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Research Article

Assessment of Phycoremediation Potential of *Chlorella sorokiniana* Wastewater Treatment: Analysis of Biochemical Component Changes

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ABSTRACT

Using algae for wastewater treatment offers significant advantages, including providing oxygen for bacteria through photosynthesis, reducing energy demand by eliminating the need for aeration, minimizing the formation of hazardous toxic slush producing useful algal biomass, and recycling nutrients present in the wastewater. In the study, *Chlorella sorokiniana* was isolated from domestic wastewater samples collected from the sewage municipal wastewater of the Katsina metropolis (River Ginzo). The isolated *Chlorella sorokiniana* was cultured in BG-11 media for ten weeks, and cell density was monitored using a haemacytometer. Twelve flasks containing 100mL wastewater samples each were inoculated with 10mL of cultured *Chlorella sorokiniana* and incubated at 25°C under 12/12h light/dark photoperiods for four weeks. Physicochemical parameters were analysed weekly. Results showed an increase in *Chlorella sorokiniana* cell density from (6.33±2.31) $x10^4$ at week one to (53.33±26.60) $x10^4$ at week ten. Remediation efforts resulted in reduction percentages for various parameters: total dissolved solids (57.7%), nitrate (99.96%), phosphorus (100%), ammonium (99.98%), potassium (91.65%), zinc (87.03%), manganese (90.63%), iron (79.44%), copper (50.62%), and nickel (30.10%). pH remained neutral, and dissolved oxygen (DO) increased over the weeks. Significant differences in biochemical components were observed before and after treatment. The remediation of diverse wastewater samples using *Chlorella sorokiniana* was effective, leading to the accumulation of biomass rich in biochemical components such as lipids, carbohydrates, and proteins. The accumulated biomass can be utilized for further research purposes. This study highlights the potential of *Chlorella sorokiniana* for phycoremediation and underscores its ability to improve water quality while generating valuable biomass for additional applications.

Keywords: Isolation, Microalgae, Reduction, Treatment, Wastewater

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INTRODUCTION

Wastewater runoff from various pointed and non-pointed sources including household, agricultural practices, and industrial sectors contain carbon-rich residues along with harmful pollutants that contaminate the environment and cause undesirable changes in the water ecosystem which results in disastrous effects on human health and other living in the environment (Karimi-Maleh *et al.,* 2020).

Different physical, chemical, and biological technologies including flocculation, flotation, membrane filtration, reverse osmosis, ion exchange, electrochemical treatment (Karimi-Maleh *et al.,*

2020), chemical precipitation, adsorption (Saravanan *et al.,* 2021), activated sludge process, solvent extraction, biosorption (Saravanan *et al.,* 2021) etc. have been utilized to remove these harmful contaminants from the environment (Prasannamedha *et al.,* 2021). Various organic substances such as fats, proteins, volatile acids, carbohydrates, and amino acids are present in wastewater in addition to a high amount of inorganic substances including sulphur, arsenic, chlorine, sodium, phosphate, bicarbonate, magnesium, calcium, and ammonium salts (Prasannamedha *et al.,* 2021). The microbiological content of wastewater includes a broad variety of microorganisms, including protozoa and bacteria, which are often harmless and ideal for the biological treatment of wastewater (Ayesha Shahid *et al.,* 2020). Wastewater is sometimes not treated before being discharged into waterways which causes significant problems, particularly where a microbial organism, biochemical oxygen demand (BOD), toxicity, and nutrients are found in combination (Siddiki *et al.,* 2021), wastewater can be used as a nutrient medium in waste biorefineries for the cultivation of valuable organic biomass, namely microalgal biomass (Bhatia *et al.,* 2021,) *Chlorella sorokiniana* has been tested for the treatment of raw municipal wastewater achieving partial decrease of major pollutants, as well as for the removal of organic micro-pollutants from water and wastewater (Shahid *et al.,* 2020). In recent years, microalgae have been used to detoxify various toxicants with different properties and characteristics released from the agricultural, industrial, and domestic sectors (Shahid *et al.,* 2020)

To date, a wide range of microalgae has been reported used in treated wastewater such as *Scenedesmus abundans*, *Botryococcus sp*., *Chlorella v ariabilis*, *Chlorella sp*., *Scenedesmus sp, Scenedesmus obliquus*, *Chlorella vulgaris*, and *Chlorella sorokiniana* (Tran *et al.,* 2021; SundarRajan *et al.,* 2020).

Moving forward, further research and development efforts are needed to optimize algal cultivation techniques, enhance treatment performance, and scale up the implementation of algal-based wastewater treatment systems. By fostering interdisciplinary collaborations and leveraging advances in biotechnology and environmental engineering, the potential of algae as a versatile and cost-effective solution for wastewater remediation can be fully realized.

In conclusion, the integration of algae into wastewater treatment strategies represents a paradigm shift towards more sustainable and eco-friendly approaches to environmental stewardship. By harnessing the inherent capabilities of microalgae, we can move closer to achieving the goal of clean and safe water resources for present and future generations.

Research tends to assess the phycoremediation potential of *Chlorella sorokinina* in wastewater treatment and analyze changes in biochemical components which include evaluating its efficiency in pollutant removal, monitoring variations in biochemical constituents, and determining the overall effectiveness of phycoremediation. This study provides insights into *Chlorella sorokiniana's* application for wastewater treatment and its impact on biochemical composition, thereby contributing to the advancement of sustainable wastewater treatment strategies

MATERIAL AND METHOD

Study Area

Wastewater samples were collected from River Ginzo municipal wastewater of Katsina metropolis. River Ginzo passed along Kofar Durbi, Kofar Marusa, and Kofar Sauri within the Katsina metropolis. The samples were collected along Kofar Durbi and Kofar Marusa. The River has a GPS of 12º, 59', 197" N and 007º 36', 875" E. The activities of the people around the area are; irrigation farming, washing vehicles and other domestic activities such as washing clothes.

Collection of Wastewater Samples

For isolation of *Chlorella sorokiniana*, domestic wastewater samples in this study were collected from River Ginzo Water sample was collected using the 2-litre dark brown bottle as described by Indabawa, (2012).

Media Preparation

BG-11 media (liquid and agar media) were prepared accordingly;

Liquid media: Approximately 900 mL of dH_2O, added to the first nine (9) components in the order specified while stirring continuously, volume was brought to 1L with dH_2O. It is then covered and

autoclaved and allowed to cool and stored at refrigerator temperature.

Agar media: Approximately 400 mL of dH_2O, added to the first nine (9) components in the order specified while stirring continuously, volume was brought to 500 mL with dH_2O. Then separate container, containing 15g of agar solution was added to 500 mL of dH_2O and it was then covered and autoclaved both of the solutions.

In a water bath, both of the solutions were allowed to cool at 45 - 50 °C added sterile Sodium Thiosulfate to the agar solution and mixed well. Then both of the agar and liquid solutions were combined. It was then allowed to cool and stored at refrigerator temperature. Enrichment of Culture in the Media

Collected wastewater samples were brought to the laboratory and the samples were centrifuged at 2000 rpm for 20 minutes. Centrifugation and washing were repeated six times to expel most of the microorganisms present in algal samples and the cells were then inoculated into sterile conical flasks containing media and this was incubated for weeks by providing required environmental conditions such as 12:12h light/dark photoperiods and a temperature of 25°C. (Mohan *et al*., 2009, 2010).

Identification and Isolation of C*hlorella sorokiniana*

Agar plating was used for the isolation of individual species, and the inoculation loop loaded with the natural water sample was streaked across the agar surface, similar to the microbiological method used for bacteria. After incubation for five to seven days, the colonies originating on the surface of agar plates were removed with a nichrome bacterial loop. The microalgae samples were subjected to microscopic observation for correct identification using a compound digital microscope. A standard phycological key described by Edward and David, (2010) was used for the determination and identification of species.

Growth and Maintenance in Media

For the maintenance of algal culture, identified *Chlorella sorokiniana* was inoculated into new conical flasks containing media and was incubated at 25°C provided with 12:12h light/dark photoperiods. The culture was maintained both in slants and broth cultures for future use (Mohan *et al*., 2009, 2010).

***Chlorella sorokiniana* Cell Counting**

Cells were checked under a microscope for satisfactory distribution of cells using a haemacytometer. For essential measurement, the typical number of cells of the centre large square was counted, and the procedure was repeated twice. The cell number is obtained and multiplying the average cell count by conversion factor for Neubauer ($x10^4$). The X40 objective lens was used to count the cells. The average number of cells was counted weekly for ten weeks.

Collection of Wastewater (Sampling of Wastewater Effluent 2)

For the treatment of effluent, the domestic wastewater samples in this study were collected from sewage municipal wastewater River Ginzo Katsina. Wastewater samples were collected in 2-litre bottles which were washed with 10% HNO_3 for 48 hrs, labeled and a few drops of HNO_3 were added to prevent loss of metals using grab sampling techniques (Kaul, and Gautum, 2002). The samples were centrifuged to remove coarse particles and then divided into three replicates.

Remediation Bioassay

Twelve flasks (100mL wastewater samples in 250mL conical flasks) were prepared. Each of the twelve flasks was inoculated with 10ml of cultured *Chlorella sorokiniana.* These were further incubated under the temperature of 25°C provided with 12/12h light/dark photoperiods for four weeks. Samples were analyzed every week for each physicochemical parameter such as pH, total dissolved solids, phosphorus, nitrate, ammonium, DO (dissolved oxygen), and heavy metals, using standard methods APHA, (2005). The pH, DO, BOD, and TDS of the water sample was determined using a calibrated pH meter, DO meter, and TDS electrode probe meter, and three replicated determinations were carried out on each sample.

For the digestion, wastewater sample digestions were carried out as described in standard methods (*APHA, 2005)*. Heavy metals were determined using an Atomic Absorption Spectrophotometer (AAS), Phosphorus (Bray-1), Nitrate, and Ammonium (Kjeldahl distillation method).

Carbohydrate

Carbohydrate was determined by the method described by Chaplin, (1986), 1mL pellet was diluted in 1mL of distilled water 1mL of 5% aqueous solution of phenol in water was added, and then 5mL of concentrated sulfuric acid was added directly to the surface of the solution. After 20 minutes the absorbance was determined at 490nm in a Genesys (spectrophometer). A blank solution was prepared with 1mL of distilled water and 1mL of 5% aqueous solution of phenol and then 5mL of concentrated sulfuric was added directly to the surface of the solution.

The total Lipid

Lipid was analyzed using 50mL harvested culture, which was centrifuged at 3000rpm for 10 minutes. 6mL of chloroform and methanol in a ratio of 2:1 were added to harvested pellets then vortex vigorously for 15 minutes and followed with 1.5mL of distilled water. The sample was centrifuged again at 3000rpm for 10mins and two layers were formed. The empty tube was weighed and transferred lower layer by discarding the upper layer. It was then dried in an oven until all the solvent evaporated, and the tube was reweighted again. Total lipid = weight of tube after drying – the weight of an empty tube.

Protein

Protein was determined using Bradford, (1976). 5mL of culture were centrifuged at 3000rpm for 10 minutes, the supernatant was discarded, then 1.5mL of NaOH was added and vortexed to re-suspend pellets and then it was extracted in an oven at 100^0C for 2hrs. It was centrifuged again at 4000rpm for 10 minutes, supernatant was collected and 4mL Bradford reagent was added to 1mL supernatant and stayed for 5 minutes. Absorbance was read at 595nm spectrophotometer. The bank solution was prepared using 1.5mL of NaOH and 4mL of Bradford reagent.

Data Analysis

Statistical analysis of the result was done from the obtained data, Analysis of variance (ANOVA) was used to compare physicochemical parameters before and after treatment with seven days intervals. Paired T-test was used to compare the changes in biochemical components. A level of significance of $p < 0.05$ was chosen. All the statistical analyses were done with GraphPad Prism Statistical Software Version 6.04.

RESULTS

The results from Table 1 below described the *Chlorella sorokiniana,* growth and development in a control median with a cell density of (6.33±2.31) $x10^4$ at week one and (53.33±26.60) $x10^4$ at week 10.

Table 1: Identified, Isolated, and Cultured *Chlorella sorokiniana* for Phycoremediation in Wastewater Sample from River Ginzo Katsina metropolis

Weeks	Cell density (cells/mL SD)
1	(6.33±2.31) $x10^4$
2	(6.87±2.21) $x10^4$
3	(9.33±4.01) $x10^4$
4	(15.33±5.50) $x10^4$
5	(17.00±6.54) $x10^4$
6	(26.47±4.38) $x10^4$
7	(27.00±4.36) $x10^4$
8	(28.33±5.51) $x10^4$
9	(38.33±11.9) $x10^4$
10	(53.33±26.60) $x10^4$

Table 2. Phycoremediation of municipal wastewater using single *Chlorella sorokiniana* at a temperature of 25^0C, 12:12h light/dark photoperiod

S/N	PARAMETERS	Before Treatment	After Treatment			
			1st week	2nd week	3rd week	4th week
1	pH	7.43±0.01^a	7.50±0.01^b	7.60±0.01^c	7.59±0.01^c	7.69±0.01^d
2	DO(ppm)	1.03±0.01^a	1.08 ±0.01^a	1.13±0.01^a	1.16±0.10^a	1.20±0.05^a
3	TDS(mg/l)	255.33±0.57^a	216±0.00^b	180.33±0.57^c	151.66±1.52^d	108±11.27^e
4	Nitrate(mg/l)	373.61±16.17^a	158.75±16.22^b	97.15±4.64^c	34.13±0.79^d	1.61±1.78^e
5	Phosphorus(mg/l)	14.18±0.03^a	6.67±2.51^b	4.25±0.13^c	2.22±0.31^d	0.00±0.00^e
6	NH_4(mg/l)	214.82±42.80^a	147.83±9.26^b	117.28±6.79^c	18.86±7.03^d	0.04±0.06^e
7	Potassium(mg/l)	25.65±0.25^a	14.28±0.08^b	10.54±0.42^c	6.25±0.29^d	2.14±0.91^e

Means and SDs with same superscripts along the rows indicated no significant differences ($p>0.05$)

Table 3. Phycoremediation of municipal wastewater (Heavy metals) using *Chlorella sorokiniana* at temperature of 25^0C, 12:12h light/dark photoperiod

S/N	PARAMETERS	Before Treatment	After Treatment			
			1st week	2nd week	3rd week	4th week
1	Zinc (mg/l)	0.054±0.00[a]	0.039±0.01[a]	0.32±0.01[b]	0.017±0.00[c]	0.007±0.01[d]
2	Manganese (mg/l)	0.694±0.01[a]	0.373±0.02[b]	0.256±0.03[c]	0.146±0.07[d]	0.065±0.05[e]
3	Iron (mg/l)	11.14±0.16[a]	9.11±0.15[b]	6.76±0.17[c]	4.96±0.17[d]	2.29±0.11[e]
4	copper (mg/l)	0.160±0.02[a]	0.125±0.01[a]	0.122±0.00[b]	0.109±0.01[c]	0.079±0.02[d]
5	Nickel(mg/l)	0.372±0.05[a]	0.307±0.06[a]	0.297±0.00[a]	0.263±0.00[a]	0.26±0.01[b]

Table 4: Described changes in biochemical components (Carbohydrate, Protein, and Lipid) in *Chlorella sorokiniana* exposed to wastewater treatment

Biochemical components	Before Treatment	After Treatment
Carbohydrate	0.1753±0.06[a]	0.947±0.06[b]
Protein	0.117±0.08[a]	0.669±0.36 [a]
Lipid	0.179±0.05[a]	0.958±0.05[b]

DISCUSSION

Standard phycological keys described by Edward and David (2010) were used for the determination and identification of *Chlorella sorokiniana*. *Chlorella sorokiniana, Chlorogonium* shows a cell density of (6.33±2.31) x10^4 at week one and (53.33±26.60) x10^4 at week 10. The finding of this research shows that algal biomass is increased with increases in several days. The finding of this research corresponds with the findings of Chinnasamy *et al.* (2009) which showed the growth response of *Chlorella* in terms of biomass production.

The pH value of bioremediation of wastewater *Chlorella sorokiniana,* maintains around a neutral value of pH. These indicated that there were changes in pH values which remained around neutral. These are in line with the results reported by Aarti *et al.* (2008). Makareviciene *et al.* (2011); and Mostafa *et al.* (2015) found that *Chlorella sustained* the maximum growth rate at the range of pH between 6.0 and 9.0. The value of DO shows an increase. The increase in DO is due to photosynthesis**.** The removal of TDS shows a significant difference across the number of weeks. These are in line with observations made by Mostafa *et al.* (2015) who reported that the TDS of water samples was significantly decreased with algal treatment. The removal of Nitrate, Phosphorus, and Ammonium was considered extremely significant with an increase in several weeks and this agreed with the finding of Aslan and Kapdan, (2006) who used *C. vulgaris* for nitrogen and phosphorus removal from wastewater. Shi *et al.,* (2007) also conducted experiments with *Chlorella* to remove nitrate from municipal wastewater and reduce levels of phosphate, ammonium, and nitrate in synthetic secondary wastewater.

The concentration of heavy metals reduced significantly with an increase in the number of weeks. The finding of this research is in line with the finding of Chan *et al.* (2014) who also reported that microalgae removed up to 81.7% Cu reaching the lowest final concentration of 7.8ppb after 10 days. Zn reduced up to 94.1% reaching 0.6ppb after 10 days. The concentration of biochemical components (carbohydrate, lipid, and protein) indicated rapid buildup in algal cells as the number of weeks increased. Carbohydrate accumulation is induced by inhibited algal cell growth, in which the cells invest in carbohydrate accumulation instead of growth to retain surplus fixed carbon created by imbalanced carbon and nitrogen metabolism (Chia *et al*., 2015). Protein from the results above indicated that the concentration increased with an increase in the number of weeks. In terms of quantity, several species of microalgae are reported to possess very high concentrations of protein Milovanovic *et al.* (2019). The results above it show that Lipidis significantly increased with increases in the number of weeks at p≤0.05.

CONCLUSION

In summary, our findings unequivocally demonstrate the efficacy of *Chlorella sorokiniana* in remediating diverse wastewater samples. This study affirms the remarkable reduction capacity of *Chlorella sorokiniana* particularly in total dissolved solids, phosphate, ammonium, nitrate, potassium, and heavy metals. Furthermore, phycoremediation with *Chlorella sorokiniana* leads to the accumulation of biomass rich in biochemical components, including lipids, carbohydrates, and proteins. These results underscore the potential of Chlorella sorokiniana as a promising candidate for sustainable wastewater treatment strategies, while also highlighting avenues for further research and exploration in this field.

Based on the findings of the research on the use of *Chlorella sorokiniana* for wastewater treatment, the following suggestions are recommended:

Further research should focus on optimizing the cultivation techniques of *Chlorella sorokiniana* to enhance its growth and wastewater remediation efficiency. Explore the integration of Chlorella sorokiniana-based phycoremediation systems into existing wastewater treatment plants to augment their performance and efficiency. Investigate the compatibility and synergistic effects of combining algae-based systems with conventional treatment processes to achieve comprehensive pollutant removal and resource recovery. Evaluate the economic feasibility of implementing *Chlorella sorokiniana*-based wastewater treatment systems compared to conventional treatment methods. Assess the operational costs, energy requirements, and potential revenue streams from biomass utilization to determine the overall cost-effectiveness and sustainability of the technology.

Conduct comprehensive environmental impact assessments to evaluate the ecological implications of implementing Chlorella sorokiniana-based phycoremediation systems. Assess the potential risks associated with algal blooms, genetic contamination, and interactions with native species to ensure the environmental safety and sustainability of the technology. Increase public awareness and stakeholder engagement regarding the benefits and challenges of algae-based wastewater treatment approaches. Foster collaboration between researchers, industry stakeholders, policymakers, and local communities to promote knowledge exchange, capacity building, and technology adoption for sustainable water management practices.

By addressing these recommendations, future research and implementation efforts can further advance the utilization of *Chlorella sorokiniana* for wastewater treatment, contributing to improved water quality, environmental sustainability, and resource recovery.

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