimal, suggesting their suitability for third-generation biodiesel production.

CONCLUSION

This study demonstrated the dual potential of NE and SS in TWW bioremediation and biodiesel production. Both cyanobacterial species exhibited significant growth in undiluted and unsterilized TWW, effectively removing pollutants while achieving high decolorization efficiencies. NE and SS exhibited notable COD removal efficiencies of 96.48% and 93.11%, respectively. SS showed superior ammoniacal nitrogen removal (96.86%), whereas NE exhibited better phosphate removal (81.14%) and nitrate removal (73%). Significant heavy metal removal efficiencies (>75%) were observed in both species. The treated wastewater significantly improved seed germination and seedling growth, highlighting their potential in TWW detoxification. Furthermore, the TWW-grown cyanobacterial biomass demonstrated high lipid accumulation, with SS yielding 25% (w/w) total lipids and NE yielding 21.5% (w/w). Fatty acid profiling confirmed the presence of key biodiesel precursors, such as palmitic acid (C16:0), oleic acid (C18:1), and stearic acid (C18:0), indicating their suitability for sustainable biodiesel production. This integrated approach presents a cost-effective and environmentally friendly alternative for TWW treatment while simultaneously producing biodiesel from wastewater-grown biomass. Future research should focus on the pilot- and field-scale applications of cyanobacteria-based bioremediation systems to evaluate their long-term stability, pollutant removal efficiency, and economic feasibility under real environmental conditions for large-scale implementation, while evaluating the safety of wastewater-grown biomass for downstream applications.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING STATEMENT

The authors declare that no funding was received for this study.

AUTHOR CONTRIBUTION

Conceptualization: **S.M.D.C.B., R.R.R., C.L.A., Y.W.;** Data collection, statistical analysis, data interpretation, and writing the original draft: **S.M.D.C.B.;** Supervision, manuscript editing, and reviewing: **R.R.R.**, **C.L.A.**, **Y.W.**

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study will be made available to the public upon request.

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