# Bioremediation Efficiency of Spirulina sp. for Treatment of Domestic Wastewater

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***Abstract*—Destruction of our surroundings, which affects the water we drink, the air we breathe, and the soil we live on, has been and still is mostly caused by industrial pollution. The rapid rise in industrialization cultivation of Spirulina sp. in wastewater systems is useful in densely populated nations like India, where a lot of garbage is produced and causes environmental issues. Spirulina species were cultivated on a large scale using the secondary effluent from a domestic wastewater treatment plant. Spirulina sp. can effectively reduce the BOD and COD of high-carbon wastewater, primarily due to its mixotropic nature, which enables it to utilize both organic and inorganic carbon present in the effluent. Spirulina sp. grown on domestic wastewater different concentrations produced better growth of DO and Chlorophyll. TDS and EC reduction of dissolved substances by Spirulina during growth. Spirulina removes heavy metals such as copper and chromium from domestic wastewater. Phosphate and phenol are reduced in treated wastewater. Protein and carbohydrates are rich in wastewater.**

***Index Terms*—Bioremediation, Wastewater, Spirulina sp., Heavy metals.**

## I. INTRODUCTION

Bioremediation has been effectively used to restore ecosystems and clean up soil, surface water, groundwater, and sediments. Numerous xenobiotic, including nitro-glycerine (explosive), can be eliminated through bioremediation, as has been clearly shown. Natural attenuation (little to no human intervention), bio-stimulation or bio-augmentation, and the intentional introduction of natural or artificial microbes to speed up the required catalytic capacities are all commonly regarded as components of bioremediation. Therefore, bioremediation, phytoremediation, and rhizoremediation can be employed to remove these undesirable substances from the biosphere and have a substantial impact on the fate of hazardous waste (Ma et al., 2011 and Schroeder et al(2004).

Industrial pollution has been and still is a significant contributor to the deterioration of our surroundings, impacting the water we drink, the air we breathe, and the dirt that we inhabit. The rapid growth of industrialization is not just devouring vast tracts of agricultural areas while also contributing to a number of environmental deteriorations. Water's source agriculture is making room for people from a variety of businesses. The difficulty lies in appropriately integrating waste disposal into a controlled management program so that applied industrial solid wastes don't cause any issues with soil, soil microbes, or the environment (Reichert et al ., 2006).

A method for growing algae in sewage oxidation pond systems has been developed in India by the National Environmental Engineering Research Institute (NEERI), Nagpur. The Central Food Technological Research Institute and the National Botanical Research Institute (NBRI) in Lucknow and (CFTRI), Mysore has set up facilities to produce "Single Cell Protein" in large quantities from Cyanobacteria. SCP is created at the NBRI using sewage, which is then used as animal feed. Therefore, microbes provide numerous opportunities for protein production in light of the protein deficit(Roth et al., 1981).

Algae on sewage not only produce useful protein but also help to clean up possible environmental contamination. Spirulina sp. can be grown in wastewater systems in densely populated nations like India, where waste is produced in large amounts and causes environmental issues.

To settle the solid particles, the wastes are put to the digester. The liquid wastewater is put to artificially built ponds as a source of fertilizers. It has been discovered that Spirulina sp. grows more readily in sewage. Modified with varying amounts of nutrients, sodium carbonate or sodium bicarbonate, and diluted sewage. When Spirulina sp. reaches full maturity, it is removed from the pond and either dried in a little solar dryer for animal feed or introduced to aquaculture to feed fish. (Vijayan et al., Kulkarni et al., 2016 RJLBPCS Life Science Informatics Publications 1988). The first research on the treatment of domestic wastewater with Spirulina maxima species was reported in Kosaric et al., 1974.

In this study, residential wastewater treatment plant secondary effluent was used to cultivate Spirulina platensis species on a big scale. According to Olguinet al. (2001) claim that Spirulina sp. has the ability to lower BOD of high carbon containing wastewater because it is mixotropic.

## II. MATERIALS AND METHODS

Collection of Spirulina Platensis culture

Spirulina sp. culture was isolated from domestic wastewater Narmadapuram M.P.

Collection of Domestic wastewater Effluent The Domestic wastewater effluent was collected in clean and dry 5-liter plastic can (stopper) from the effluent discharge point of Narmadapuram Dist. Narmadapuram M.P. The effluent was collected and then it was brought to laboratory and taken for analysis.

Enrichment of Spirulina sp.

After being incubated for 8 to 10 days in a Zarrouk's Media growth medium, the pure culture of Spirulina sp. was further multiplied. Zarrouk's media was autoclaved for 15 minutes at 121°C under 15 psi pressure, whereas salt $NaHCO_3$ was autoclaved separately. The media's pH was maintained all sterile conditions and was adjusted within the range of 8 to 9. Next, 5 millilitres of Spirulina platensis pure culture were added to the media. For eight to ten days, the flask was incubated.

Experimental Setup

The experiment was done for 25 days conducted in a 250 ml conical flask. The entire treatment procedure was completed in 28 $^0$ C temperature. Photoperiod by utilizing a 25-watt light bulb in each flask and 16/8 hours dark/light cycle to keep the conditions ideal for the cultivation of Spirulina platensis. The physico-chemical parameters was measured at different periods (0 day, 5$^{th}$ day, 10$^{th}$ day, 15 $^{th}$day, 20 $^{th}$ day and 25 $^{th}$ day ) and different concentration in 20%, 40 %, 60 %, 80 %, and 100 % of domestic wastewater with Zarrouk's media and compared control .The chemical parameter like, Total Dissolved Solids (TDS), Electrical Conductivity, Dissolve oxygen (DO) Winkler method, 1888, Biological oxygen demand ( BOD) Winkler method 1888, Chemical oxygen demand (COD) J. Noguerol-Arias 2012, Phosphate (Mahadevaiah, 2007), Phenol (Slinkard-Singleton method,1977), Copper(K. Ravindhranath, 2012), Chromium (Onchoke and Sasu 2016)and Growth parameters like, biomass density (OD), Chlorophyll a (G. Mackinney ,1941), Protein (Lowry et al.1951) and Carbohydrates (Dubois Method,1956) were estimated.

## III. RESULTS AND DISCUSSION

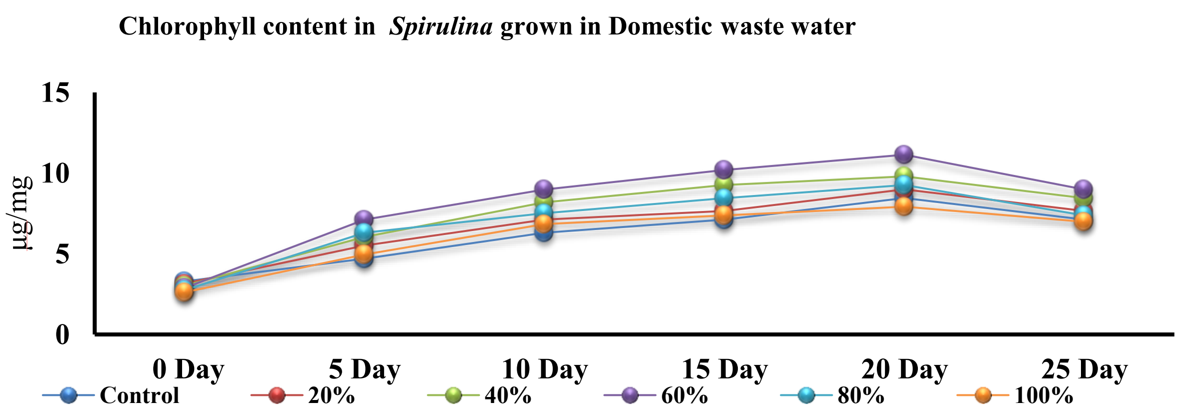


Fig. 1Chlorophyll content in Spirulina grown in Domestic waste water

The chlorophyll content of Spirulina sp. was estimated at 0 day in control and different concentrations (20% –100%) of domestic wastewater. The highest chlorophyll content was observed in the control sample (3.28 mg/mL), indicating optimal pigment levels in the standard growth medium. With 20% wastewater, the chlorophyll content slightly decreased to 3.15 mg/mL, followed by further reductions at 40% (3.01 mg/mL) and 60% (2.88 mg/mL). A more pronounced decrease was recorded at 80% (2.75 mg/mL). At 100% wastewater concentration, chlorophyll content was the lowest (2.61 mg/mL).

The 20-day chlorophyll data clearly demonstrate that Spirulina sp. adapted well to the nutrient-rich environment of domestic wastewater. The control sample recorded a chlorophyll content of 8.45 mg/mL, which was lower than most wastewater treatments. At 20% wastewater, chlorophyll content increased to 8.99 mg/mL, suggesting that the nutrients present in diluted domestic wastewater supported pigment synthesis. A further enhancement was observed at 40% wastewater (9.79 mg/mL). The maximum chlorophyll content was recorded at 60% concentration (11.13 mg/mL), demonstrating that this level provided the most favourable combination of nutrients such as nitrogen, phosphate, and trace elements which stimulated chlorophyll biosynthesis and algal growth. At 80% wastewater, chlorophyll declined slightly to 9.25 mg/mL, and at 100%, the value decreased further to 7.91 mg/mL. This reduction at higher concentrations may be attributed to excessive organic load, limited light penetration, and increased physiological stress due to accumulation of pollutants.

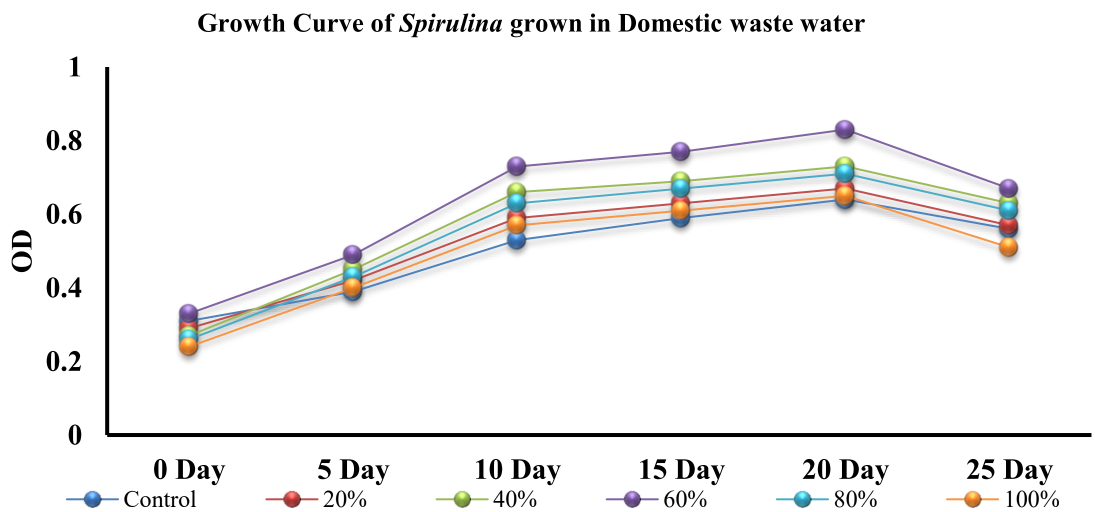


Fig. 2 DO in Spirulina grown Domestic waste water

The dissolved oxygen (DO) level at 0 day showed a decreasing trend with increasing domestic wastewater concentration. The control sample recorded a DO value of 6.5 mg/L. A slight improvement in DO was observed at 20% wastewater (6.9 mg/L), which may be attributed to partial dilution and lower organic load at this concentration. At 40% wastewater, the DO value slightly decreased to 6.7 mg/L, followed by further reductions at 60% (6.3 mg/L) and 80% (5.9 mg/L). The lowest DO was recorded at 100% wastewater (5.3 mg/L), indicating a higher oxygen demand and lower available dissolved oxygen in highly concentrated wastewater.

After 25 days of cultivation, the dissolved oxygen (DO) levels increased significantly in all treatments compared to their initial (0-day) values. The maximum DO was recorded in the control (9.9 mg/L), followed by 20% (9.5 mg/L) and 40% wastewater (9.1 mg/L). DO levels gradually decreased at higher concentrations, with 60% (8.7 mg/L), 80% (8.3 mg/L) and the minimum at 100% wastewater (7.9 mg/L). Despite this reduction at higher concentrations, all wastewater treatments exhibited a substantial improvement in DO over 25 days. According to Yati Prabha (2016) the DO levels are increased biological agents present in waste water resistant to Spirulina maxima.

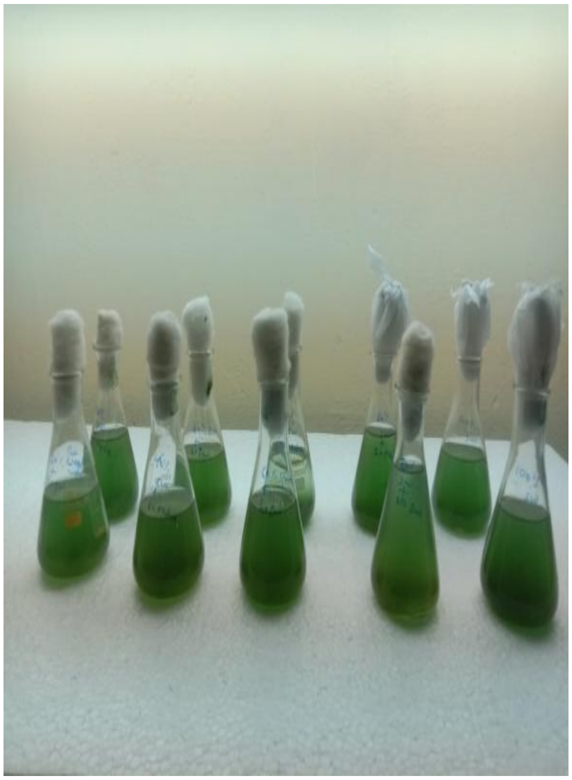

Photo: Domestic wastewater on different concentration treated of Spirulina sp.

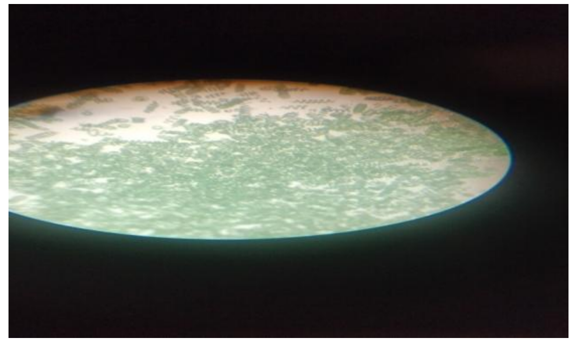

Photo: Domestic wastewater growth before treatment in Spirulina sp.

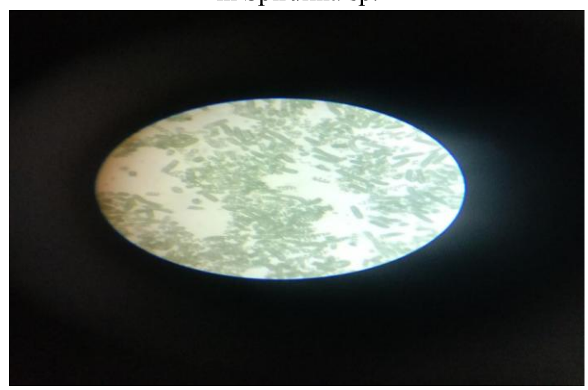

Photo - Domestic wastewater growth after treatment in Spirulina sp.

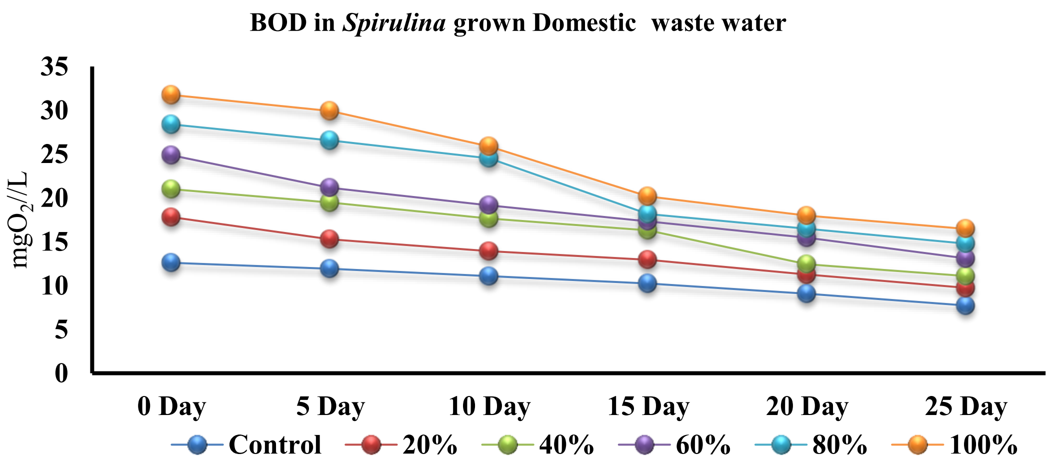


Fig. 3 BOD in Spirulina grown Domestic wastewater

At 0 Day, the BOD increased progressively with the increasing concentration of domestic wastewater. The control contained the lowest organic load (12.6 mg/L), whereas the 100% wastewater sample exhibited the maximum BOD value (31.75 mg/L). The increasing trend from control to 100% wastewater reflects the high biodegradable organic content naturally present in domestic effluents.

A remarkable reduction in BOD was observed across all treatments after 25 days of Spirulina growth. The BOD values decreased from 12.6 to 7.72 mg/L in the control and from 31.75 to 16.46 mg/L in 100% wastewater. Similarly, intermediate concentrations also showed significant reductions (20%: 17.8 → 9.74 mg/L; 40%: 21 → 11.08 mg/L; 60%: 24.86 → 13.10 mg/L; 80%: 28.39 → 14.78 mg/L). The viability of treating pharmaceutical wastewater with the salt water algae Spirulina platensis was investigated by Kshirsagar et al., (2010). The COD/BOD, sulphates, and chlorides were all well reduced, according to the results.

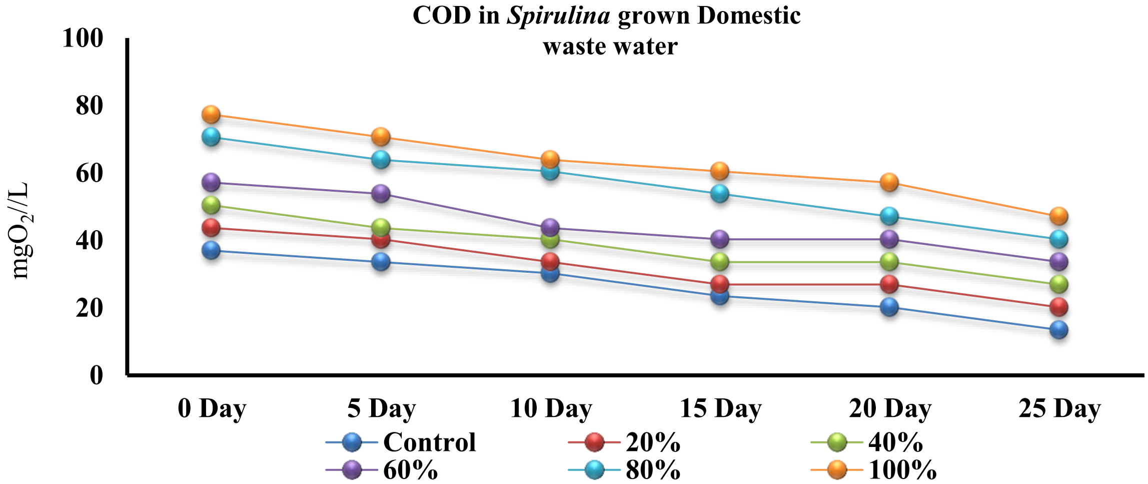


Fig. 4 COD in Spirulina grown Domestic wastewater

The initial COD values increased proportionally with the concentration of domestic wastewater. The control sample showed the lowest COD value (36.96 mg/L), whereas the highest COD was recorded in 100% wastewater (77.28 mg/L). This indicates that higher wastewater concentrations contain a greater load of chemically oxidizable organic matter.

A substantial reduction in COD was observed after 25 Days in all wastewater concentrations, indicating the effective bioremediation capacity of Spirulina. The lowest COD value was recorded in the control (13.44 mg/L), while the highest COD after treatment was seen in 100% wastewater (47.04 mg/L). The COD in the different therapies in this study was drastically reduced. This is consistent with the findings of the investigation carried out by Zainab et al., (2012), as they further more revealed a strong 90% drop in COD.

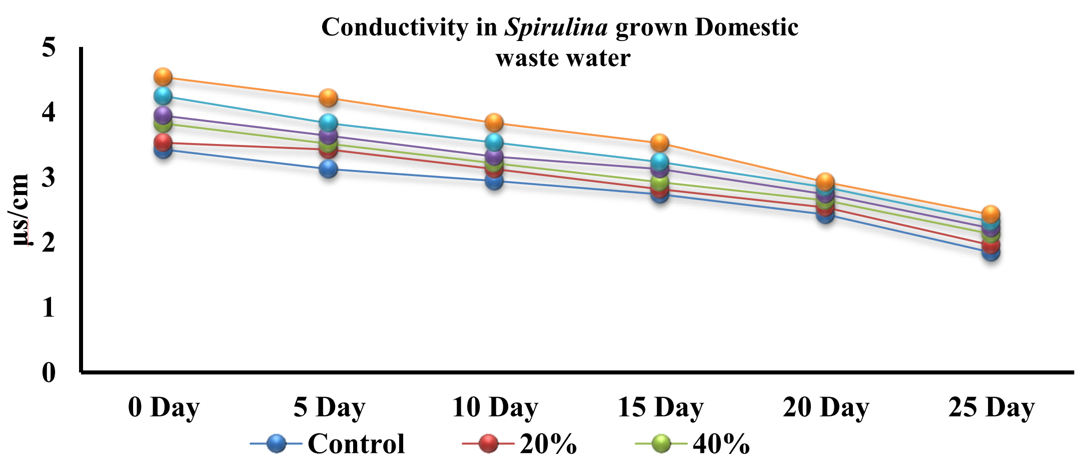


Fig. 5 Conductivity in Spirulina grown Domesticwaste water

The initial EC values increased gradually with increasing wastewater concentration. The lowest EC was recorded in the control (3.423 mS/cm), while the highest EC (4.534 mS/cm) was observed in 100% wastewater. This indicates a higher ionic load in more concentrated wastewater samples. A significant reduction in EC was observed after 25 Days across all treatments. The lowest EC value was recorded in the control (1.843 mS/cm), while the highest EC after treatment was in the 100% wastewater sample (2.423 mS/cm).

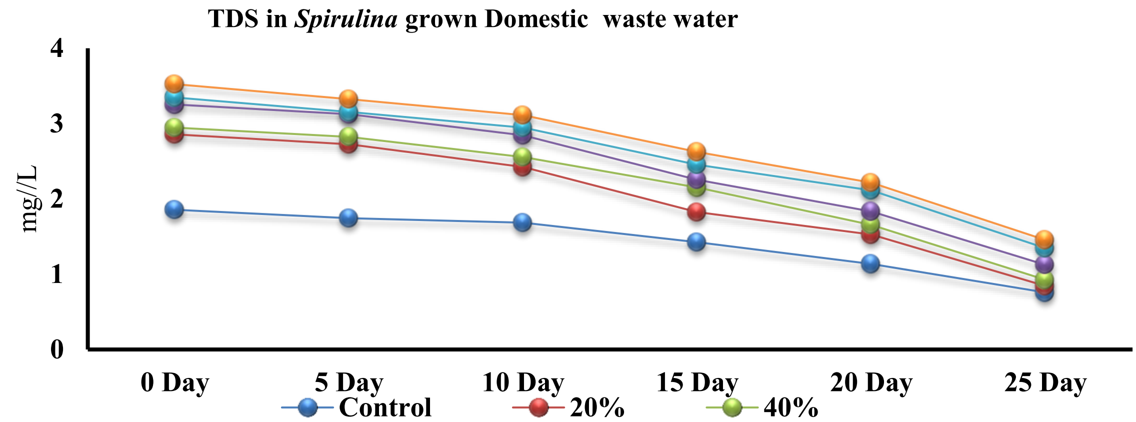


Fig. 6 TDS in Spirulina grown Domesticwaste water

At 0 day, TDS values showed a clear increasing trend with increasing domestic wastewater concentration. The control sample exhibited the lowest TDS (1.85 µS/ppm), while the highest value was observed in 100% wastewater (3.52 µS/ppm). This confirms that domestic wastewater contains high levels of dissolved organic and inorganic solids.

After 25 days of Spirulina cultivation, TDS values decreased significantly across all treatments. The lowest TDS was observed in the control (0.75 µS/ppm), whereas the highest value remained in the 100% wastewater sample (1.45µS/ppm). The reduction in TDS indicates active uptake and removal of dissolved substances by Spirulina during growth.

TDS and EC is a good indicator of aesthetic characteristics of drinking water and as an aggregate indicator for the presence of different types of chemical contaminants (Dolatabadi et al., 2016).

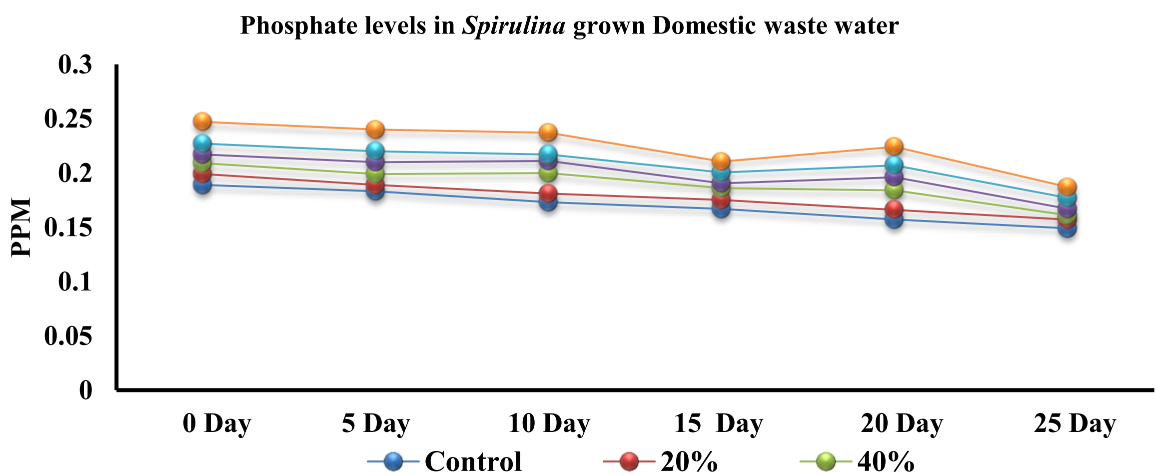


Fig. 7 Phosphate levels in Spirulina grown Domestic waste water

Phosphate ($PO_4^{3-}$) is an essential nutrient and a key indicator of eutrophication potential in wastewater. Elevated phosphate levels promote algal blooms and deteriorate water quality. At the initial stage, phosphate concentrations increased steadily with the increasing proportion of domestic wastewater. The control sample recorded the lowest phosphate concentration (0.189 mg/L), while the highest concentration (0.247 mg/L) was observed in the 100% wastewater treatment.

A notable decrease in phosphate concentration was recorded across all wastewater concentrations after 25 Days of Spirulina cultivation. The lowest phosphate level was seen in the control (0.149 mg/L), whereas the 100% wastewater sample showed 0.187 mg/L after treatment.According to Vijayan et al. (1988), Spirulina sp. grows more readily in sewage that has been diluted and modified with nutrients and sodium carbonate in varying amounts. In the experiment conducted by Lodi and Binaghi et al. (2003), the biomass of Spirulina platensis was utilized to lower the levels of phosphate and nitrate in wastewater. It has been reported that the entire nitrate removed was utilized for biomass development (biotic removal), while phosphate seems to be mostly eliminated by chemical precipitation (abiotic removal).

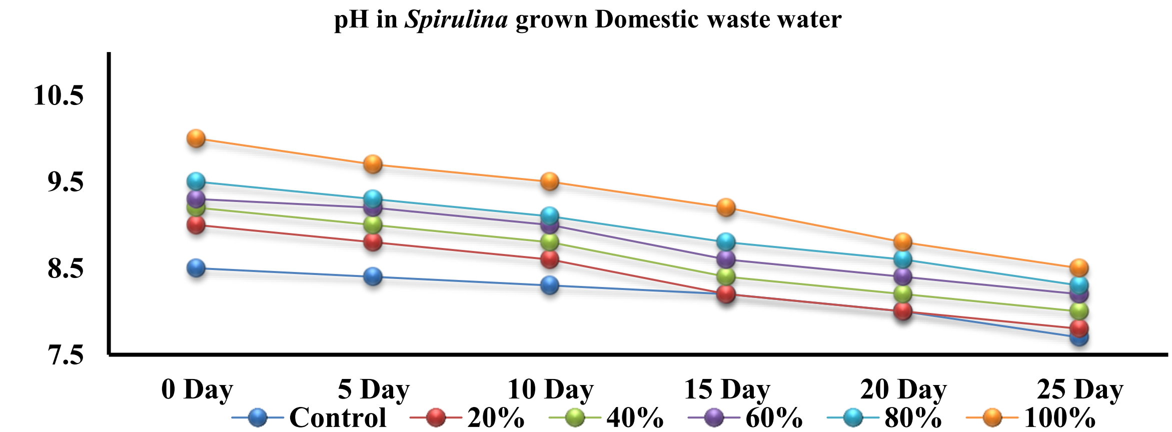

Fig. 8 pH in Spirulina grown Domestic wastewater

At 0 day, the pH of the culture medium increased progressively with higher concentrations of domestic wastewater, ranging from 8.5 in the control to 10.0 at 100% wastewater. This indicates the naturally alkaline nature of domestic wastewater.

After 25 days of Spirulina cultivation, the pH values showed a noticeable decrease across all treatments. The final pH ranged between 7.7 (control) and 8.5 (100% wastewater). Although the wastewater initially exhibited high alkalinity, the pH stabilized to moderately alkaline conditions over time. According to research by Wetzel et al., (1968), when photosynthetic rate increases, more biocarbonates are consumed, which causes an increasing amount of carbonates to develop and raise the pH of wastewater. An increase in pH and Algae's photosynthetic activity was the cause of the treated effluent's alkalinity.

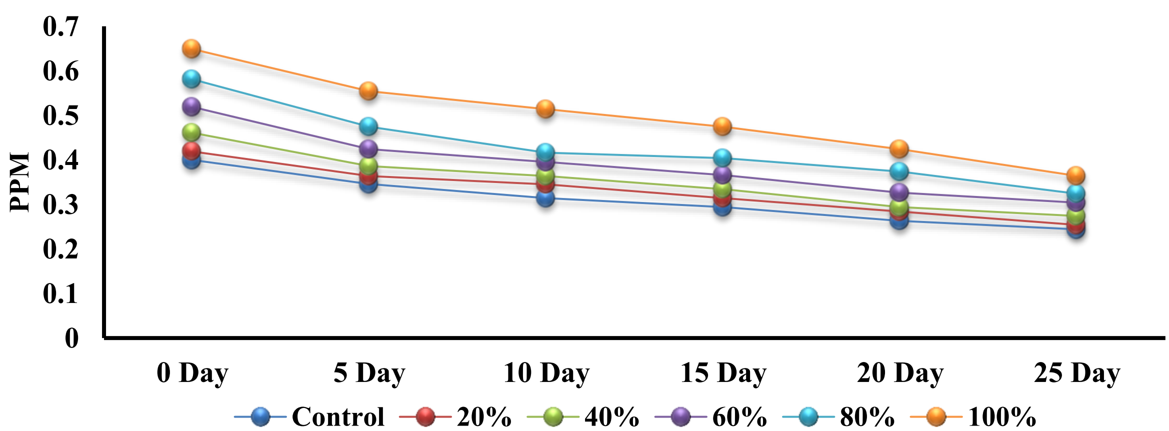

Fig. 9 Copper levels in Spirulina grown domestic waste water

At the beginning of the experiment, copper concentration increased progressively with increasing proportions of domestic wastewater. The control sample contained 0.399 mg/L of copper, whereas cultures grown in 20%, 40%, 60%, 80%, and 100% wastewater showed 0.418, 0.460, 0.518, 0.580, and 0.648 mg/L, respectively.

After 25 days of incubation with Spirulina, a remarkable reduction in copper concentration was observed in all treatments. Copper levels decreased to 0.278 mg/L (control) and 0.288, 0.308, 0.338, 0.358, and 0.398 mg/L in 20%, 40%, 60%, 80%, and 100% wastewater, respectively. This consistent decline clearly demonstrates the ability of Spirulina to remove or biosorb copper from the wastewater environment.According to research by Converti A. et al., (2006) Spirulina platensis biomass was utilized as a biosorbent for Cu adsorption in order to replicate the removal of copper from wastewater contaminated by this metal in the food industry.

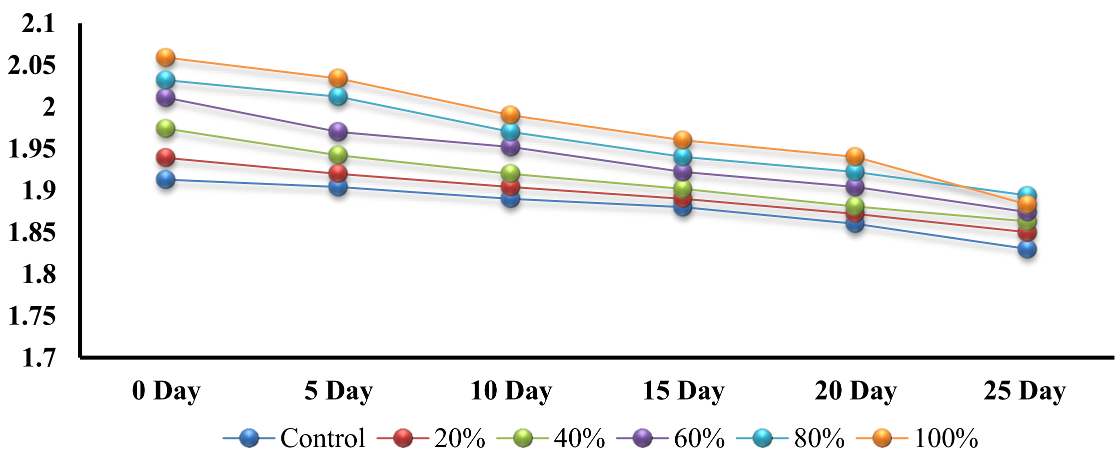


Fig. 10 Chromium levels in Spirulina grown domestic waste water

At the start of the experiment, chromium concentration showed a gradual increase with increasing wastewater percentage. The control sample recorded 1.912 mg/L, while the 20%, 40%, 60%, 80%, and 100% domestic wastewater concentrations showed 1.93, 1.973, 2.010, 2.031, and 2.058 mg/L, respectively.

This progressive rise reflects the higher chromium load associated with greater proportions of untreated domestic wastewater. After 25 days of treatment with Spirulina, chromium concentrations declined across all treatments. The values reduced to 1.829 mg/L (control) and 1.849, 1.862, 1.873, 1.893, and 1.882 mg/L in 20%, 40%, 60%, 80%, and 100% wastewater, respectively. Although the reduction is moderate compared to other metals (e.g., copper), the consistent downward trend indicates clear chromium removal over time.According to Babuet al., (2013) the heavy metals lead (Pb (II)) and chromium (Cr (VI)) was extracted from the aqueous samples in this work using live Spirulina platensis as a biosorbent.

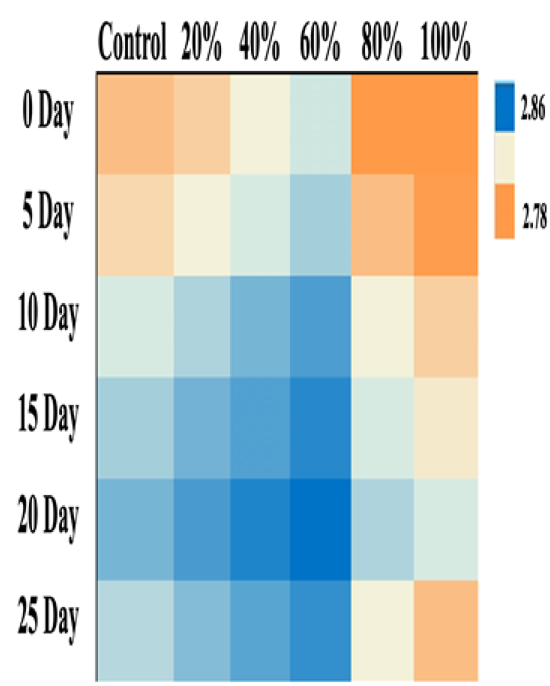


Fig. 11Protein content in Spirulina grown domestic waste water

The highest protein content was recorded at 60% wastewater concentration, where Spirulina exhibited a value of 2.86 mg/ml, represented by a dark blue colour on the heat map. The dark blue shade signifies the maximum intensity, indicating that 60% concentration was the most favourable condition for protein synthesis during the 20-day cultivation period.

Slightly lower protein content was observed at 80% concentration, with a value of 2.78 mg/ml, which appeared as a brown colour in the heat map. The brown shade corresponds to a comparatively lower magnitude than dark blue, suggesting that while Spirulina was still able to synthesise protein at higher wastewater concentration, the efficiency was reduced relative to the 60% treatment.

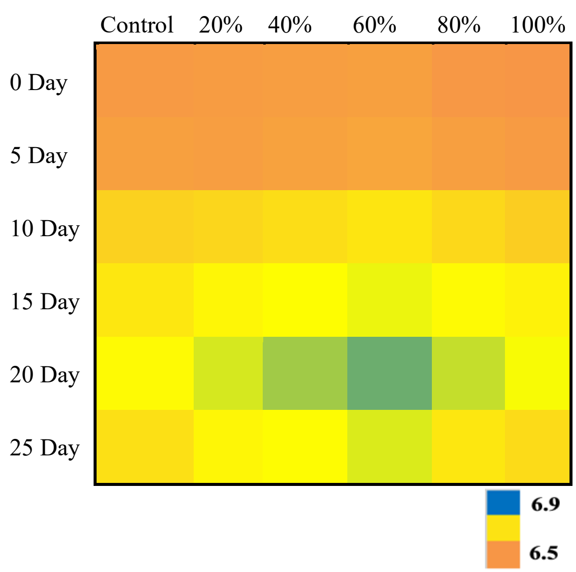


Fig.12Carbohydrate content in Spirulinagrown domestic wastewater

The highest carbohydrate content was recorded at 60% wastewater concentration, where Spirulina showed a value of 6.9 mg/ml, represented by a blue colour in the heat map. The blue shade indicates maximum intensity, signifying that 60% concentration created the most favourable conditions for carbohydrate synthesis during the 20-day cultivation period. At 80% concentration, the carbohydrate content slightly decreased to 6.50 mg/ml, marked by a brown colour on the heat map. This brown coloration reflects a lower magnitude compared to the blue region, suggesting that higher wastewater concentration imposes mild stress on the culture, resulting in a moderate decline in carbohydrate accumulation.

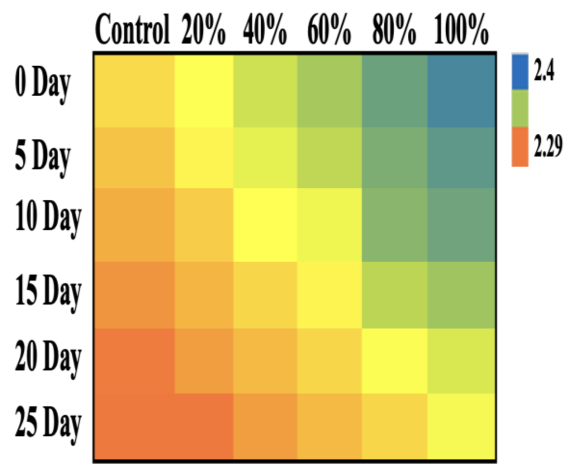


Fig. 13 Phenol content in Spirulina grown Domestic wastewater

The highest phenol content was observed at 100% wastewater concentration, where the value reached 2.4 mg/ml, represented by a blue colour on the heat map. The blue shade indicates the maximum intensity within the dataset, suggesting that Spirulina cultured in full-strength wastewater experienced enhanced phenol uptake or biosorption due to higher pollutant availability. In contrast, the 20% wastewater concentration exhibited a phenol content of 2.29 mg/ml, which appeared as a brown colour on the heat map. The brown coloration corresponds to a lower magnitude, indicating reduced phenol levels at lower concentrations of domestic wastewater.

## IV. CONCLUSION

The chlorophyll content of Spirulina sp. showed a clear concentration-dependent response when cultivated in varying proportions (20–100%) of domestic waste water. These results indicate that 60% domestic wastewater is the most suitable concentration for maximizing chlorophyll production and promoting robust Spirulina growth. All wastewater treatments exhibited a substantial improvement in DO over period. The reduction in TDS and Electrical conductivity indicates active uptake and removal of dissolved substances by Spirulina during growth. The decline in BOD and COD is attributed to the active assimilation of organic matter by Spirulina, which utilizes carbon, nitrogen, and other organic nutrients for metabolic activities. A consistent reduction in Phosphate and Phenol levels across all treatments indicates that Spirulina effectively assimilated phosphate from the culture medium. This consistent decline clearly demonstrates the ability of Spirulina to remove or biosorbent Copper and Chromium from the wastewater environment. Thehighest Protein and Carbohydrate content was recorded at 60% wastewater concentration.

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