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Phycoremediation Efficacy of Different Microalgae Species in Treating Poultry Slaughterhouse Wastewater in Bauchi Local Government Area, Bauchi State, Nigeria

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Abstract

The study explores the phycoremediation efficiency of two freshwater microalgae species in poultry slaughterhouse wastewater (PSWW) of Bauchi Poultry House. The research aimed to isolate and identify these species based on their morphological appearance, determine their efficiency based on physicochemical parameters, and molecularly characterize the species with the best performance. The data was analyzed using an independent t-test in SPSS version 27. *Chlorella* and *Chlorogonium* species were identified based on morphological appearance. The results showed significant differences in nitrate phycoremediation between days 0 and 7, and 14 for *Chlorella* and *Chlorogonium* species ($p < 0.05$), while no significant difference was observed between days 14 and 21 ($p > 0.05$). The phycoremediation efficacy of phosphate showed significant differences between days 0 and 7, while only *Chlorella* species showed significant differences between days 7 and 14 ($p<0.05$). Total dissolved solids (TDS) showed significant differences in phycoremediation efficiency ($p<0.05$) between day 0 and day 21 in PSHWW effluents for Chlorella and *Chlorogonium* species. Biological Oxygen demand (BOD) phycoremediation efficacy showed no significant difference in the PSWW effluent between day 0 and day 7, but a significant difference ($p<0.05$) was recorded between days 7 to 21 in the two algal species. The phycoremediation efficacy of the two species in PSWW effluents showed increased pH levels between days 0 and 21. However, comparing the phycoremediation efficacy of *Chlorella* and *Chlorogonium* species in PSWW effluents showed no significant differences in nitrates, phosphates, TDS, BOD, and pH. The sequence and phylogenetic tree analysis of the microalgae species with the best phycoremediation was found to be *Chlorella vulgaris* using the 18S gene. In conclusion, *Chlorella vulgaris* and *Chlorogonium* species were all efficacious and should be recommended for public health precautions for PSWW.

Keywords: *Poultry slaughterhouse wastewater, Chlorella vulgaris, Chlorogonium spp, Phycoremediation, Microalgae*

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Introduction

The poultry sector ranks as the second largest contributor to global meat production (Vladić *et al*., 2023). Poultry effluents are generated significantly at agro-industrial farms and slaughterhouses globally, resulting from animal slaughter and meat processing activities (Ferreira *et al*., 2019). In 2019, the poultry sector had significant growth, with global chicken meat output nearing 128 million metric tons, reflecting a 3% rise compared to the yearly production in 2018. Freshwater is utilized in this industry for multiple processes, such as bird washing, cleaning, cooling, waste transport, and slaughtering (Yaakob *et al*., 2018). Poultry slaughterhouse wastewater (PSWW) is identified as a highly polluting effluent, characterized by substantial levels of organic matter, mixture of fats, suspended solids, proteins, blood, as elevated levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and nutrients, including nitrogen and phosphorus, derived from slaughter and cleaning processes, which require specialized treatment before environmental disposal (Hilares *et al*., 2021, Pérez-Guzmán *et al*., 2024). This type of wastewater is regarded as one of the most polluted. The elevated levels of nitrogen and phosphorus are the main contributors to the eutrophication phenomenon in open waters, which has emerged as a significant environmental concern in recent decades (Mujtaba *et al*., 2017).

The elevated organic content presents multiple challenges, such as unpleasant odours, decomposition, and the establishment of conditions conducive to the growth and accumulation of insects and vectors (Jindal *et al*., 2019). Consequently, discharging this wastewater into the environment without adequate treatment may present a significant risk (Yaakob *et al*., 2018; Cui *et al*., 2020). It may also lead to substantial environmental and health issues, including deoxygenation of rivers, groundwater contamination, and the proliferation of waterborne infections (Fatima *et al*., 2021).

Before wastewater treatment, it is essential to characterize the wastewater to determine pollutant levels using various equipment and tests. The metrics typically employed to characterize PSWW are nitrogen, phosphate, pH, biochemical oxygen demand (BOD), and total suspended solids (TSS) (Rinquest *et al*., 2019). The nutrients in wastewater are total nitrogen (TN) and total phosphorus (TP); nitrogen exists in wastewater in both organic forms, predominantly in proteins and inorganic form, which encompasses nitrite (NO2−) and nitrate (NO3−). The most stable form of nitrogen in water is nitrate, which derives from the natural decomposition of living material. Elevated nitrates in wastewater can result in detrimental algal blooms and oxygen depletion.

Furthermore, orthophosphate (PO_4^{3-}) is the predominant form of phosphorus derived from disinfectants and cleaning agents in wastewater. Chemical precipitation is a practical and effective method for reducing phosphorus in wastewater (Yaakob *et al*., 2018). BOD denotes the biological oxidation of organic substances, and elevated BOD levels indicate substantial amounts of organic contaminants in wastewater (Fatima *et al*., 2021). The parameters differ among slaughterhouses due to several factors, including system type, operational approach, and processing capacity.

Multiple techniques, such as chemical coagulation, aerobic-anaerobic digestion, and electrocoagulation, have been conventionally utilized to treat wastewater originating from poultry slaughterhouses. The limitations of physical and chemical methods encompass the need for significant space, reliance on chemicals, the demand for complex and expensive equipment, and the production of hazardous byproducts (Dehghan Banadaki *et al*., 2024). Consequently, these traditional methods are considered ineffective and costly. There is increasing interest in investigating the potential of biological treatment using microalgae species as a viable alternative for the future (Dehghan Banadaki *et al*., 2024, Adou *et al*., 2020). Microalgae-based biological treatment methods typically demonstrate reduced energy consumption relative to conventional methods. This may result in lower energy costs, operational expenses, and a diminished requirement for chemical additives. These advantages can reduce chemical costs in the wastewater treatment process (Bilińska *et al*., 2016).

The use of microalgae in wastewater treatment is regarded as more environmentally sustainable and effective compared to bacteria-based methods, as it significantly decreases pollutants and pathogens (de Wilt *et al*., 2016). This method also allows for the potential use of sludge produced during the treatment process for fertilizer production and the development of other bioproducts, which is currently under investigation. Numerous studies have investigated various species of algae for wastewater treatment.

It is essential to assess the efficacy of two microalgae species in treating PSWW. Based on their morphological characteristics, this study aimed to isolate and identify two potential microalgae species, *Chlorella* and *Chlorogonium*. It also sought to evaluate their efficiency through various physicochemical parameters and conduct molecular characterization of the most effective isolated microalgae.

Materials and Methods

Poultry Slaughterhouse Wastewater (PSWW) sampling

Poultry Slaughterhouse Waste Water (PSWW) samples were aseptically collected from Yan Kaji Muda Lawan, Bauchi Local Government Area, Bauchi State. The collected samples were preserved, transported to the laboratory, and stored at 4ºC in the refrigerator before analysis.

Isolation of Microalgae

Water samples from the Bauchi State University Gadau, Nigeria's fishwater ponds, were suspended in 500 mL of distilled water. The supernatant was transferred to BG-11 solid culture medium (Zamani *et al*., 2012), and the petri dishes were incubated at 25ºC room temperature, which leads to optimum growth of microalgae, and placed next to a glass window in the laboratory to provide natural light for two weeks incubation period. After colonization, the isolation and purification were performed using the plate agar method to obtain unialgal cultures. The microalgal cells were grown at room temperature in a liquid BG-11 medium. For the spread-plate technique, 1 mL of diluted sample was transferred to an agar plate using the pipette technique and spread evenly on the media surface with the applied aseptic technique. For the streak-plate technique, grown microalgae colonies were streaked on new agar plates under sterile conditions for further isolation. The streaking method was repeated until a single algal species was obtained.

Identification of microalgae

Algae strains were isolated and differentiated based on the morphological examinations of colonies, such as colour, shape, and size, once they grew well on the agar plate. The two different microalgae were morphologically identified, according to Prescott (1982).

Pre-cultivation of Microalgae

The microalgae were pre-cultured in a 500 mL Erlenmeyer flask of BG-11 culture medium. pH was adjusted at 10.1. The culture was cultivated under light conditions with a regimen of 24 h at 27ºC of temperatures. The culture was shaken by hand twice a day. The culture was transferred into a new 2 L Erlenmeyer flask, and BG-11 medium was added until the total culture volume reached 1 L.

Poultry Slaughterhouse Wastewater (PSWW) and algal mixture preparation

The PSWW sample and cultivated microalgae were mixed in certain amounts. The PSWW samples were diluted with each microalga. Each microalgae was added to a 250 ml conical flask at 10% concentration (25 mL of microalgae and 225 mL of PSWW). The total mixture of microalgae and PSWW was 250 mL and measured using a measuring cylinder.

Determination of the phycoremediation efficacy of the two algal strains on the physicochemical parameters of PSWW

The initial physicochemical analysis of the PSWW samples was made before and after the inoculation of the specific algae. The wastewater indices measured in this study include total nitrates (TN), total phosphorus (TP), total dissolved salts (TDS), biochemical oxygen demand (BOD) and hydrogen ion concentration (pH) ((APHA), 2005).

Determination of Percentage Nutrient Removal

The percentage removals of the nitrate, phosphate, TDS, and BOD were computed using the formula.

$$P = \frac{C_i - C_f}{C_i} \times 100$$

where *P*= Percentage removal, *Ci*= Initial concentration and *Cf*= is the final concentration

Molecular Characterization

DNA extraction and PCR amplification

Genomic DNA was isolated from the microalgae with the best efficacy in treating the PSWW following the manufacturer's instructions, utilizing the Accu prep genomic DNA extraction kit from Bioneer. The study amplifies the 18SrRNA region. The amplification process involved the use of the P45 forward primer (5′- ACCTGGTTGATCCTGCCAGT -3') and the P47 reverse primer (5′- TCTCAGGCTCCCTCTCCGGA -3') (Bérard *et al*., 2005). The PCR reaction of the genomic DNA extracted was performed in a 25µl response in a Mastercycler gradient PCR machine. The 25µL PCR reaction mixture comprised of 5µL PCR buffer at 5X, 2µL of 25mM MgCl2, 0.4µL dNTP's from 10mM stock, 0.63U/25µL reaction mixture of Taq polymerase (Stock 5U/µL), 1µL of 18SrRNA forward and reverse primers (10mM), 1µL genomic DNA template of concentration between 20-100ng/µL and the volume was made up to 25µL with sterile distilled water. The negative control was performed by adding sterile water to the PCR mixture to ensure no exogenous DNA was introduced to the PCR reaction. The thermocycler program for the 18S rRNA region of the microalgae was set up with an initial denaturation step at 95ºC for 2 minutes, followed by denaturation at 95ºC for 5 minutes, 1-minute annealing at 45ºC, and 2 minutes extension at 72ºC repeated for 30 cycles and a final extension cycle of 5 min at 72ºC. The PCR products were observed on a 1.5% agarose gel stained with ethidium bromide and illuminated with UV light using a gel documentation system (Syngene, USA). The PCR product was purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) following the directions provided by the manufacturer.

Sequence Analysis and construction of Phylogenetic tree

The 18S gene sequencing results were confirmed using BLAST software analysis in the NCBI GenBank database, available at http://www.ncbi.nlm.nih.gov/. The gene sequences were aligned using the Clustal W multiple sequence alignment tools. A phylogenetic tree was then constructed based on the sequence information using the neighbour-joining tree method in MEGA6 software.

Data Analysis

After data collection, entry was done using Microsoft Excel, 2000 for Windows and later exported to SPSS version 27 for the analysis. Descriptive statistics, means percentages and standard deviations were used to summarise the physio-chemical parameters of PSWW. An independent t-test was used to assess and compare the pH, BOD, TDS, nitrates, and phosphates levels in all the periods, that is, day 0, day 7, day 14, and day 21 in the algae-treated effluents of PSWW. T-tests were also used to compare the pollutant removal efficacy of the two algae species in the PSWW. In all the analyses, p<0.05 was considered significant, and interpretations were done appropriately. Results were presented in the form of tables and figures.

Results

Phycoremediation efficacy of *Chlorella* spp. and *Chlorogonium* spp. on nitrate of PSWW effluents

The phycoremediation of nitrate in PSWW effluents is shown in Table 1. At the initial concentration range of 28.95 mg/L, about 64.52-77.42% of nitrate was removed by *Chlorella* spp., and 45.16-67.74% of nitrate was removed by *Chlorogonium* spp. from the PSWW effluents. In the phycoremediation efficacy of nitrate content of PSWW effluent between day 0 and day 7, there was a significant difference in phycoremediation using *Chlorella spp. and Chlorogonium* spp. (p-value=0.001 and p-value=0.002) respectively. Between day 7 and day 14, *Chlorogonium* spp. had no significant difference (p=0.07), with *Chlorella* having a significant difference (p-value=0.001). Between day 14 and day 21, a non-significant difference of p-value= 0.621 and p-value= 0.152 was recorded for *Chlorella* spp. and *Chlorogonium* spp., respectively. However, the phycoremediation efficiency between *Chlorella* spp. and *Chlorogonium* spp. in treating PSWW effluent showed no significant difference (p=0.396) in nitrate removal.

Table 1: Phycoremediation efficacies of C*hlorella* spp. and *Chlorogonium* spp. on the nitrate of PSWW effluent

Parameter	Days	*Chlorella* spp.	% removal	*Chlorogonium* spp.	% removal
Nitrate	0	28.954±2.140	0%	28.954±2.140	0%
	7	10.274±1.617	64.52%	15.878±2.140	45.16%
	14	7.472±2.140	74.22%	11.675±2.088	59.68%
	21	6.538±2.140	77.42%	9.340±2.140	67.74%

Phycoremediation efficacy of *Chlorella* and *Chlorogonium* species on phosphate of PSWW effluents

The phycoremediation of phosphate in PSWW effluents is shown in Table 2. At the initial concentration range of 98.06 mg/L, about 30.97-73.32% of phosphate was removed by *Chlorella* species, and *Chlorogonium* species removed 13.05-59.36% of phosphate from the PSWW effluents. In the phycoremediation efficacy of phosphate content of PSWW effluent between day 0 and day 7, there was a significant difference in phycoremediation using *Chlorella* species and *Chlorogonium* species (p-value=0.001 and p-value=0.045), respectively. Between day 7 and day 14, Chlorella species had a significant difference (p-value 0.001), while *Chlorogonium* species had non-significant differences (p-value 0.0953). Between day 14 and day 21, a significant difference of (p-value 0.001) and (p-value 0.032) was recorded for *Chlorella* and *Chlorogonium* species, respectively. However, the comparison of phycoremediation efficacy of *Chlorella* spp. and *Chlorogonium* spp. of PSWW effluent was determined and found not to vary significantly (p-value 0.140) in the phosphate removal.

Table 2: Phycoremediation efficacies of C*hlorella* and *Chlorogonium* species on the phosphate of PSWW effluent

Parameter	Days	*Chlorella* spp.	% removal	*Chlorogonium* spp.	% removal
Phosphate	0	98.06±3.20	0%	98.06±3.20	0%
	7	67.69±0.42	30.97%	85.26±24.19	13.05%
	14	49.09±4.07	49.94%	84.33±7.86	14.00%
	21	26.16±1.04	73.32%	39.85±3.40	59.36%

Phycoremediation efficacy of *Chlorella* and *Chlorogonium* species on TDS of PSWW effluents

The phycoremediation of TDS in PSWW effluents is shown in Table 3. At the initial concentration range of 3.25 mg/L, about 0.92-10.77% of TDS was removed by *Chlorella* species, and *Chlorogonium* species removed 0.92-4.31% of TDS from the PSWW effluents. In the Phycoremediation efficacy of TDS content of PSWW effluent between day 0 and day 7, there was a significant difference in phycoremediation using *Chlorella* spp. and *Chlorogonium* spp. (p-value= 0.108 and p-value=0.008) respectively. While between days 7 and 14, both *Chlorella* species and *Chlorogonium* species have a significant difference (p-value=0.001, p-value=0.010), respectively. Between day 14 and day 21, a significant difference of p-value= 0.001 and p-value 0.006 was recorded for *Chlorella* spp. and *Chlorogonium* species, respectively. However, the comparison of phycoremediation efficacy of *Chlorella* species and *Chlorogonium* species PSWW effluent was determined and found not to vary significantly (p-value=0.087) in the phosphate removal.

Table 3: Phycoremediation efficacies of C*hlorella* and *Chlorogonium* species on the TDS of PSWW effluent

Parameter	Days	*Chlorella* spp.	% removal	*Chlorogonium* spp.	% removal
TDS	0	3.25±0.07	0%	3.25±0.07	0%
	7	3.22±0.02	0.92%	3.22±0.01	0.92%
	14	3.06±0.007	5.85%	3.17±0.01	2.46%
	21	2.9±0.01	10.77%	3.11±0.0	4.31

Phycoremediation efficacy of *Chlorella* and *Chlorogonium* species on BOD of PSWW effluents

The Phycoremediation of BOD in PSWW effluents is shown in Table 4. About 22.22-55.56% of BOD was removed from the PSWW effluents by *Chlorella* species and 11.11-44.44% by *Chlorogonium* species at the initial concentration range of 0.9 mg/L. There was no significant difference in the efficiency of phycoremediation employing either *Chlorella* species or *Chlorogonium* species between days 0 and 7 in the BOD content of PSWW effluent (p-value = 0.101). Between days 7 and 14, there is a significant difference between *Chlorella* and *Chlorogonium* species (p-values of 0.001 and 0.010, respectively). For *Chlorella* and *Chlorogonium* species, there was a significant difference between days 14 and 21 with p-values of 0.001 and 0.006, respectively. However, when the phycoremediation efficacy of *Chlorella* species and *Chlorogonium* species for treating PSWW effluent was compared, there were no significant differences (p-value = 0.087) in the phosphate removal.

Table 4: Phycoremediation efficacies of *Chlorella* and *Chlorogonium* species on the BOD of PSWW effluent

Parameter	Days	*Chlorella* spp.	% removal	*Chlorogonium* spp.	% removal
BOD	0	902±72.28	0	902±72.2	0%
	7	772±78.23	22.22%	834±58.38	11.11%
	14	721±79.31	33.33%	648±45.36	33.33%
	21	448±49.28	55.56%	524±5.24	44.44%

Phycoremediation efficacy of suspected *Chlorella* and *Chlorogonium* species on pH of PSWW effluents

Table 5 displays the impact of *Chlorella* and *Chlorogonium* species on phycoremediation on the pH of PSWW effluents. After Phycoremediation, the pH of all PSWW effluents increased from day 0 to day 21 without decreasing. *Chlorella* and *Chlorogonium* species showed a significant variation in the PSWW effluent between day 0 and day 7 with p-values of 0.001 and 0.002, respectively. However, there was no statistically significant variation in the amount of phycoremediation between days 7 and 14, with p-values for *Chlorella* spp. and *Chlorogonium* species being 0.132 and

0.27743, respectively. The phycoremediation efficacy of pH by the two algae species exhibited a consistent non-significant difference between days 10 and 15, with a p-value of 1.0000.

Table 5: Phycoremediation efficacies of *Chlorella* and *Chlorogonium* species on the pH of PSWW effluent

Parameter	Days	*Chlorella* spp.	*Chlorogonium* spp.
pH	0	5.66±0.01	5.66±0.01
	7	7.57±0.04	7.54±0.04
	14	7.77±0.2	7.69±0.04
	21	7.79±0.2	7.87±0.07

In the current work, the 18S region was used to identify the *Chlorella* species, which shows the best phycoremediation efficiency in treating PSWW through a molecular approach. The appearance of a single distinct band indicates that the genomic DNA amplified is of good quality. The fragment size of the 18S region amplified was approximately 400bp, as shown in Figure 1.

The phylogenetic tree analysis using the neighbour-joining (NJ) tree method showed that the *Chlorella* species sequences from the present study formed a strong monophyletic clade with other *Chlorella vulgaris* sequences retrieved from the GenBank database, as shown in Figure 2.

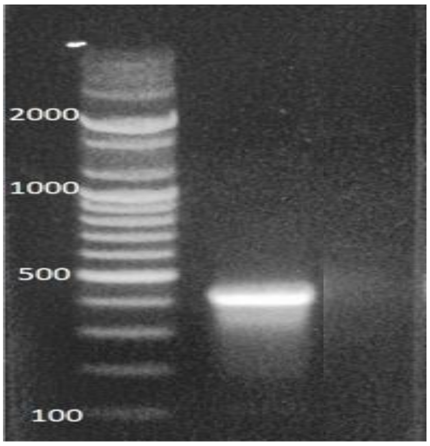

Figure 1: Amplified PCR product of 18S region from the genomic DNA extracted from *Chlorella* species. Lane 1: Isolated *Chlorella* species A, Lane M: 1kb DNA ladder (Promega).

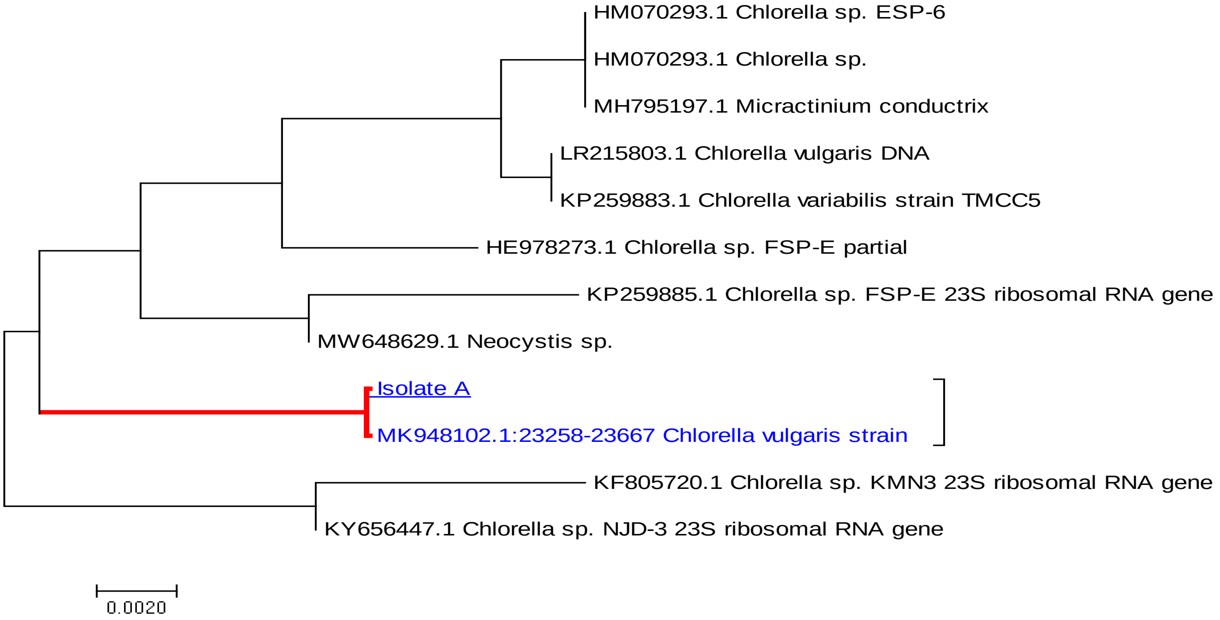

Figure 2: A phylogenetic tree showing the relationship between the 18S region isolated *Chlorella* strains and other sequences retrieved from GenBank. Highlighted in bold red was the moderately supported (BS=65) monophyletic clade. Isolate A: *Chlorella* species.

Discussion

To protect the public from possible exposure to pollutants that may have adverse effects, applying the phycoremediation technique can accumulate and degrade the pollutants in the PSWW. The analysis of various physicochemical parameters, mainly nitrate, phosphate, TDS, BOD, and pH, revealed that

Chlorella and *Chlorogonium* species can effectively reduce the pollutants found in PSWW. The physicochemical parameters were therefore measured for 21 days, with the initial concentrations of each parameter being recorded on day 0 (zero), and these were used as the controls for each parameter.

The specific use of micro-algae in efficiently removing different forms of combined nitrogen and phosphorus has been reported successfully in many studies globally (Shi *et al*., 2007). The polluted PSWW samples were treated with *Chlorella* and *Chlorogonium* species in the present study. The nitrates content was reduced from 28.9mg/l to 6.5mg/l, while *Chlorogonium* species were reduced from 28.9mg/l to 9.3mg/l. In the phycoremediation of the phosphates content in the PSWW effluents, the *Chlorella* and *Chlorogonium* species also showed varied phycoremediation efficacies. The *Chlorella* species reduced the phosphate content from 98.06 mg/l to 26.1 mg/l. At the same time, *Chlorogonium* species also reduced the phosphate from 98.06 mg/l to 39.85 mg/l. The gradual reduction of the phosphorous and nitrates from the wastewater was also attributed to the fact that nutrients had been absorbed from the wastewater by the *Chlorella* and *Chlorogonium* species, mainly for their growth.

Sivasubramanian *et al*. (2012) noted that phosphorous and nitrate concentrations in the wastewater mediums were related to the growth of the microalgae and the eventual reduction in the wastewater without establishing their origin. The phycoremediation of nitrates from industrial effluents by Kshirsagar (2013) showed that nitrate reductions using *Chlorella* species are always high compared to other algae. The high percentage removal of phosphate by the *Chlorella* species observed in the present study could be attributed to the fact that phosphorous nutrients are highly required for their growth, as Rao *et al*. (2011) reported. The present study, conducted under laboratory conditions, established that *Chlorella* and *Chrogonium* species could phycoremediate the PSWW with higher efficiency observed in *Chlorella* spp. Following phycoremediation with *Chlorella* and *Chlorogonium* species, the concentrations of the total dissolved solids (TDS) from the PSWW effluents were shown to decrease for all two algae species utilized. The environmental adjustment of the algae species in the mixture inside the effluents can be attributed to the modest reduction percentages seen on day 7 (Ahmad *et al*., 2013). As the days progressed, particularly on day 14, the exponential phase began to take hold, which caused the reduction percentages to rise, and on day 21, the stationary phase was finally seen.

The TDS removal rate from the effluents was higher in *Chlorella* than in *Chlorogonium* species, and this may be because *Chlorella* species had more functional groups on their cell wall responsible for the high absorption and increased phycoremediation brought about by the various ion exchange potential than *Chlorogonium* species. This may explain why the *Chlorella* species had the highest percentage of removal compared to the *Chlorogonium* species. These reductions in TDS were also reported in another similar study by Kshirsagar (2013), who showed varied wastewater TDS phycoremediation success with *Chlorella* species compared to other types of algae.

The BOD concentrations in the PSWW effluents showed the various levels of toxicities within the effluents and the amounts of oxygen needed by *Chlorella* and *Chlorogonium* species to break down the organic matter found in the wastewater samples. According to the study, the PSWW had a high demand for oxygen because of the high organic matter, as it had an initial BOD of 902 mg/l. This meant that the PSWW had more organic and inorganic pollutants, thus requiring more oxygen molecules to break them down, thus releasing energy for the growth of the algal species, which in turn will drive the process of photosynthesis.

Differences in phycoremediation efficacies were also noted, with *Chlorella* species showing a better phycoremediation efficacy than *Chlorogonium* species in the PSWW. However, these changes in phycoremediation among the algal species could be attributed to the different functional groups found within the algal species, which are essential in the bioabsorption of various wastewater pollutants through the ion exchange mechanism (Elumalai *et al*., 2013). Therefore, the current study showed a marked decline in initial BOD values from high to lower levels at day 14. The progressive reduction in BOD was due to high photosynthetic activities and increased algal growth rate. The high oxidation of carbons releasing carbon dioxide also reduced BOD values. Similarly, the enhanced biological conversion of the wastewater organic matter and the increased biodegradation due to algae might have been the other reason (Elumalai *et al*., 2013).

The phycoremediation effect of *Chlorella* and *Chlorogonium* species on the pH of PSWW increased between day 1 and day 21 without any decrease. The phycoremediation efficacy was significantly different ($p<0.05$) only on day 0 and day 7, while all the remaining days were not statistically significant ($p>0.05$). From the current phycoremediation findings on the pH of the PSWW, the study established a progressive increase in pH from neutral to alkaline across all the different effluents. During the phycoremediation process, while the other physicochemical parameters were decreasing, the pH levels increased initially before remaining at a mean of 8.0 across all the two algal species used in the treatments. The rise in pH levels was attributed to the reduction in the dissolved CO2 concentrations through photosynthesis, which, in turn, raised the pH level (Rao *et al*., 2011).

The phycoremediation efficacy of *Chlorella* and *Chlorogonium* species in PSWW effluent was determined, and the comparison of phycoremediation efficacy between and within groups was found not to vary significantly in all the physicochemical parameters. This implied that the phycoremediation efficacies of *Chlorella* and *Chlorogonium* species in PSWW effluent were almost the same and that the two species were all efficacious in the phycoremediation of PSWW effluent. Therefore, the phycoremediation efficacy exhibited might be due to the structural cell wall similarities of *Chlorella* and *Chlorogonium* species (Lesmana *et al*., 2009).
Also, the phycoremediation efficacy demonstrated by *Chlorella* and *Chlorogonium* species in the present study can be attributed to their biosorbent and adsorption properties

originating from their porous cell walls. Therefore, microalgae *Chlorella* and *Chlorogonium* species improved water quality by removing nitrates and phosphates, with their results showing that all two algae were highly efficacious in pollutant reductions of the wastewater. From the current results, however, the *Chlorella* species was more efficacious in the phycoremediation of physicochemical parameters of PSWW than the *Chlorogonium* species. However, all the two algae showed high efficiency in reducing and removing nutrients. For molecular identification, the identity of the *Chlorella* species isolates was confirmed by DNA sequencing, which also enabled classification at the species level. The species level that was identified was *Chlorella vulgaris*.

Conclusion

The results indicate that both microalgal species possess significant capacity to reduce all assessed physicochemical parameters (nitrate, phosphate, TDS, BOD, and pH) over a 21-day treatment period. However, the comparison of phycoremediation efficacy between and within groups did not vary significantly in all the physicochemical parameters. *Chlorella vulgaris* has shown greater efficacy in the phycoremediation of physicochemical parameters of PSWW compared to the *Chlorogonium* species. Consequently, *Chlorella vulgaris* and *Chlorogonium* species ought to be utilized for the phycoremediation of nitrate and phosphate pollutants, as well as other physicochemical characteristics in PSWW, to mitigate potential contaminants.

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