

# Fungi and Algae: A Synergistic Duo for Wastewater Treatment

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**Abstract**—Innovation and sustainability are being explored due to the growing demand for efficient wastewater treatment techniques worldwide. Modern wastewater treatment methods often face limitations in efficiency and complete pollutant removal. To overcome the shortcomings of modern technology, this research explores the combined potential of fungi and algae in wastewater treatment. The research employed a comprehensive methodology, utilizing a carefully selected combination of fungi and algae in a controlled experimental setup. Four genera of organisms, two species of fungi (*Phanerochaete chrysosporium*, *Trichoderma asperillum*), and two species of algae (*Spirogyra maxima*, *Gloeocapsa rupicola*) are used to react. Examinations are done on water treated with algae and fungi since there are eight treatments applied as opposed to just water in the control treatment. Measured are the bicarbonate ions, sulfates, copper, and iron concentrations. The findings illustrate the symbiotic link between fungi and algae and show a notable improvement in wastewater treatment efficiency. When compared to separate treatments, the synergistic pair showed significant pollution reduction. This study provides important insights into a new, environmentally friendly approach to wastewater treatment, revealing the potential to simultaneously utilize the unique abilities of fungi and algae in tandem.

**Keywords**—wastewater treatment, Fungi, Algae, symbiotic relationship, pollutant removal

## I. INTRODUCTION

The quantity and quality of water sustain ecological equilibrium, which has an impact on human lifestyle [1], yet its quality is often compromised by contamination, particularly through wastewater discharge. Mitigating these risks requires effective wastewater treatment, which entails a variety of procedures meant to eliminate or neutralize pollutants before releasing water back into the environment. Traditional methods of wastewater treatment often rely on chemical processes that can be costly and have negative

environmental impacts [2]. In recent years, there has been interest in the use of green technologies in wastewater treatment. One such green technology approach is the combined use of fungi and algae. Fungi and algae have been found to form a synergistic duo in the treatment of wastewater. They complement each other's abilities and contribute to the overall effectiveness of the treatment process. The growing popularity of co-cultivating fungal and micro algal cells stems from the great efficiency of micro algal cell bio-flocculation, which eliminates the need for additional chemicals and minimal energy inputs. Certain types of mold and microalgae are known for their outstanding ability to effectively filter wastewater due to biochemical processes that break down contaminants. Molds such as *Aspergillus* and *Penicillium* are excellent at metabolizing organic compounds and absorbing heavy metals, while microalgae such as *Chlorella* and *Spirulina* are excellent at absorbing nutrients such as nitrogen and phosphorus [3]. In particular, these microorganisms produce biomass rich in proteins, lipids, and carbohydrates that not only treat wastewater but also serve as renewable feedstock for biofuel production. These dual benefits are consistent with sustainability goals by providing circular solutions that reduce pollution and promote the use of renewable energy. Additionally, the use of fungi and microalgae can reduce the environmental impact of wastewater treatment processes by minimizing reliance on traditional treatment methods, which are energy-intensive and chemical-dependent. This integration highlights promising opportunities to advance both environmental remediation and biofuel production as part of sustainable development. Fungi can break down complex organic compounds and remove pollutants, such as heavy metals from wastewater [4]. Algae, on the other hand, are capable of photosynthesis and can absorb nutrients and carbon dioxide from the wastewater, thereby reducing excess nutrients and

organic matter. When fungi and algae are used together in wastewater treatment, they create a balanced ecosystem where fungi break down organic compounds and pollutants, and algae absorb nutrients and CO<sub>2</sub>, leading to a more efficient and environmentally friendly treatment process. This synergistic approach not only offers a sustainable and cost-effective solution for wastewater treatment but also reduces the reliance on chemical treatments, minimizing the negative impact on the environment. Modern water treatment facilities often face limitations in their ability to restrict cyanobacterial toxins. Cell viability tests prove useful in assessing potential threats to drinking water quality by measuring membrane integrity using DNA-binding labeling agents [5].

One of the key advantages of using fungi and algae in wastewater treatment is their adaptability to diverse environments, which makes them suitable for a wide range of wastewater applications. Furthermore, the byproducts generated during the treatment process offer additional utility. For instance, the biomass derived from fungi and algae can be repurposed for biofuel production or as a high-quality organic fertilizer. This capacity for resource recovery enhances the sustainability of wastewater treatment. Notably, plants have maintained a close symbiotic relationship with fungi and cyanobacteria since diverging from green algae over 400 million years ago. This association gave rise to lichens, which currently cover 6% of the Earth's terrestrial surface [6]. This enduring natural partnership has not only lasted for millennia but has also been instrumental in influencing ecosystems all around the world. Because lichens are composed of both fungal and algal or cyanobacterial elements, they are among the first organisms to colonize a variety of habitats, including urban areas and desolate landscapes. They also play a major role in the creation of soil and the cycling of nutrients. This long-lasting symbiosis demonstrates the durability and flexibility of living forms throughout evolutionary time scales and emphasizes the complex interactions between various organisms in the natural world. Among their many diverse characteristics are the synthesis of a wide range of extracellular enzymes and the complex hyphal network they generate, which together give fungi an impressive ability to remediate wastewater. Cellulases, ligninases, and lipases are among the enzymes that catalyze the breakdown of complex organic molecules found in wastewater, making them more microbially degradable. In addition, the delicate organelles within fungal cells are shielded from the damaging effects of toxins and pollutants by the thick hyphal meshwork. A potential approach to resolving environmental issues related to contamination and pollution is provided by the special combination of fungi's enzymatic ability and structural durability, which positions them as highly effective and sustainable agents for wastewater treatment remediation operations. *Cephalosporium aphidicola*, *Pleurotus pulmonarius*, *Stachybotrys* sp., *Candida* sp., *Aspergillus parasitica*, *Verticillium terrestre*, *Acremonium* sp., *Glomus* sp., *Minimedusa* sp., *Talaromyces*, *Hydnobolites*, *Peziza*, and other fungal species can be employed to remediate wastewater [7]. Numerous studies have demonstrated that yeast is capable of removing pollutants containing heavy metals from the environment [8]. Moreover, yeast can

eliminate mono and polyphenols and reduce COD levels as yeast has the ability to accumulate, absorb, and break down harmful substances into harmless forms [9]. Textile wastewater can be treated with it. Wastewater can be treated by *Saccharomyces cerevisiae*, *Galactomyces geotrichum*, *Trichosporon beigellii*, and *Candida krusei* to break down dyes [10]. Algal biomass is more effective in removing heavy metals than membranes [11]. Phycoremediation involves employing various types of algae to aid in the process of bioremediation. This method harnesses the capabilities of algal species to facilitate environmental cleanup and restoration efforts. Wastewater treatment involves the employment of strains of algal and cyanobacteria. Algal such as *Chlorella* sp., *Tetraselmis* sp., *Picochlorum* sp., and *Scenedesmus* sp. and cyanobacterial strains like *Anabaena* sp., *Oscillatoria* sp., *Chroococcus* sp., *Spirulina* sp., *Pseudosporangium* sp., *Scytonema* sp., and *Dolichospermum* are used for this [12]. Since algal biomass grows regardless of the environment, it can be generated all year round [13]. A high sorption capacity is another feature of algae biosorbents [14]. By harnessing the unique capabilities of both organisms, we can achieve enhanced purification of wastewater while minimizing environmental impact.

Although traditional techniques of treating wastewater are vital to maintaining the quality of the water, they frequently have limitations when it comes to efficacy and total removal of pollutants, especially when dealing with complex organic molecules. Chemical-based methods have the potential to be expensive and detrimental to the environment. They frequently rely significantly on chemicals for a variety of processes, which raises expenses and may have negative environmental effects from chemical use and manufacture. Furthermore, additional processes could be necessary to remove complex organic compounds or develop pollutants using these approaches. In addition, conventional treatment methods can produce large amounts of sludge that are difficult to dispose of and can be energy-intensive. Due to these drawbacks, novel and environmentally responsible methods such as fungi-algae synergy are required to treat wastewater effectively and sustainably. Algae effectively absorb surplus nutrients and break down products for development, whereas fungi are the best at breaking down complex organic contaminants. This mutually beneficial interaction presents a viable strategy for improving therapy efficacy. We examine the combined impact of particular fungal and algal species (*Phanerochaete chrysosporium*, *Trichoderma asperallum*, *Spirogyra maxima*, *Gloeocapsa rupicoig*) on the elimination of important contaminants from wastewater, through a controlled experiment. This study is innovative since it examines the combination of fungi and algae to effectively target contaminants based on copper as well as other pollutants. It may open the door to a new, eco-friendly approach to wastewater treatment by utilizing the combined capabilities of algae and fungi.

## II. MATERIALS AND METHODS

The use of photobioreactors for the cultivation of algae and fungi is important in the field of bioengineering and wastewater treatment. Ten-liter and eight-glass containers make up the basic photobioreactors that are used to cultivate

fungi and algae in sewage water. In order to create an environment that is favorable for the growth and multiplication of microorganisms necessary for sewage treatment, each component is needed. A control treatment system that measures the process's effectiveness under particular circumstances is added to these main vessels. An aeration system strengthens this control system, which sets it apart by depending only on wastewater. The microbial communities' metabolic activities depend on the medium having the right amount of oxygen, which is ensured by adding aeration.

Fungal Species: *Phanerochaete chrysosporium*, *Trichoderma asperallum*. Algal Species: *Spirogyra maxima*, *Gloeocapsa rupicoig*.

Equipment: Incubator, pH meter, analytical balance, atomic absorption spectrophotometer, filtration apparatus, 500 mL glass beakers for culturing the fungi and algae, 3500 cc glass containers, buffer solution, distilled water, stirring apparatus, and water bath for temperature control.

Type of wastewater: Municipal wastewater, secondary effluent.

The introduction and dosage of microbial biomass are critical to the culture process's effectiveness. For each genus of algae in the system, precisely 300 milliliters of fresh algae pollen are delivered, providing the essential nutrients and organic matter needed for further growth. Similar to other species, fungi are given specific care, with 100 milliliters of fungal pollen from every genus being meticulously mixed into the medium. The meticulous methodology employed ensures the diversity and robustness of the microbial communities, which are essential for the breakdown of organic contaminants found in wastewater. The glass containers, which have a 3500 cc capacity, are the ideal vessels for combining wastewater and biomass. Algal and fungal cultures were carefully added to a glass containers, which served as each bioreactor. In addition to facilitating the spread of microorganisms, this tactical arrangement guarantees effective nutrient exchange and metabolic activity in the system. An air pump similar to those used in aquaculture systems is used to support aeration and preserve ideal environmental conditions. This configuration, when combined with a flexible rubber tubing system, ensures the even distribution of oxygen throughout the medium, promoting aerobic microbial processes that are crucial for the treatment of wastewater. To further optimize growth conditions, these bioreactors are placed within a regulated culturing environment. A constant temperature of  $25^{\circ}\text{C} \pm 2$  and 2500 lux of illumination replicate the natural environment that is favorable for the growth of microorganisms. A well-timed light schedule of 16 hours of light and 8 hours of darkness guarantees the best possible photosynthetic activity and metabolic cycles in microbial populations [15]. A systematic extraction protocol is used to track the development and effectiveness of the therapy procedure. To assess the microbial community's growth and capacity to break down organic contaminants, microbial growth was measured. To evaluate the effectiveness of nutrient uptake and removal from the wastewater, nutrient concentrations (such as bicarbonate, sulfate, iron, and copper) were measured. We sought to learn more about the treatment

process and spot any possible bottlenecks or restrictions by monitoring these metrics over time. The evaluation of microbial growth dynamics, nutrient usage, and pollutant degradation efficiency is made possible by this strict monitoring program, which offers insightful information for improving wastewater treatment methods in the future. Weekly 300 ml sample extraction allows for comprehensive water analysis for 30 days. To determine the most effective treatment for eliminating contaminants, as indicated in Table 1, a pair of sexes of fungi and a pair of sexes of algae are employed and separated into containers for use in combinations and individually.

Table 1. Different Treatment combinations

| Cases | Treatment materials (strains)                                |
|-------|--|
| 1     | Phanerochaete chrysosporium only                             |
| 2     | Trichoderma asperallum only                                  |
| 3     | Spirogyra maxima only  |
| 4     | Gloeocapsa rupicoig only                                     |
| 5     | Spirogyra maxima and Phanerochaete chrysosporium together    |
| 6     | Spirogyra maxima and Trichoderma asperallum together         |
| 7     | Gloeocapsa rupicoig and Phanerochaete chrysosporium together |
| 8     | Gloeocapsa rupicoig and Trichoderma asperallum together      |
| 9     | Control  |

According to the APHA (2017) procedure, the pH level is measured using a pH meter in the laboratory after setting up the device with buffer solutions. Meanwhile, the bicarbonate ion concentration is determined by using the APHA (1985, 1998) method, which involves the following equation:

$$\text{HCO}_3 \text{ (mg.L}^{-1}\text{)} = \frac{V \times N \times 61 \times 1000}{\text{ml of sample}}$$

The sulfate ions are measured using a 420 nm wavelength spectrophotometer following the protocol described in APHA (1985), and the result is displayed in mg/l. As per APHA (1985, 2017), measurements of the elements are made using an atomic absorption spectrophotometer, and the results are given in milligrams per volume. This study highlighted the potential of green microalgae cultivated in nutrient-rich wastewater for resource recovery and bioenergy production [16]. The authors emphasize the critical role of mutualistic interactions between bacteria and Chlorella algae in enhancing biodegradation efficiency and bio-hydrogen production. Their genomic analysis underscores the importance of microalgae in nitrogen and phosphorus removal, confirming their value in optimized hybrid systems that combine waste treatment with renewable energy generation.

### III. RESULT AND DISCUSSION

When compared to the average concentration seen in the control treatment, the pH function test findings show a significant increase in concentration. This result highlights a notable change in the acidity or alkalinity levels in the investigated environment. This kind of divergence may indicate different changes in the chemical makeup or biological activity. The rate of acidity function, however, drops when *P. chrysosporium* and *T. asperallum* are treated; over the incubation period, the average concentration is 7.52 as opposed to 7.70 for the control treatment. As opposed to this, *M. racemosus* and *G. rupicoig* show a high rate of mixed

culture concentration, with an incubation period of 7.85. As like the Buffer solution,



Some fungi can modulate the pH of their surroundings to facilitate their growth, reproduction, and pathogenicity. Species are sometimes forced to adapt to pH changes caused by their host species or external factors in a variety of ecological circumstances. Maintaining adequate physiological functioning and survival depends on this adaptability. Living in both aquatic and mammalian digestive tracts, organisms have to deal with pH fluctuations, which can have a significant impact on biochemical reactions and ecosystem dynamics as a whole. Complex systems, like as ion transport, acid-base balance, and modulation of enzyme activity, are frequently involved in this type of adaptation to control internal pH. Thus, the capacity to adjust to changes in pH is essential to an organism's ability to survive and thrive in a variety of ecological niches. The extent of the fungi's metabolic alterations can be the cause of the decrease since fungi have a unique pH-regulating system based on the environment of their original habitat and the initial pH levels. Detergents can increase the pH of wastewater and make it more alkaline. This can affect the biodegradation of detergents and other organic pollutants by microorganisms. According to one study, the optimum pH range for biodegradation of detergents in wastewater is 6.9–8.8 [17].

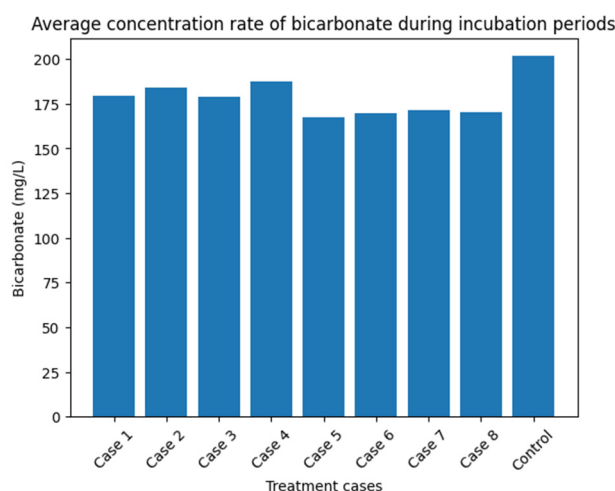


Fig. 1. Average concentration rate of bicarbonate during incubation periods.

Table 2. Analysis table for bicarbonate

| Cases  | Bicarbonate (mg/L) | % Difference from Control |
|--|--------------------|---------------------------|
| Case 1 (Phanerochaete chrysosporium only)                    | 179.3              | -11.07%                   |
| Case 2 (Trichoderma asperallum only)                         | 184.2              | -8.14%                    |
| Case 3 (Spirogyra maxima only)                               | 179.0              | -11.22%                   |
| Case 4 (Gloeocapsa rupicoig only)                            | 187.4              | -6.55%                    |
| Case 5 (Spirogyra maxima and Phanerochaete chrysosporium)    | 167.2              | -17.07%                   |
| Case 6 (Spirogyra maxima and Trichoderma asperallum)         | 169.6              | -15.88%                   |
| Case 7 (Gloeocapsa rupicoig and Phanerochaete chrysosporium) | 171.5              | -14.95%                   |
| Case 8 (Gloeocapsa rupicoig and Trichoderma asperallum)      | 170.2              | -15.61%                   |

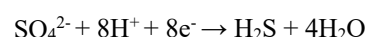
The combination of Spirogyra maxima and Phanerochaete

chrysosporium cultures is the most efficient biological treatment for bicarbonates, based on the results of laboratory experiments. The average concentration during incubation is 167.2 mg/L, which is lower than the average control coefficient of 201.6 mg/L.

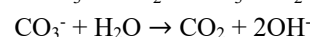
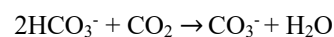
Additionally, the concentration of the bicarbonate for the fungus Trichoderma asperallum alone reached 184.2 mg/L and the concentration of the bicarbonate for the algae Gloeocapsa rupicoig alone reached 187.4 mg/L. This demonstrates that the removal rate has reached 17.07%, showing an increase in removal efficiency when the fungus Phanerochaete chrysosporium and the algae Spirogyra maxima work together. Furthermore, Fig. 1 displays the alga Gloeocapsa rupicoig coefficient with the lowest efficiency.

Bicarbonate removal is greatly improved by the combination of two different microorganisms. While the other organism efficiently extracts bicarbonate from the breakdown products, the first uses enzymes to break down complex organic molecules. Furthermore, maximizing the elimination of bicarbonate depends heavily on the exchange of nutrients and metabolites between these microorganisms. Combining them results in the perfect pH balance that for effective bicarbonate removal. These two microorganisms also create a biofilm, which creates a favorable microenvironment that speeds up the bicarbonate elimination process.

The sulfate concentration has decreased, which can be explained by the self-purification processes that are present in aquatic environments. As particulate matter containing sulfate ions falls out of the water, processes like sedimentation play a crucial role. Further aiding in sulfate reduction is the consumption of bicarbonate ions, which results in their transformation into precipitated carbonates. Several self-purification processes govern the decrease in sulfate concentration in aquatic environments. One important procedure is microbial sulfate reduction, in which anaerobic microbes transform sulfate ions into sulfide by using them as an electron acceptor:



Additionally, as these processes demonstrate, bicarbonate and carbonate ion transformations help to regulate ionic balances:



These carbonate-related processes enhance the self-purification of aquatic systems, demonstrating the interdependence of chemical and biological processes, even if they do not directly involve sulfate ions.

One possible explanation for the observed growth is the increase in the content of organic compounds, which are easily broken down by aerobic microbes like algae. Carbon dioxide ( $\text{CO}_2$ ) and carboxylic acids that are produced as a result of this process might interact with the calcium carbonate that is present in sediments and suspended particles. The product of this chemical reaction is calcium bicarbonate, which dissolves easily in water. Thus, increased amounts of organic matter could be a factor in the rise in dissolved calcium bicarbonate concentrations in aquatic environments, as shown by the following equations [18]:

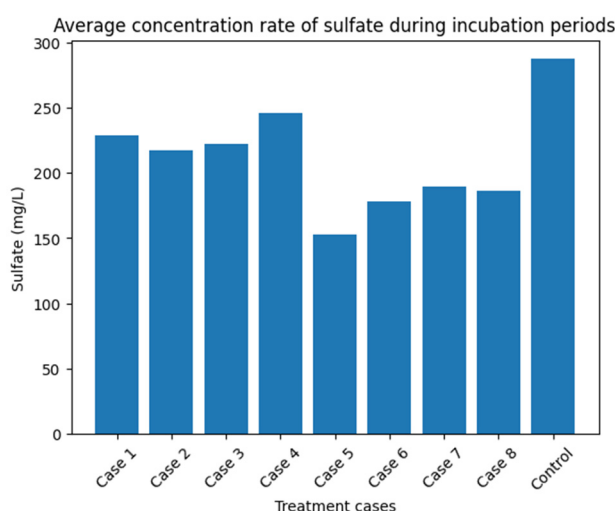
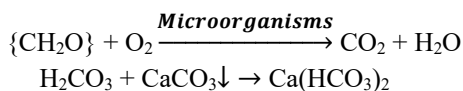


Fig. 2. Average concentration rate of sulfate during incubation periods.

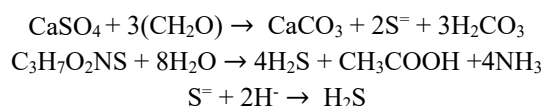
Table 3. Analysis table for sulfate

| Cases  | Sulfate (mg/L) | % Difference from Control |
|--|----------------|---------------------------|
| Case 1 (Phanerochaete chrysosporium only)                    | 287.2          | -0.01%                    |
| Case 2 (Trichoderma asperallum only)                         | 228.4          | -20.51%                   |
| Case 3 (Spirogyra maxima only)                               | 216.8          | -24.73%                   |
| Case 4 (Gloeocapsa rupicoig only)                            | 222.3          | -22.99%                   |
| Case 5 (Spirogyra maxima and Phanerochaete chrysosporium)    | 245.8          | -14.43%                   |
| Case 6 (Spirogyra maxima and Trichoderma asperallum)         | 152.7          | -46.75%                   |
| Case 7 (Gloeocapsa rupicoig and Phanerochaete chrysosporium) | 178.2          | -37.76%                   |
| Case 8 (Gloeocapsa rupicoig and Trichoderma asperallum)      | 189.3          | -34.05%                   |

Regarding sulfate concentration, all treatments resulted in a measurable decrease in sulfate ion levels relative to the control group. This finding emphasizes how well the interventions that were put in place to reduce sulfate contamination in the experimental system worked. Over a period, the average sulfate ion concentration in the treatments is found to be 152.7 mg/L, and the removal rate is 46.75% higher than that of the control of 287.2 mg/L when *Spirogyra maxima* and *Trichoderma asperallum* together used and it is the most effective biological therapy. The single actions of any algae or fungi could not manage this kind of higher efficiency. The average concentration rate of sulfate during incubation periods for different cases have been displayed in Fig.2.

There are several efficient ways to remove sulfate from water. Bioaccumulation, in which sulfate ions are actively taken up by microbial cells, is one significant process. Sulfate ions stick to the surface of these cells through a passive process called biosorption, which complements this. Because of their unique cell wall architectures, fungi and algae in particular are renowned for their remarkable biosorption properties. We can increase the effectiveness of sulfate removal from water and open the door to better water treatment solutions by utilizing the advantages of both bioaccumulation and biosorption, particularly within synergistic microbial communities [19].

It is possible to explain the observed rise in values to the presence of proteinaceous and organic waste in the water matrix. A series of biological processes are triggered by this inflow of organic materials, which acts as a substrate for microbial metabolism. Enzymatic activities are carried out by living organisms in the system, converting complex organic compounds into simpler forms through respiration and fermentation, for example. The interaction between oxidation and reduction reactions supported by microbial communities also plays a role in changing the chemical composition of water. Various chemicals are generated and transformed by these dynamic biogeochemical processes, which ultimately increase observed levels; as shown in the example below [20]:



Algae can absorb sulfate from the water by passive diffusion or active transport, depending on the sulfate gradient and the energy demand of the cells. Passive diffusion is faster and more efficient, but it requires a higher value of sulfate in the water than inside the cells. Active transport is slower and more costly, but it allows algae to absorb sulfate even when the value of sulfate in the water is lower than inside the cells [21].

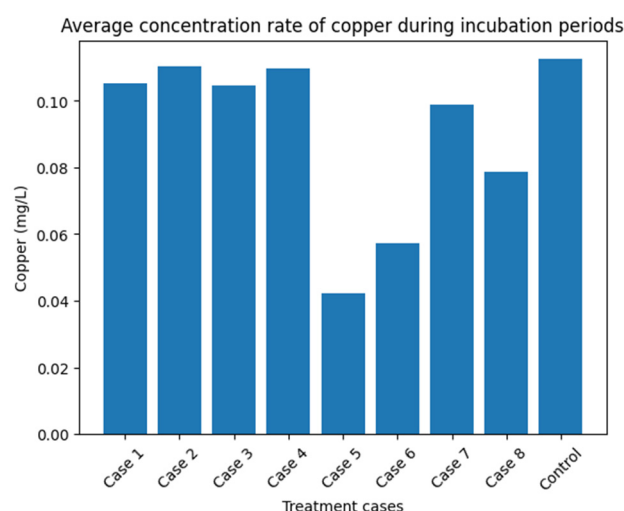


Fig. 3. Average concentration rate of copper during incubation periods.

Table 4. Analysis table for copper

| Cases  | Copper (mg/L) | % Difference from Control |
|--|---------------|---------------------------|
| Case 1 (Phanerochaete chrysosporium only)                    | 0.1125        | -0.44%                    |
| Case 2 (Trichoderma asperallum only)                         | 0.1052        | -6.54%                    |
| Case 3 (Spirogyra maxima only)                               | 0.1102        | -1.96%                    |
| Case 4 (Gloeocapsa rupicoig only)                            | 0.1046        | -22.99%                   |
| Case 5 (Spirogyra maxima and Phanerochaete chrysosporium)    | 0.1098        | -2.41%                    |
| Case 6 (Spirogyra maxima and Trichoderma asperallum)         | 0.0422        | -62.53%                   |
| Case 7 (Gloeocapsa rupicoig and Phanerochaete chrysosporium) | 0.0573        | -49.11%                   |
| Case 8 (Gloeocapsa rupicoig and Trichoderma asperallum)      | 0.0987        | -12.31%                   |

According to the research findings, copper offers the most effective biological treatment when used in conjunction with *Spirogyra maxima* and *Trichoderma asperallum*. The



concentration after the incubation period is 0.0422 mg/l and the clearance is 62.53%, contraindications for the control coefficient, the average concentration after the incubation period is 0.1125 mg/l.

The procedure entails copper ions attaching themselves to particular functional groups on the cell surface, including hydroxyl, carboxyl, and amino groups. The concentration rate of copper in wastewater can have both positive and negative effects on algae used in wastewater treatment. Algae are exceptionally effective at treating wastewater by eliminating a wide range of contaminants from water bodies, including pesticides, heavy metals, chemical fertilizers, and other pollutants [22, 23]. These tiny creatures absorb and retain these pollutants in their cellular structures through a process called bioaccumulation, which successfully rids the water of dangerous materials. Algae can also produce oxygen and biomass that can be converted into biofuels such as biohydrogen [24]. However, algae are also sensitive to copper, which a common metal is found in wastewater. Copper can act as an algicide, killing or inhibiting the growth of algae, but it can also cause stress, damage, and toxicity to algae cells [25]. Even the production of insoluble copper compounds can result from the biomineralization of copper ions, which is facilitated by some microbes. Because these processes frequently cooperate, microbes are useful agents for the bioremediation of water contaminated with copper.

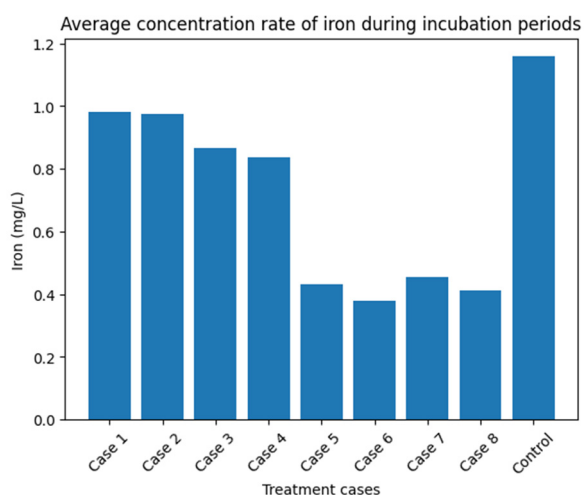


Fig. 4. Average concentration rate of iron during incubation periods.

Table 5. Analysis table for iron

| Cases  | Iron (mg/L) | % Difference from Control |
|--|-------------|---------------------------|
| Case 1 (Phanerochaete chrysosporium only)                    | 1.1587      | -0.47%                    |
| Case 2 (Trichoderma asperallum only)                         | 0.9806      | -15.40%                   |
| Case 3 (Spirgyra maxima only)                                | 0.9745      | -15.91%                   |
| Case 4 (Gloeocapsa rupicoig only)                            | 0.8672      | -25.21%                   |
| Case 5 (Spirgyra maxima and Phanerochaete chrysosporium)     | 0.8380      | -27.39%                   |
| Case 6 (Spirgyra maxima and Trichoderma asperallum)          | 0.4306      | -62.70%                   |
| Case 7 (Gloeocapsa rupicoig and Phanerochaete chrysosporium) | 0.3806      | -67.23%                   |
| Case 8 (Gloeocapsa rupicoig and Trichoderma asperallum)      | 0.4562      | -60.55%                   |

Regarding iron content, the result for *Gloeocapsa rupicoig* and *Phanerochaete chrysosporium* combination shows a significant decline, suggesting a significant drop in iron

concentration in the experimental system. In particular, the observed value is 0.3806 mg/L, which indicates a noteworthy clearance rate of 67.23%. The iron content in the control group is 1.1587 mg/l, a markedly different level from this notable reduction. These noticeable variations emphasize how well this mutualism of algae and fungi reduces iron levels and points to the plant's potential as an efficient remediation tool for areas contaminated with iron.

Iron removal from water is largely dependent on microorganisms, especially iron-oxidizing bacteria, which oxidize soluble ferrous iron ( $\text{Fe}^{2+}$ ) to insoluble ferric iron ( $\text{Fe}^{3+}$ ), which precipitates out of solution. Additionally, microorganisms can actively transport iron ions into their cells through bioaccumulation or adsorb them onto their cell surfaces through biosorption. In certain situations, microorganisms can also induce biomineralization, which results in the formation of iron-containing minerals. All of these mechanisms make microbial processes a promising method for the bioremediation of iron-contaminated water. In wastewater treatment, the relationship between iron concentration and algae is a complex phenomenon that is influenced by several variables. These variables include the particular kind and amount of algae used in the treatment process, the water's pH and temperature, the presence of additional metals and contaminants at the same time, and the length and severity of the treatment plan. The complex interplay between these variables determines the overall efficacy and result of the wastewater treatment mediated by algae, highlighting the significance of taking into account holistic environmental aspects while improving treatment approaches. Some studies have shown that algae can reduce iron concentration in wastewater by up to 90% [24]. However, other studies have reported that algae can increase iron concentration in wastewater due to the release of organic acids and chelating agents [25]. Therefore, the optimal conditions for algae-based wastewater treatment need to be carefully determined and monitored.

Some elements are more likely to experience concentration amplification in water matrices due to the breakdown of organic molecules and suspended particulate debris. Furthermore, these substances might change so that they become indigestible to marine life or that they attach to biological waste products, changing back into a less absorbable form. This phenomenon emphasizes the dynamic nature of elemental transformations and nutrient cycling within natural water bodies, as well as the intricate interactions between biotic and abiotic variables in aquatic ecosystems. Aquatic species' ability to absorb ions is closely related to environmental variables such as pH and temperature. These factors have a direct impact on how heavy metals behave, which impacts how organisms absorb them. The kinetics of ion absorption can be changed by changes in pH and temperature, which can affect ecological dynamics and rates of bioaccumulation. Therefore, precise control over these environmental factors is essential to comprehending and regulating the interactions of heavy elements in aquatic systems. Acidic pH increases solubility and hence increases absorbable ion concentrations, but alkaline pH makes it easier for heavy elements to be absorbed and precipitated. These two effects on pH ion concentrations are mutually exclusive. By absorbing and securing ions, microorganisms additionally

participate in the remediation of the surrounding environment. Mycoremediation is emerging as an eco-friendly and promising solution for managing environmental contaminants. Through their unique capabilities, fungi, via their mycelium, play a pivotal role in the removal of organic and inorganic pollutants. Their mechanisms of biosorption and bioaccumulation enable the fixation and metabolism of substances such as heavy metals, industrial dyes, and recalcitrant compounds present in wastewater [26–31].

#### IV. CONCLUSION

This study highlights the great potential for effectively treating wastewater by utilizing the synergistic properties of fungi and algae. We have clarified the transformative effect of these organisms on several parameters essential to water quality repair through a series of laboratory tests. Firstly, *P. chrysosporium* and *T. asperallum* treatments showed a considerable reduction in acidity levels, with an average pH concentration of 7.52 compared to 7.70 in the control treatment, according to the pH function tests. Additionally, our examination of the concentrations of bicarbonate highlights the effectiveness of the co-cultures of *Spirogyra maxima* and *Phanerochaete chrysosporium* in lowering bicarbonate levels by 17.07%, with an average concentration of 167.2 mg/L as opposed to 201.6 mg/L in the control. These findings demonstrate the potential of combined fungal-algal treatments for wastewater bicarbonate reduction. Furthermore, the treatments involving *Spirogyra maxima* and *Trichoderma asperallum* showed the greatest reduction in sulfate, with an average concentration of 152.7 mg/L and a removal rate of 46.75%, as opposed to 287.2 mg/L in the control. This highlights how well these biological treatments work to reduce sulfate pollution in aquatic environments. In terms of heavy metal removal, our results show that co-cultures of *Spirogyra maxima* and *Trichoderma asperallum* were most effective in removing copper, with a concentration of 0.0422 mg/L and a clearance rate of 62.53%, as opposed to 0.1125 mg/L in the control. In the case of iron removal, a noteworthy clearance rate of 67.23% by *Gloeocapsa rupicoig* and *Phanerochaete chrysosporium* combination, with an average concentration of 0.3806 mg/L as opposed to 1.1587 mg/L in the control. This study has shed important light on the feasibility of treating wastewater with a fungal-algal partnership. The observed decreases in the quantities of iron, copper, and bicarbonate demonstrate how effective this method is at eliminating different types of pollutants. This research is novel since it targets copper-based contaminants and other pollutants by investigating fungal and algae combinations and achieved a high efficiency. Compared to conventional approaches, this synergistic treatment strategy has several benefits, such as a lower dependency on chemicals, a possibly wider ability to remove pollutants, and a more sustainable methodology.

#### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

#### AUTHOR CONTRIBUTIONS

Likhon Chandra Roy: Writing: Original Draft; Conceptualization; Methodology; Formal Analysis; Yassine

Ezaier: Writing: Original Draft; Methodology; Ahmed Hader, Khalid Ansari, Abderrahim Maftouh: Writing: Review & Editing, Formal Analysis, Methodology; Hesam Kamyab, Hussameldin Ibrahim: Writing: Review & Editing, Resources; Mohammad Yusuf: Writing: Review & Editing, Formal Analysis, Supervision.

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