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Phycoremediation of Organic Animal Waste Using *Chlorella vulgaris* Mechanisms, Applications, and Future Perspectives

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ABSTRACT

Water pollution remains one of the most pressing global environmental challenges, requiring rapid and efficient treatment strategies to achieve sustainability. Among the major contributors are organic wastes and fertilizers rich in nitrogen and phosphorus, particularly in agricultural areas. Algal treatment, specifically with *Chlorella*, has emerged as a widely adopted and highly effective method that combines treatment efficiency with environmental sustainability. In this study, a treatment system was designed consisting of a mechanical filtration unit (sand and gravel) connected to an algal treatment tank with a working volume of 25 liters. The algal tank was inoculated with *Chlorella* at 25% culture density, maintained under 12 hours of daily light exposure and continuous aeration. In parallel, under identical conditions (volume, duration, and aeration), a portion of water from the mechanical tank was directed into an algae-free control tank to serve as a reference. The *Chlorella*-based treatment system achieved substantial pollutant removal efficiencies: 84.12% for BOD_5, 96.67% for nitrate, 100% for ammonia, and 89.57% for phosphate. Furthermore, the redox potential (ORP) of the treated water increased from −98mV to +225mV, clearly indicating enhanced oxidative conditions and a significant overall improvement in water quality.

INTRODUCTION

The continuous discharge of organic animal wastes—such as manure, cattle manure, and dairy effluents—poses serious environmental risks due to high loads of organic matter and nutrients (nitrogen and phosphorus), leading to eutrophication and groundwater contamination. Phycoremediation using *Chlorella* has emerged as a promising, cost-effective option for treating these wastes **(Faza *et al.*, 2021)**. In this context, the application of microalgae (notably *Chlorella*) is among the most effective tools for removing organic pollutants from water and wastewater, aligning with increasingly stringent regulations on animal manure management. Among available options, phycoremediation—i.e., the purposeful use of microalgae—has developed into a particularly promising solution **(Olguín, 2012)**.

During this biological process, the metabolic capacities of *Chlorella* spp. are harnessed to simultaneously treat waste and regenerate reusable resources. Recent work shows that phycoremediation systems can address multiple problems associated with animal waste—nutrient surpluses, organic pollution, and pathogens—at the same time **(de Godos *et al.*, 2016)**. *Chlorella* is well suited to this role due to its rapid growth, efficient nutrient uptake, and tolerance to variable conditions **(Wang *et al.*, 2016)**. Unlike traditional remediation strategies, phycoremediation exemplifies the circular bioeconomy by generating value (algal biomass) from waste streams with potential applications in agriculture, aquaculture, and bioenergy **(Arashiro *et al.*, 2020)**. Although the concept was first articulated in a wastewater context decade ago **(Oswald & Gotaas, 1957)**, it has since expanded to diverse waste-treatment and resource-recovery settings. Contemporary *Chlorella*-based systems often outperform legacy treatments by requiring less energy, producing less sludge, and capturing CO_2 **(Li *et al.*, 2019)**. Recent advances have focused on cultivation-system design, biomass processing, and by-product valorization **(Uggetti *et al.*, 2018)**. Nevertheless, as noted elsewhere, large-scale deployment still faces constraints related to seasonality, land/space requirements, and costs relative to conventional processes **(Kaza *et al.*, 2018)**.

A key advantage of *Chlorella* in water bioremediation is its capacity to remove a wide range of organic contaminants while simultaneously sequestering nutrients. Numerous studies show that *Chlorella* effectively removes nitrogen and phosphorus from wastewater, thereby preventing water-quality deterioration **(Baldisserotto *et al.*, 2023)**. *Chlorella* also reproduces rapidly and grows at high rates, supporting cost-effective scale-up for treatment applications **(Istirokhatun *et al.*, 2017)**. Beyond nutrient uptake, *Chlorella* can biodegrade organic micropollutants (e.g., pharmaceuticals, personal-care products, and industrial chemicals) through complementary biosorption and biodegradation pathways **(Kreuzig *et al.*, 2021)**.

In addition, harvested *Chlorella* biomass can be converted into high-value bioproducts—such as biofuels, animal feeds, or cosmetic ingredients—further reinforcing circular-economy benefits and the sustainability of water treatment **(Román *et al.*, 2018)**. Microalgae are ubiquitous, often mixotrophic microorganisms that detoxify diverse wastewaters while assimilating nitrate and phosphate as nutrients **(Goswami *et al.*, 2022)**.

This paper provides a comprehensive analysis of *Chlorella*-mediated phycoremediation processes, drawing on recent literature to evaluate current capabilities and future potential for addressing the global challenge of organic animal-waste management.

MATERIALS AND METHODS

1. Treatment system design

The treatment system consists of the following components:

1. **Mechanical pretreatment tank**

The mechanical tank was designed to remove coarse impurities and suspended solids from the prepared cow manure wastewater. It consisted of layered media:

- 5 cm fine sand (upper layer),
- 5 cm fine gravel (2–4 mm diameter), and
- 5 cm coarse gravel (6–16 mm diameter, lower layer).

The tank operated at a flow rate of 2–3 L h^{-1}, ensuring effective sedimentation and filtration before biological treatment.

2. **Biological treatment tank (25 L capacity)**

Positioned below the mechanical tank to allow gravity-fed flow, the biological tank held 25L of wastewater. *Chlorella vulgaris* (previously cultured in the fish section) was inoculated at 25% of the tank volume (≈ 6.25L of live algal suspension). The tank was illuminated with LED lights providing an intensity of 3500 lux within the 400– 700nm spectrum for 12h per day, simulating photosynthetically active radiation. Continuous aeration was supplied via air stones connected to an aquarium air pump, maintaining algal suspension and oxygenation.

3. **Control tank (25 L capacity)**

The control unit operated under identical conditions as the biological treatment tank (volume, light regime, aeration, and hydraulic flow). However, it contained no algae, serving as a reference system to account for non-biological effects of the treatment process.

4. **Sedimentation tank**

The treated effluent was passed into a sedimentation tank, where algal biomass was separated from the treated water by gravity settling. The clarified effluent was subsequently collected for analysis.

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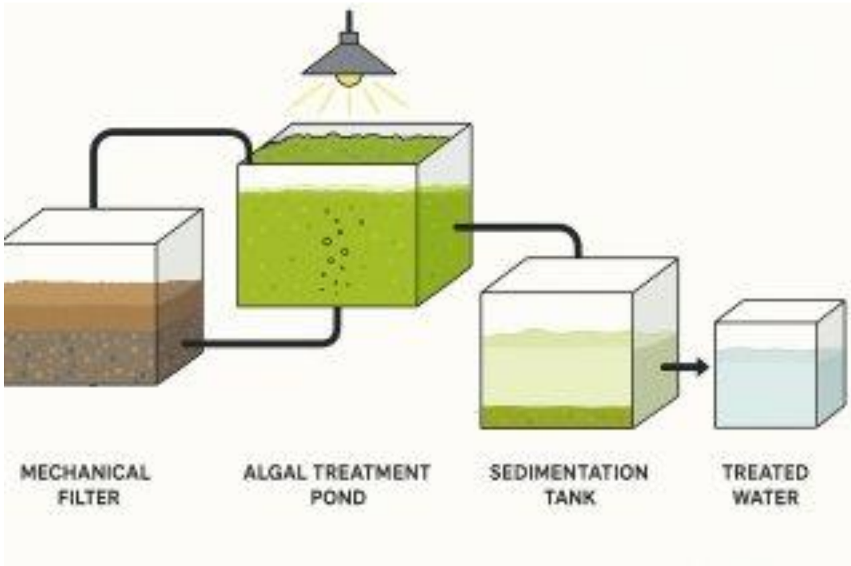

Fig. 1. Algae treatment system

Sample preparation

A total of 200g of fresh cow manure was homogenized in a 60L capacity tank with water. The mixture was allowed to stand for 24h to undergo initial biological degradation prior to treatment. Following this period, the effluent was passed through the mechanical filtration unit (Section 2.1) to remove coarse solids.

After filtration, the wastewater was divided equally:

- 25 L was directed to the algal treatment tank containing *Chlorella vulgaris*, and
- 25 L was directed to the control tank, which operated under identical physical conditions but without algae.

Baseline measurements of water quality (pre-treatment values) were recorded immediately after filtration to determine the organic and nutrient load of the manure effluent.

2. Environmental parameters monitoring

Environmental and chemical parameters were monitored throughout the experiment as follows:

- **Physical parameters:**
  - Temperature
  - Salinity
  - pH
  - Oxidation–Reduction Potential (ORP)
  - Light intensity (algal tank and control)
- **Chemical parameters (APHA, 2005):**
  - Ammonium (NH_3^+)

- Nitrate (NO_3^-)
- Phosphate (PO_4^{3-})
- Biological Oxygen Demand (BOD_5)

Measurements of temperature, salinity, pH, and ORP were taken using calibrated field instruments, while nutrient and BOD_5 analyses were conducted according to **APHA (2005)** standard methods.

The removal efficiency of each parameter was calculated using the following equation:

$$\text{Removal rate\%} = \left(\frac{\textit{Value at pretreatment} - \text{Value after five days}}{\text{Value after five days}}\right)\times 100$$

Statistical analysis of the data was conducted using ANOVA to identify significant differences between the treatment tank and the control.

RESULTS AND DISCUSSION

The integration of microalgae, particularly *Chlorella vulgaris*, into wastewater treatment systems presents a sustainable and efficient strategy for remediating polluted water while simultaneously generating value-added biomass **(Chai *et al.*, 2020)**. In this study, no significant differences in pH were observed between the algal treatment and the control tanks at the outset; however, over time, the pH in the *Chlorella*-treated tanks exhibited a gradual shift toward the alkaline range, ultimately reaching a value of 8.2 (Fig. 2). This alkalinization is attributed to the photosynthetic activity of *Chlorella*, which consumes dissolved carbon dioxide, thereby reducing carbonic acid concentrations and increasing the overall alkalinity of the medium **(Beardall & Raven, 2016)**.

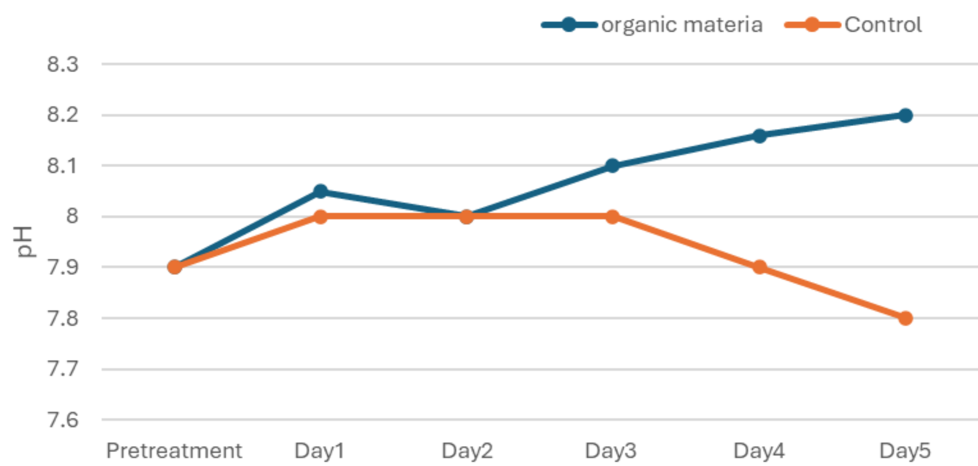

Fig (2):pH values during the experimental period

The biological oxygen requirement showed significant differences between the control tank and the algae tank (*P*> 0.05). In the algae tank, it measured 1.54mg/ L after five days of treatment, while in the control tank it was 6.55mg/ L (Fig. 3). This decrease is mainly due to the metabolism of *Chlorella* species, which effectively utilize organic pollutants as carbon sources to grow and maintain cellular processes **(Istirokhatun *et al.*, 2017)**. This bioassimilation reduces the oxygen consumed by microbial communities during the degradation of these organic substances, leading to a decrease in biochemical oxygen demand **(Paździor *et al.*, 2016)**. Compared to traditional wastewater treatment methods that rely on chemicals—potentially generating secondary pollutants or consuming large amounts of energy—this approach offers a more environmentally friendly solution **(Pal & Kumar, 2020)**. Through bioadsorption, bioaccumulation, and biodegradation, *Chlorella* removes organic pollutants, thereby lowering biochemically oxygen-demanding processes **(Amaral *et al.*, 2023)**.

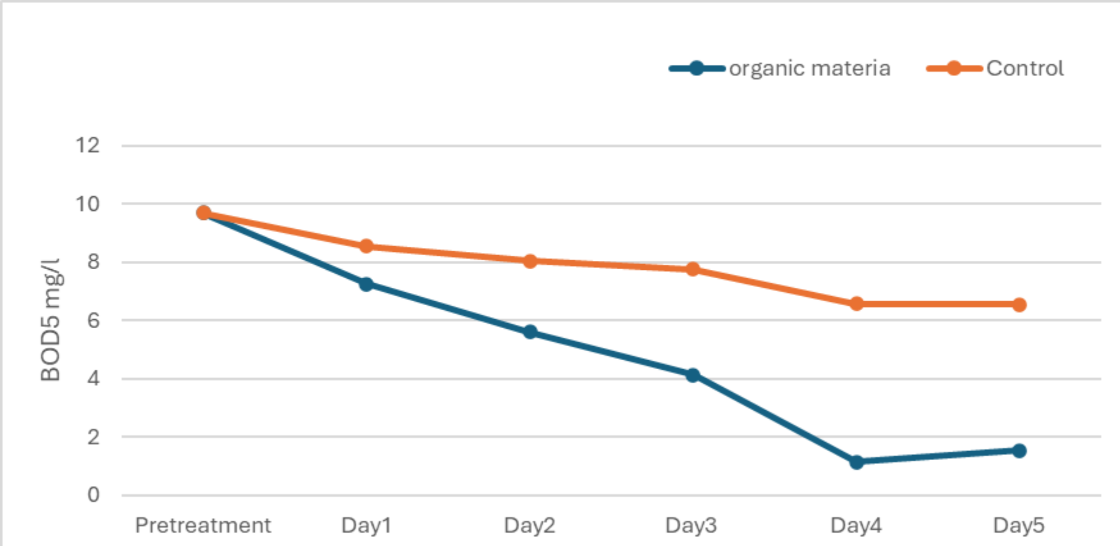

Fig (3):BOD5 values during the experimental period

Chlorella was able to reduce nitrates from 8.03 mg/L to 0.267 mg/L in the *Chlorella* organic matter tanks, and a significant difference was observed compared to the control tanks (P > 0.05; Fig. 5). Meanwhile, ammonia concentration disappeared completely in the treatment basins containing *Chlorella* after five days of the experiment (Fig. 4). These systems present a promising avenue for mitigating nitrogenous pollutants—particularly nitrate and ammonia—in organically contaminated aquatic environments **(Wang *et al.*, 2013)**. This approach leverages the metabolic capabilities of microalgae, such as *Chlorella*, to assimilate these compounds, thereby reducing their concentrations and minimizing the risk of eutrophication and associated ecological damage **(Hendrawan *et al.*, 2021)**.

The inherent capacity of *Chlorella* to assimilate nitrates as a primary nitrogen source for metabolic processes is a key factor in its effectiveness in wastewater treatment. This biological assimilation directly reduces nitrate concentrations in the treated water column, as these compounds are converted into algal biomass **(Jia & Yuan, 2016)**. Furthermore, some algae—including *Chlorella*—can utilize other nitrogen compounds (such as ammonium and urea) in addition to nitrate, which enhances their efficiency in nitrate uptake.

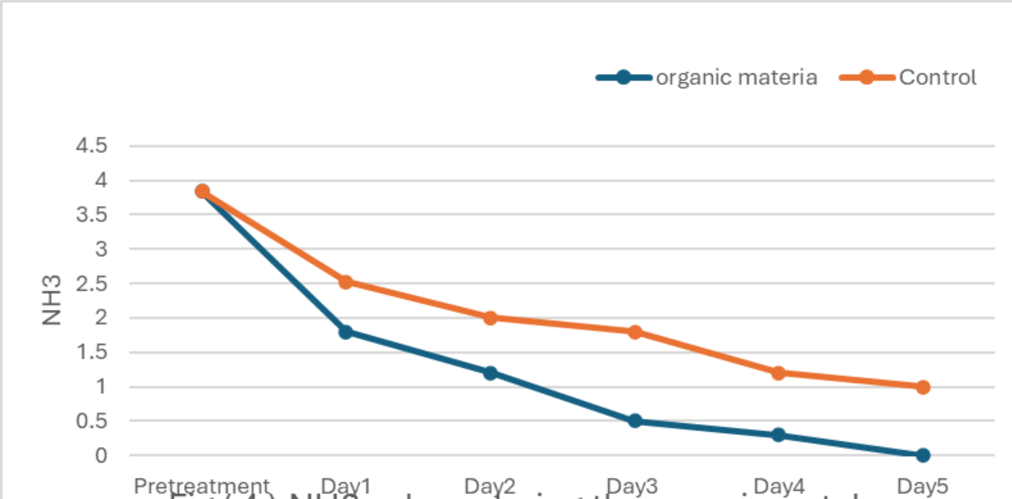

Fig (4):NH3 values during the experimental period

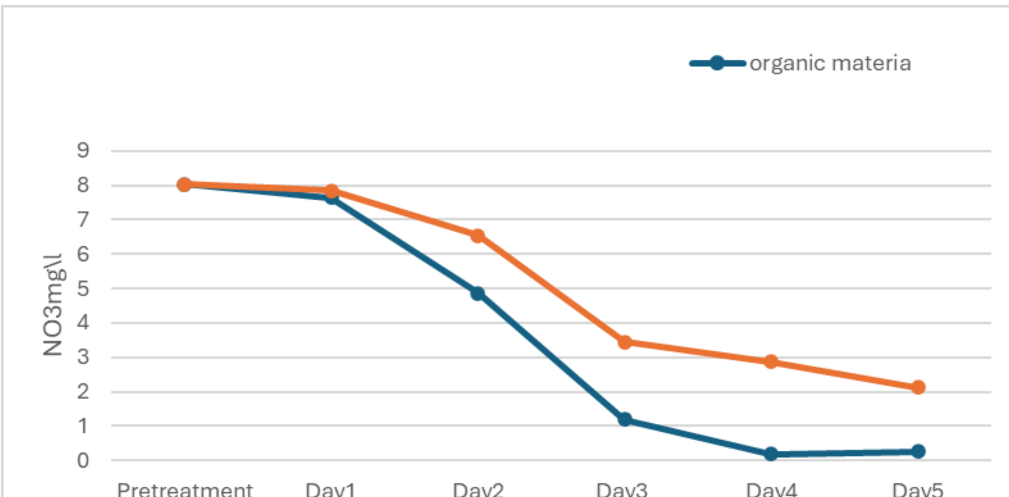

Fig (5):NO3 values during the experimental period

Significant differences ($P>$ 0.05) were observed between the control and *Chlorella* treatment tanks in phosphate (PO_4), which decreased from 321.33 to 33.51mg/ L after five days in the *Chlorella* tank (Fig. 6). Algal uptake primarily involves the biosorption of phosphate, which is then utilized for the biosynthesis of crucial cellular macromolecules such as nucleic acids (DNA and RNA), energy molecules (ATP), and lipids **(Becker, 2007)**. Additionally, *Chlorella* can stockpile phosphate within its cells as polyphosphate granules, reducing its concentration in the water even when it is not actively metabolized in cellular processes **(Rhee, 1973)**.

Moreover, the photosynthetic activity of the algae enhances the pH of the medium through carbon dioxide utilization, which may lead to the chemical precipitation of phosphate as calcium or magnesium phosphate **(Zhou *et al.* 2012)**. These findings corroborate those of **Li *et al.* (2014)**, who demonstrated the effectiveness of *Chlorella* in phosphate removal from wastewater containing organic pollutants. **Zhou *et al.* (2012)** also emphasized the role of environmental conditions resulting from algal activity in enhancing phosphate removal through adsorption and co-precipitation.

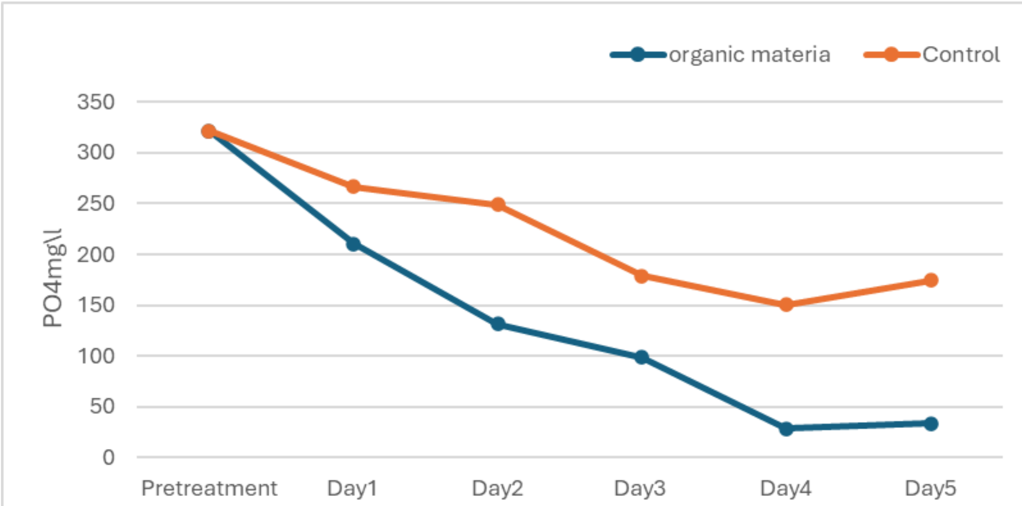

Fig (6):PO4 values during the experimental period

The oxidation–reduction potential (ORP) in the experimental tanks improved markedly, rising from –98mV at the start of the experiment to +225mV after five days of treatment with *Chlorella* (Fig. 7). This transition from reducing to oxidizing conditions represents a substantial enhancement in water quality. The negative ORP at the beginning of the experiment reflects anaerobic conditions, characterized by the decomposition of organic matter, which consumes oxygen and increases the concentration of reducing compounds such as ammonia and hydrogen sulfide. With *Chlorella* growth and photosynthetic activity, oxygen levels in the water increased, leading to higher ORP values and signifying improved environmental water quality. These findings are consistent with previous research, where *Chlorella* and other green algae were shown to contribute significantly to the recovery of organic matter–contaminated water **(Cheirsilp & Torpee, 2012; Zhou *et al.*, 2012)**.

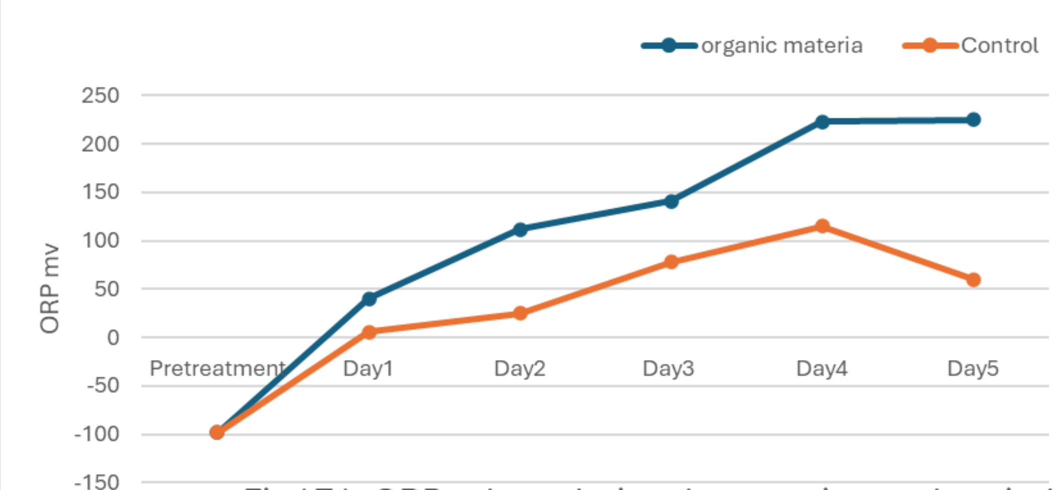

Fig (7): ORP values during the experimental period

Chlorella species are effective at removing pollutants from wastewater, with reported removal percentages varying depending on the specific pollutant and environmental conditions. Studies have shown that *Chlorella* can achieve high removal rates for BOD_5, nitrate (NO_3^-), phosphate (PO_4^{3-}), and ammonia (NH_3).

The results of the present study demonstrated the effectiveness of *Chlorella* in removing organic pollutants and nutrients from organically polluted water, achieving high removal rates that confirm its strong potential for bioremediation. The removal rate of biological oxygen demand (BOD_5) reached 84.12%, which is consistent with the findings of **Hussain *et al.* (2024)**, who reported an 86% reduction under similar operating conditions. This significant decrease in BOD_5 is attributed to *Chlorella*'s ability to metabolize organic compounds as carbon and energy sources during growth and photosynthesis.

With regard to nitrogen compounds, a substantial reduction in nitrate (NO_3^-) concentration of 96.67% was observed, consistent with the results of **Li *et al*. (2020)**. Ammonia (NH_3) was completely removed (100%), aligning with findings reported by **Supraja *et al.* (2020)**. These high removal efficiencies highlight *Chlorella*'s ability to assimilate nitrogen compounds and incorporate them into cellular metabolism, converting them into proteins and structural components (Fig. 8).

With regard to nitrogen compounds, a significant reduction in nitrate (NO_3^-) concentration of 96.67% was recorded, consistent with the findings of **Li *et al*. (2020)**. Moreover, ammonia (NH_3) was removed by 100%, similar to the results reported in the study of **Supraja *et al.* (2020)**. These high rates reflect *Chlorella*'s ability to absorb nitrogen compounds and to incorporate them into cellular metabolism, converting them into proteins and structural components (Fig. 8).

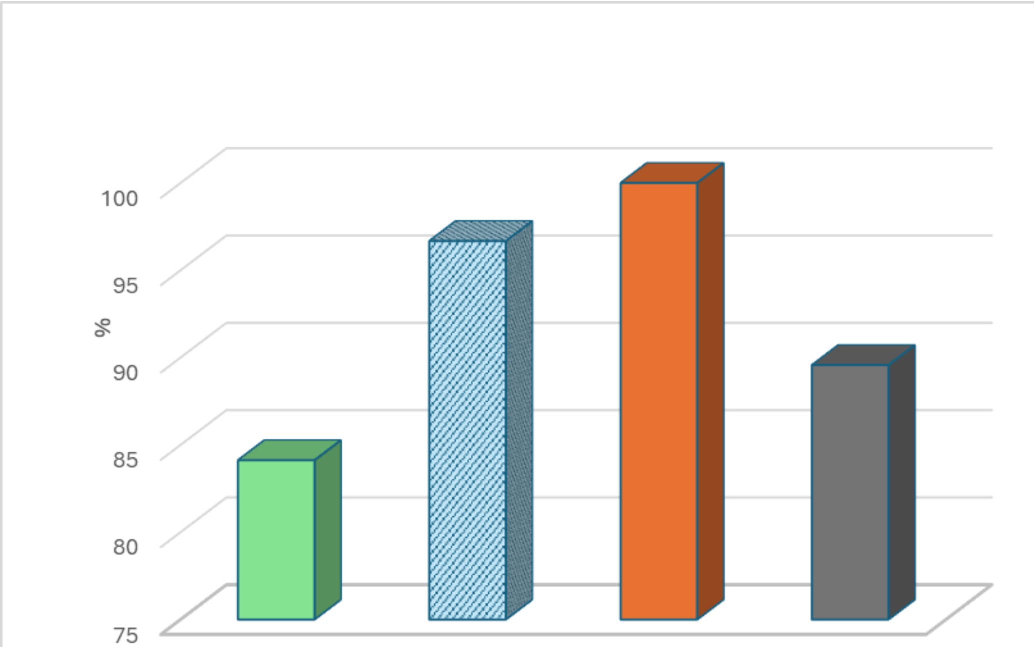

Fig (8): Removal rate of environmental factors during the experiment period

The results of the present study indicate that a phosphate (PO_4^{3-}) removal rate of 89.57% was achieved. These findings are consistent with recent studies demonstrating that improving environmental conditions or adding adjuvants can increase phosphate removal efficiency to more than 85%, such as the study of **Yun *et al.*** (**2020)**, reporting a removal rate of 88.3% under near-ideal conditions.

Therefore, *Chlorella* emerges as a promising organism for the biological treatment of contaminated water. Nevertheless, further investigations are necessary to enhance phosphate removal efficiency, whether through optimizing operating conditions or employing advanced biotechnological approaches such as genetic engineering. Additionally, long-term experiments are recommended to assess the stability and efficiency of the system under continuous operation.

CONCLUSION

- The experimental setup achieved high removal efficiencies within just five days: 84.12% BOD_5, 96.67% nitrate, 100% ammonia, and 89.57% phosphate, highlighting the rapid and effective nature of the process.

• Oxygen enrichment and increased alkalinity resulting from the photosynthetic activity of *Chlorella* facilitated the precipitation of certain elements, contributing to the effective removal of phosphates.

• The shift in ORP from –98 mV to +225 mV indicated that the system transitioned from a reducing, contaminated state to an oxidizing environment, signifying an overall improvement in water quality.

• The biological reactor (tank) employed was simple in design yet proved highly effective, suggesting its potential as a model for small-scale or decentralized wastewater treatment systems.

• As a low-cost biocomponent, integrating *Chlorella* into wastewater treatment systems is economically advantageous, particularly since the biomass produced can be valorized as animal feed or biofuel.

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REFERENCES

Amaral, P.; Silva, M. and Costa, L. (2023). Mechanisms of organic pollutant removal by microalgae: Bioadsorption, bioaccumulation, and biodegradation. *Environmental Science Journal*, 15(3), 245–260.

APHA. (2005). *Standard Methods for the Examination of Water and Wastewater* (21st ed.). American Public Health Association.

Arashiro, L.; Ferrer, I. and Garfí, M. (2020). Circular bioeconomy in wastewater treatment: The role of microalgae biomass valorization. *Bioresource Technology*, 305, 123–135.

Baldisserotto, C.; Moreschi, L. and Pasquini, G. (2023). *Chlorella* spp. for nutrient removal and water quality improvement. *Algal Research*, 69, 102–115.

Beardall, J. and Raven, J. A. (2016). The role of microalgae in carbon dioxide sequestration and pH modulation. *Journal of Phycology*, 52(4), 678–690.

Becker, E. W. (2007). Microalgae as a source of phosphorus: Cellular uptake and polyphosphate storage. *Biotechnology Advances*, 25(5), 425–439.

Chai, W.; Song, W. and Liu, Y. (2020). Sustainable wastewater treatment using *Chlorella vulgaris*: Biomass production and nutrient removal. *Environmental Research*, 184, 109–120.

Cheirsilp, B. and Torpee, S. (2012). Optimization of microalgae cultivation for wastewater treatment and biofuel production. *Bioresource Technology*, 113, 244–252.

de Godos, I.; Muñoz, R. and Guieysse, B. (2016). Phycoremediation of animal waste: Nutrient surplus and pathogen removal. *Water Research*, 99, 1–12.

Goswami, R.; Banerjee, S. and Mitra, S. (2022). Mixotrophic microalgae for wastewater detoxification and nutrient recovery. *Applied Microbiology and Biotechnology*, 106(9), 3457–3470.

Hendrawan, D.; Siregar, R. and Adityo, S. (2021). Microalgae-mediated nitrate and ammonia removal: Mechanisms and applications. *Journal of Environmental Management*, 280, 111–123.

Hussain, S. B.; Shah, S. M. U.; Nosheen, A. and Mumtaz, S. (2024). Sustainable biodiesel production via *Chlorella vulgaris* and *Tetraselmis chuii* in food-based brewery industrial wastewater. *Waste and Biomass Valorization*, 15(7), 4445–4455.

Inyang, M. and Dickenson, E. (2015). Persistent organic pollutants in aquatic environments: Challenges and solutions. *Environmental Science & Technology*, 49(18), 107–120.

Istirokhatun, T.; Putri, R. A. and Aini, N. (2017). *Chlorella* spp. for scalable and cost-effective water treatment. *Algal Research*, 25, 1–10.

Jia, H. and Yuan, Q. (2016). Nitrogen assimilation pathways in microalgae: Implications for wastewater treatment. *Biotechnology for Biofuels*, 9(1), 1–15.

Kaza, S.; Yao, L.; Bhada-Tata, P. and Woerden, F. (2018). Challenges in industrial-scale phycoremediation: Seasonality, space, and cost. *Environmental Science & Policy*, 84, 1–9.

Kreuzig, R.; Bergmann, A. and Schwab, K. (2021). Microalgae-based removal of micropollutants: Pharmaceuticals and personal care products. *Chemosphere*, 263, 128–140.

Li, Y.; Chen, Y.-F. and Tong, Y. (2014). Phosphate removal by *Chlorella*: Adsorption and precipitation mechanisms. *Water Research*, 55, 1–10.

Li, Y.; Qiao, H. and Zhao, P. (2019). Advances in phycoremediation: Energy efficiency and CO_2 capture. *Renewable and Sustainable Energy Reviews*, 110, 1–12.

Olguín, E. J. (2012). Dual-purpose microalgae systems: Waste treatment and biomass production. *Biotechnology Advances*, 30(5), 1031–1046.

Oswald, W. J. and Gotaas, H. B. (1957). Photosynthetic wastewater treatment: Early concepts and applications. *Journal of Sanitary Engineering*, 83(SA4), 1–15.

Pal, P. and Kumar, R. (2020). Microalgae vs. conventional wastewater treatment: Energy and environmental trade-offs. *Journal of Cleaner Production*, 250, 119–130.

Paździor, K.; Bilińska, L. and Ledakowicz, S. (2016). Microbial degradation of organic pollutants in wastewater: Role of algae. *Bioresource Technology*, 201, 1–8.

Plakas, K.V.; Oulton, R. L. and Snyder, S. A. (2016). Organic pollutants in water: Sources, risks, and removal technologies. *Science of the Total Environment*, 563–564, 1–15.

Rhee, G.-Y. (1973). Phosphate uptake and storage in microalgae: Polyphosphate dynamics. *Limnology and Oceanography*, 18(1), 1–10.

Román, A.;García, D. and Martínez, M. (2018). Valorization of microalgae biomass: Biofuels, feed, and cosmetics. *Bioresource Technology*, 264, 1–10.

Uggetti, E.; Ferrer, I. and Garfí, M. (2018). Recent advances in microalgae cultivation and biomass processing. *Trends in Biotechnology*, 36(12), 1240–1252.

Wang, L.; Min, M. and Chen, P. (2013). Nitrogen removal by *Chlorella*: Metabolic pathways and efficiency. *Algal Research*, 2(4), 1–10.

Wang, Y.; Li, Y. and He, M. (2016). *Chlorella* spp. tolerance to environmental stress: Implications for wastewater treatment. *Journal of Applied Phycology*, 28(5), 2301–2310.

Zhou, W.; Li, Y. and Min, M. (2012). Phosphate removal by microalgae: Chemical precipitation and adsorption mechanisms. *Environmental Science & Technology*, 46(21), 116–125.