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Original article

Bioremediation of Synthetic Prepared Domestic Wastewater with *P. chrysosporium*

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Abstract

In this study, the removal efficiencies were evaluated based on key environmental parameters such as chemical oxygen demand (COD), Total Organic Carbon (TOC), Total Nitrogen (TN), and Total Phosphorus (TP), using P. chrysosporium white rot fungus to treat synthetically prepared domestic wastewater. The research aimed to assess the suitability of bioremediation results with respect to the Urban Wastewater Treatment Regulation. The experiment was carried out over a span of 11th days, employing static 1/1 and 1/5, as well as dynamic 1/1 and 1/5 dilution ratios. The outcomes revealed varying removal efficiencies, with the highest rates observed at dynamic conditions and a 1/5 dilution ratio: 84% for COD, 81% for TOC, 73% for TN, and 56% for TP. Conversely, the lowest removal efficiencies were determined under static 1/1 conditions, reaching 48% for COD, 33% for TOC, 31% for TN, and 45% for TP. Based on the results, it is evident that *P. chrysosporium* exhibited effective bioremediation capabilities on synthetic domestic wastewater within a reasonable 11th day period. Furthermore, the results aligned with the specified limit values outlined in the Urban Wastewater Treatment Regulation. Consequently, the study highlights the efficacy of *P. chrysosporium* as a valuable species for biological treatment stages in urban wastewater management.

Keywords: Bioremediation, Domestic wastewater, *P. chrysosporium,* Total Nitrogen, Total Phosphorus.

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INTRODUCTION

Wastewater refers to water discarded or previously used by municipalities or industries. Domestic wastewater, originating from household activities like kitchen, shower, toilet, and laundry, constitutes wastewater produced by human activities in households (Samwel, 2005). Municipal wastewater primarily comprises water (99.9%) along with minor concentrations of suspended and dissolved organic and inorganic solids. These solids include carbohydrates, lignin, fats, proteins, synthetic detergents, and both natural and synthetic organic chemicals from industrial processes. Among wastewater streams, municipal wastewater stands out as a prevalent type with low organic strength and high particulate organic matter content (Van, 2008). Characteristically, domestic wastewater is grey in color, presents a musty odor, and contains around 0.1% solids. This solid material includes faeces, food particles, grease, oil, soap, salts, metals, detergents, and other components, both suspended (30%) and dissolved (70%).

Domestic wastewaters, rich in carbon, nitrogen, and phosphorus, are produced in substantial volumes and offer potential nutrient sources for fungal growth. Nitrogen and phosphorus are pollutants of concern due to their role in algal growth and water body eutrophication. Nitrogen, present as organic nitrogen in fresh sewage, transforms into ammonia upon bacterial decomposition. In aerobic conditions, ammonia nitrogen converts into nitrites and nitrates, which can be utilized by algae to form plant proteins. Phosphorus typically exists as orthophosphate, polyphosphate, and organic phosphate forms. While organically bound phosphorus has minimal significance in domestic sewage, polyphosphate forms revert to orthophosphates over time. COD serves as a measure of organic strength in wastewater, which reduces after treatment due to enhanced oxygen availability for organic matter oxidation (Raju et al., 2010). Similarly, TOC serves as an indicator of organic pollution and is commonly analyzed in wastewater control (Visco et al., 2005).

Presently, various techniques like reverse osmosis, ozonation, and chemical precipitation are used for wastewater treatment, but they are often expensive and energy-intensive, especially for diverse wastewater compositions (Singh et al., 2020). This necessitates exploring economical and sustainable approaches aligning with discharge standards (Upadhyay et al., 2021). Bioremediation, utilizing microorganisms to degrade pollutants into less toxic forms, offers a cost-effective alternative. White-rot fungus *Phanerochaete chrysosporium* is recognized for degrading persistent pollutants using extracellular enzymes. The fungus employs oxidative and reductive mechanisms along with redox mediators to effectively degrade various chemicals (Cameron et al., 2001).

In literature studies, there are too much studies for *P. chrysosporium* to treate organic and inorganic pollutants, so the authors selected this white rot fungi as a bioremediator tool for treate a synthetic domestic wastewater. This is the first study about treatment of synthetic domestic wastewater with *P.chrysosporium.*

This study focuses on using the white-rot fungus *P. chrysosporium* for remediating synthetically prepared domestic wastewater. The primary objective is to assess the feasibility of employing *P. chrysosporium* for remediating this type of wastewater.

MATERIALS and METHODS

Preparation of synthetic wastewater

In first stage, a total of 5 liters of synthetic domestic wastewater was meticulously prepared. To achieve this, 800 mg of peptone and 550 mg of malt extract medium were combined with 140 mg of urea (K_2HPO_4), 35 mg of sodium chloride (NaCl), 20 mg of calcium chloride dihydrate ($CaCl_{22}H_2O$), and 10 mg of magnesium sulfate heptahydrate ($MgSO_4.7H_2O$). These components were added to a sterile plastic bottle, following the procedural guidance presented in Krismastuti and Hamim (2019), and then stirred to achieve uniformity.

The composition of this synthetic wastewater formulation approximates 106 mg/L of organic carbon (OC), 46 mg/L of nitrogen, and 5 mg/L of phosphorus. The methodology applied to create this synthetic wastewater was informed by the approaches detailed by Krismastuti and Hamim (2019).

Preparation of *P. chrysosporium* individuals

P. chrysosporium strains, already present in the culture collection of Munzur University's Department of Environmental Engineering Microbiology Laboratory, were initially transferred to Sabouraud dextrose agar medium under aseptic conditions. Subsequently, three small samples of approximately 1 cm in diameter were collected from juvenile individuals that had developed over a span of 3 days at a temperature of 27°C. These samples were then introduced into 250 ml flasks, each containing freshly prepared 200 ml of Sabouraud dextrose broth medium.

The flasks were placed in an incubator equipped with a shaker, set to operate at 25°C and 150 rpm. The intention was to induce pellet formation within the culture. After a 72-hour incubation period, these pellets, which had formed by the end of the process, were selected as the individuals for subsequent bioremediation activities.

Bioremediation procedures

Pellets generated by *P. chrysosporium*, cultivated at 1/1 and 1/5 dilution ratios, were introduced in amounts of approximately 5 grams each into two sets of 250 ml synthetic domestic wastewater. These two sets comprised both static and dynamic conditions, each at 1/1 and 1/5 dilution ratios. All manipulations were performed under sterile conditions.

During this phase, the dynamic sets were placed in a shaker incubator model ZHWY-2008 by Ttyitech, set to 27°C. Conversely, the static sets were incubated at a constant temperature of 27°C.

Chemical Oxygen Demand (COD) determinations

COD analyses were conducted on samples collected every 48 hours. For these analyses, HACH DRB 200 model thermoreactor and Hach DR 890 Colorimeter were employed, with the capability to measure within the range of 0-1500 ppm. The COD assessments utilized COD kits with Cat. 23459-52 model, which were compatible with the instruments.

The experiments spanned a total of 11^{th} days. Analysis of total phosphorus and ammonium nitrogen was carried out using a Schimadzu brand Ion Chromatography (IC) device. To determine these parameters, the device was configured with 2.5mM phthalic acid (pH=4) and 2.5mM oxalic acid, respectively.

During analysis, Shim-pack IC-C4 and IC-A1 columns were employed, each with flow rates of 1.5 ml/min and 1 ml/min. Injection volumes of 30µL and 50µL were used for anionic and cationic columns, respectively.

Total Organic Carbon (TOC) Analysis

The determination of Total Organic Carbon (TOC) involved the high-temperature combustion method outlined in Standard Method 5310B, which was executed using the SHIMADZU TOC-V device. The experiments were conducted over an 11-day duration.

Total Phosphorus (TP) and Total Nitrogen (TN) Analysis

Total phosphorus and total nitrogen analysis were performed using a Schimadzu brand Ion Chromatography (IC) device. For these analyses, the device was set with 2.5mM phthalic acid (pH=4) and 2.5mM oxalic acid. The analytical process employed Shim-pack IC-C4 and IC-A1 columns, operating with flow rates of 1.5 ml/min and 1 ml/min, respectively. Injection volumes were 30µL for anionic columns and 50µL for cationic columns.

RESULTS and DISCUSSION

Bioremediation Study Results

The outcomes derived from the bioremediation activities conducted using the *P. chrysosporium* are presented between Figure 1 - Figure 4 for the COD parameter, and in Figure 5 - Figure 8 for the TOC parameter. For, TN parameter, the results were presented between Figure 9 – 12. The overall results pertaining to phosphorus are illustrated between Figure 13 and Figure 16 for all synthetic and dynamic conditions.

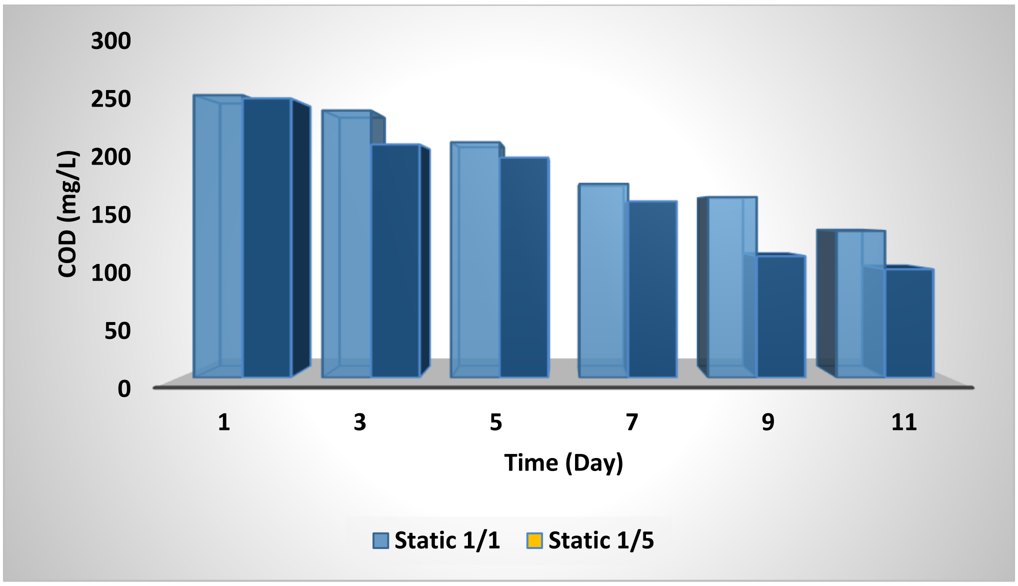

Figure 1. COD reduction of synthetic wastewater on *P. chrysosporium* in static 1/1 and 1/5 conditions.

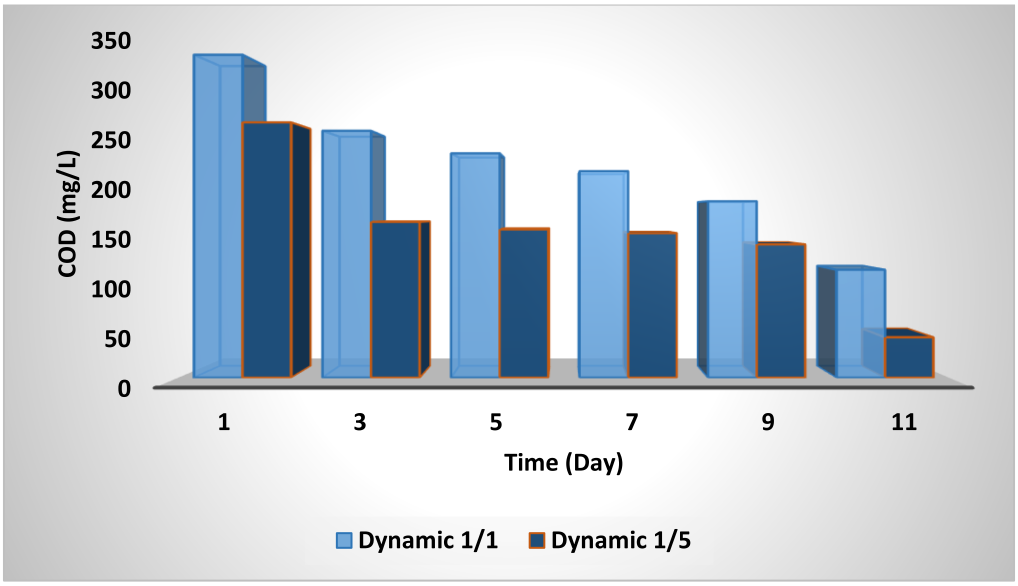

Figure 2. COD reduction of synthetic wastewater on *P. chrysosporium* in dynamic 1/1 and 1/5 conditions.

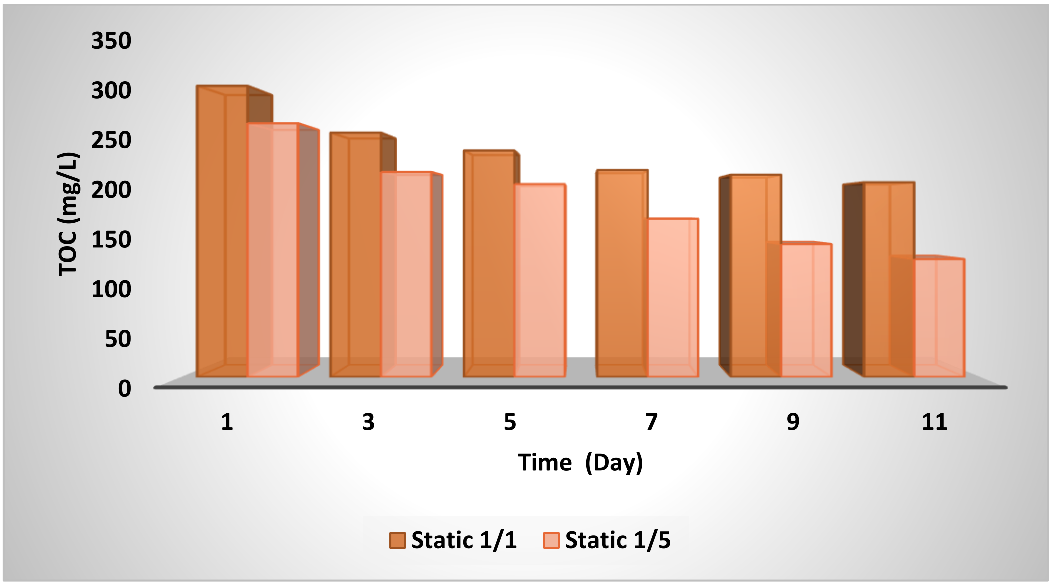

Figure 3. TOC reduction of synthetic wastewater on *P. chrysosporium* in static 1/1 and 1/5 conditions.

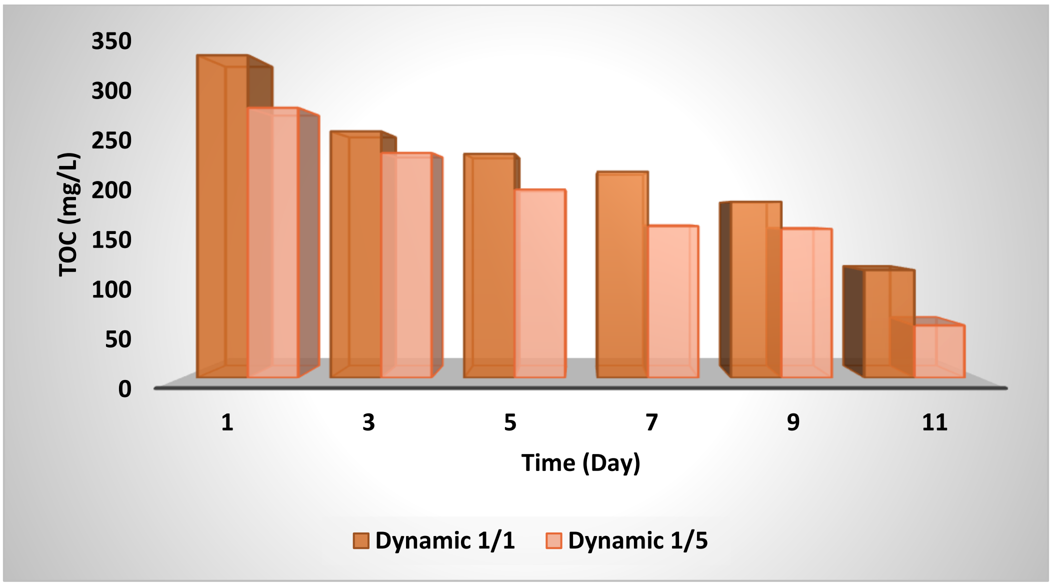

Figure 4. TOC reduction of synthetic wastewater on *P. chrysosporium* in dynamic 1/1 and 1/5 conditions.

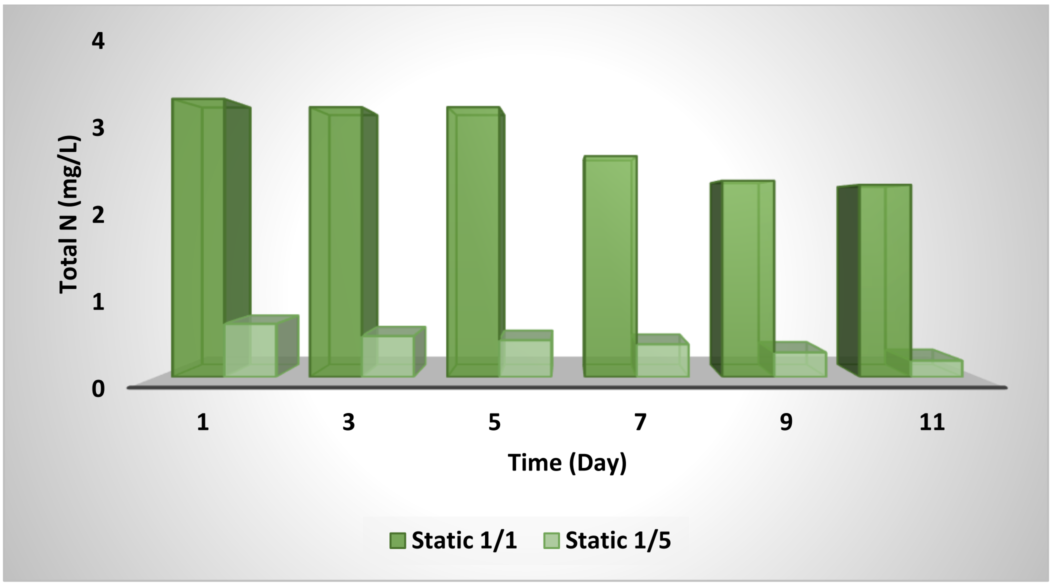

Figure 5. Total N reduction of synthetic wastewater on *P. chrysosporium* in static 1/1 and 1/5 conditions.

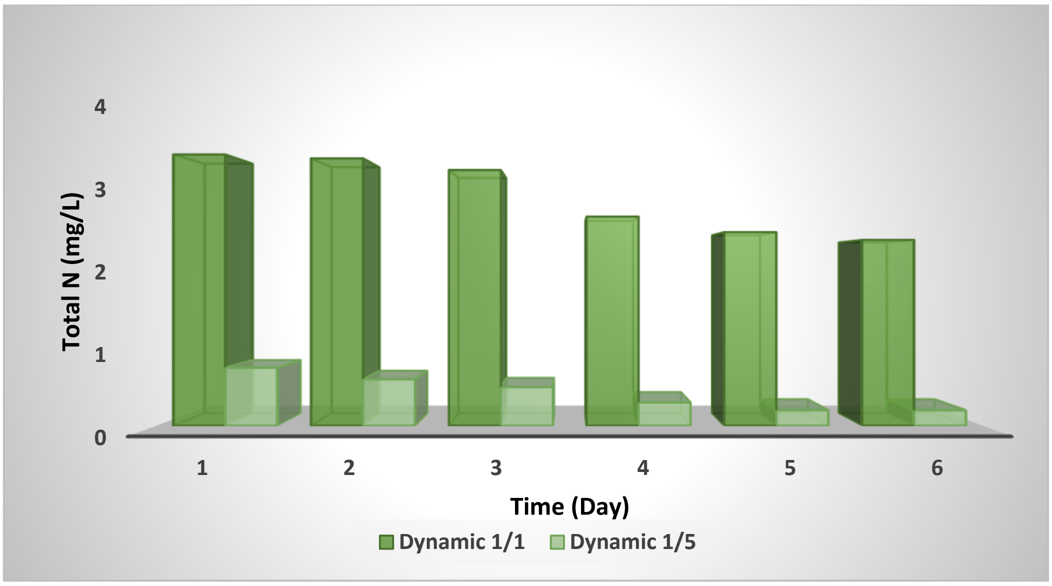

Figure 6. Total N reduction of synthetic wastewater on *P. chrysosporium* in dynamic 1/1 and 1/5 conditions.

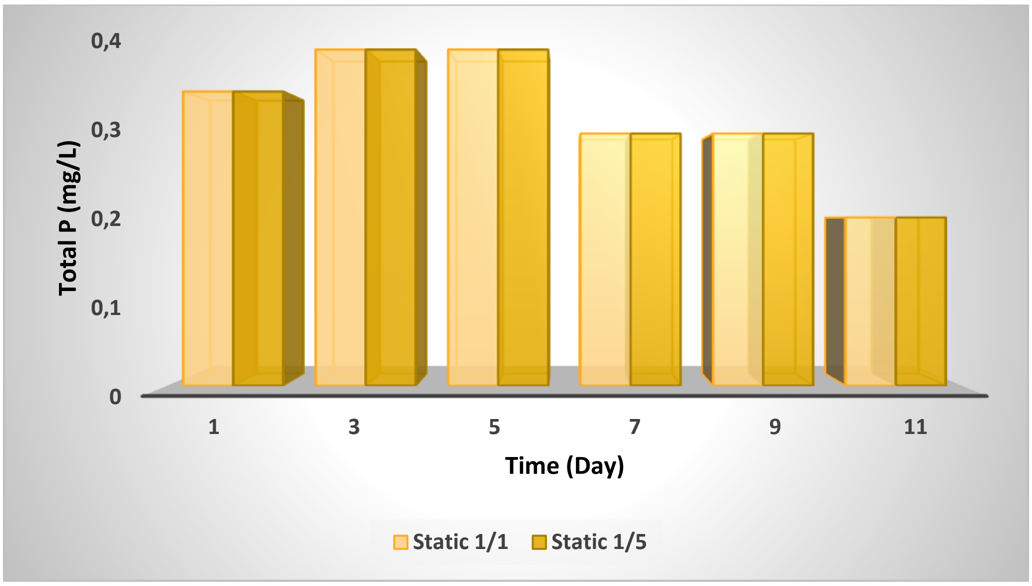

Figure 7. Total P reduction of synthetic wastewater on *P. chrysosporium* in static 1/1 and 1/5 conditions (Total Phosphorus = Total organic phosphorus + total inorganic phosphorus)

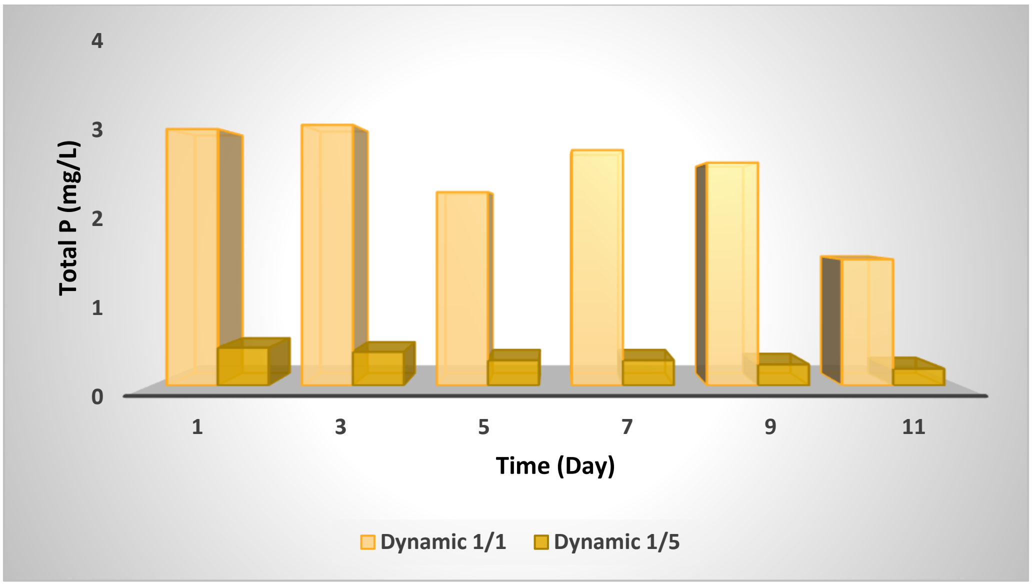

Figure 8. Total P reduction of synthetic wastewater on *P. chrysosporium* in dynamic 1/1 and 1/5 conditions

The results of the study revealed removal efficiencies of 48, 33, 31, and 45% for COD, TOC, total nitrogen, and total phosphorus, respectively, by the end of the 11th day in a static 1/1 conditions. Specifically, these removal efficiencies were from 258 mg/L to 134 mg/L for COD, from 311 mg/L to 208 mg/L for TOC, and from 3.4 mg/L to 2.35 mg/L for total nitrogen, along with a trend from 3.2 mg/L to 1.75 mg/L for total phosphorus.

In the context of 1/5 diluted media, which represents another static condition, significant COD, TOC, total nitrogen, and total phosphorus reductions were observed. COD levels decreased from 255 mg/L to 99 mg/L, reflecting a notable removal efficiency of 61%. Likewise, TOC exhibited a decrease from 271 mg/L to 126 mg/L, with a corresponding removal efficiency of 53%. Additionally, total nitrogen demonstrated a considerable reduction of 69%, declining from 0.65 mg/L to 0.2 mg/L. The decrease in total phosphorus ranged from 0.35 mg/L to 0.2 mg/L, signifying a reduction of 43%.

The bioremediation investigation was concurrently carried out under dynamic conditions with dilution ratios of 1/1 and 1/5. Analyzing the bioremediation efficiencies in these dynamic conditions, the removal efficiencies achieved by the end of the 11th day within the 1/1 medium ranged from 278 mg/L to 58 mg/L, resulting in a notable efficiency of 79%. Furthermore, concerning TOC, there was a decline from 344 mg/L to 115 mg/L, indicating a substantial reduction of 67%. In terms of total nitrogen, the study observed a reduction from the initial level of 3.5 mg/L to 2.4 mg/L, representing a removal efficiency of 31%. Conversely, the total phosphorus parameter exhibited a decreasing trend from 3.05 mg/L to 1.5 mg/L, corresponding to a significant decrease of 51%.

As the culmination of the dynamic study phase, the COD and TOC values within the 1/5 dilution medium witnessed a substantial reduction. Specifically, the initial values of 272 mg/L for COD and 288 mg/L for TOC decreased significantly to 43 mg/L and 56 mg/L, respectively, yielding impressive removal efficiencies of 84% and 81%.

Furthermore, the parameter for total nitrogen showcased a decrease from 0.75 mg/L to 0.2 mg/L by the end of the 11th day, with a significiant removal efficiency of 73%. Similarly, the total phosphorus exhibited a notable decline of 56%, dropping from 0.45 mg/L to 0.2 mg/L.

Upon scrutinizing the reductions of COD, TOC, total nitrogen, and total phosphorus across various settings including dynamic 1/1 and 1/5, as well as static 1/1 and 1/5 conditions, it is evident that the highest removal rates were achieved in the dynamic 1/5 medium, reaching an impressive 84% for COD and 81% for TOC. Furthermore, for total nitrogen and total phosphorus, removal efficiencies of 73% and 56% were respectively attained in this dynamic 1/5 context, underscoring its effectiveness.
On the other hand, the lowest removal rates were observed in the static 1/1 medium, with COD and TOC experiencing 48 and 33% reduction, respectively. Notably, the removal rate for total nitrogen remained consistent at 31% in both static 1/1 and dynamic 1/1 conditions. Similarly, the total phosphorus parameter exhibited a minimal removal rate of 43 and 45% in the static 1/5 medium, indicating close alignment with this value respectively. According to these results, it can be conducted that the static 1/1 condition is suitable for achieving the minimum removal efficieincy.

Furthermore, the results of this study were compared with the Urban Wastewater Treatment Regulation standards. Notably, the calculated removal efficiencies in the static 1/5, dynamic 1/1, and dynamic 1/5 conditions all demonstrate adherence to the specified limits. Specifically, the observed COD values in these medium do not surpass the required maximum discharge value of 125 mg/L. In the case of total phosphorus, the maximum permissible level of 2 mg/L for cities with populations ranging from 10,000 to 100,000 remains unexceeded. For cities with populations exceeding 100,000; the stringent threshold of 1 mg/L for total phosphorus is not breached, whether in static 1/5 or dynamic 1/5 settings. Encouragingly, the recorded levels of total phosphorus consistently remain below 0.2 mg/L across these medium, signifying compliance with regulatory standards.

Upon comparison of the total nitrogen values obtained in this study with established limits, it is evident that the upper threshold of 15 mg/L for total nitrogen is not exceeded within cities encompassing a population range of 10,000 to 100,000. While regulatory guidelines do not explicitly encompass the TOC parameter, its values can be correlated with those of COD. By evaluating both treatment efficiency and final values in line with existing literature, this study demonstrates alignment with urban wastewater discharge limits. The escalating issues of rapid urbanization, industrialization, and sanitation challenges are resulting in the escalation of water pollution. This disturbing trend intensifies the risk associated with consuming contaminated water, impacting both underdeveloped and developing nations alike. As societies experience heightened water demand, available water resources dwindle due to the depletion of non-renewable sources, amplified water system pollutants, declining groundwater reservoirs, shifts in climate patterns, and the accumulation of pollutants in water bodies. Consequently, a confluence of factors contributes to elevated instances of drought, increased evaporation, diminished precipitation, and transformative changes in aquatic ecosystems (Ali et al., 2021).

Diverse physicochemical methods are commonly employed to mitigate environmental pollutants. However, the feasibility of these techniques is often hindered by factors like exorbitant maintenance and operational expenses, coupled with the generation of hazardous secondary byproducts. In contrast, a promising strategy for pollutant removal is bioremediation, which offers distinct advantages over alternative approaches. It boasts superior removal efficiency, an ecologically conscious process, and notably lower operational and maintenance costs. Bioremediation hinges on harnessing the capabilities of microorganisms, such as fungi and bacteria, to eradicate or substitute contaminants (Giovanella et al., 2020).

Among microorganisms, bacteria emerge as particularly well-suited for biological processes. Their inherent attributes facilitate rapid growth and replication at a heightened rate compared to other counterparts. This characteristic renders bacteria uniquely equipped to efficiently cleanse organic pollutants, presenting significant potential for purifying environmental substrates (Bhatia et al., 2017).

Numerous types of fungal spores hold potential for bioremediation applications, including species like *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus niveus*. However, certain strains may be constrained by extended spore formation cycles. Notably, the Ascomycetes fungi group has gained prominence for its efficacy in mitigating COD in industrial wastewater, particularly from industries such as distilleries (Mohammad et al., 2006).

In a study by Bardi et al. (2017), the impact of a white root fungus strain *named Bjerkandera adusta* MUT 2295 was investigated on synthetic persistent waste samples containing tannic and humic acids. The outcomes revealed substantial promise, with tannic acid waste witnessing a 61% COD reduction and tannic and humic acid solutions experiencing 89 and 75% reductions in biochemical oxygen demand (BOD_5), respectively.

Within domestic wastewater, there is an inclusion of detergent effluents, and phosphorus loads stemming from human waste and household cleaning items measure 0.30, 0.60, and 0.10 kg phosphorus per capita, respectively. These insights have proven instrumental in gauging the cumulative burden on wastewater treatment facilities. In a study by Larsen and Maurer (2011), they proposed that the amalgamated composition of domestic wastewater, with a volume of 130 L/person/day, encompasses 120 g/person/day of COD, 14 g/person/day of N, and 2 g/person/day of P. The evaluation of contaminant removal was conducted under varying COD concentrations to assess microbial competence during the experimentation.

When subjected to high COD levels (970 – 1200 mg/L), the percentage of COD removal remained consistently within the range of 91.9 to 94.7%, yielding an average effluent of 54–80 mg/L from day 71 to day 75. Conversely, under low COD conditions (236 – 320 mg/L), the percentage of COD removal remained stable, ranging from 57.9 to 59.9%, with the mean effluent falling between 67–135 mg/L from day 80 to day 85 (Zulkifli et al., 2023). These microorganisms are believed to exhibit remarkable adaptability across varying COD concentrations. While modest removal rates are achieved under low COD levels, the extended adaptation period fosters more favorable outcomes in such conditions.

In a distinct study, the *Botryococcus sp.* NJD-1 strain exhibited remarkable traits as a lipid producer within the wastewater milieu, demonstrating lipid contents that reach up to 61.7% and TN levels reaching as high as 64.5%. Moreover, this strain exhibited notable removal capabilities, achieving rates of 89.8% for TN and 67.9% for TP and TOC (Shen et al., 2017). *Parachlorella kessleri*, on the other hand, displayed an impressive removal capacity by eliminating 99% of nitrogen compounds and 82% of phosphorus compounds from municipal wastewater (Aketo et al., 2020).

Domestic wastewater is a complex mixture containing both organic compounds and nutrients. The BOD/COD ratio, calculated as 0.5, signifies its biodegradability, indicating the potential for microbial degradation (Zhang et al., 2020). Additionally, domestic wastewater harbors elevated levels of ammonia and nitrites. The abundance of nitrites may be attributed to the reduction of nitrates, a process occurring under anaerobic conditions, as evidenced by the low dissolved oxygen (DO) value of 0.06 mg/L in domestic wastewater (Jeannote, 2014).

Nitrogen sources within domestic wastewater originate from various contributors, including organic matter and food waste from cesspool wastewater and kitchen-related activities (Cao et al., 2022). The primary origins of domestic water pollution are traced back to gray water and activities within the kitchens (Boano et al., 2020). According to Al-Ajalin et al. (2022), COD concentrations in domestic wastewater reach 234 mg/L, accompanied by concentrations of 12 mg/L for ammonia and 5 mg/L for phosphate. In a similar vein, Dinh et al. (2021) reported concentrations of COD, ammonia, nitrate, and phosphate as 231, 42, 0.16, and 6.5 mg/L, respectively. While the organic constituents in domestic wastewater are primarily contributed by gray water (Widyarani et al., 2022), nutrients such as N and P originate from activities like kitchen use, car washing, and laundry (Al-Ajalin et al., 2020).

In a study by Huo et al. (2020), it was found that the optimal N/P ratio for the growth of *Tribonema sp.* was 30:1, leading to a maximum biomass of 2.04 g/L after 14 days of cultivation. Similarly, *C. sorokiniana* demonstrated a final mean biomass concentration of 12 g/L, where N/P ratios ranged from 15 to 26 (Fernandes et al., 2017). This underscores the pivotal role of the N/P ratio in wastewater, as microalgae thrive and assimilate nitrogen and phosphorus at a specific stoichiometric balance (Beuckels et al., 2015).

The noteworthy removal of COD through co-culture is attributed to increased biomass production. This phenomenon is attributed to the assimilation of organic matter present in domestic wastewater, stimulating the growth of the microalgae culture. Nevertheless, the presence of persistent organic compounds poses challenges for microalgae decomposition, potentially leading to incomplete COD reduction (Xia and Murphy, 2016).

Furthermore, the effective elimination of nitrogen and phosphorus from wastewater through co-culture is attributed to the attainment of a specific stoichiometric N/P ratio. This balanced ratio fosters the co-culture's growth, resulting in heightened cell density and augmented nutrient uptake from the wastewater (Zhang et al., 2020). However, the role of phosphate concentration is crucial, as low levels can hinder nitrate absorption within an ideal concentration range of 10–75 mg/L, potentially impeding bioremediation and microalgae growth (Suthar and Verma, 2020).

CONCLUSION

This study explores the efficacy of pellets generated by the *P. chrysosporium* in removing COD, TOC, total nitrogen, and total phosphorus parameters from synthetic domestic wastewater, considering dynamic and static conditions at 1/1 and 1/5 dilution ratios. The compliance of these removals with the Urban Wastewater Treatment Regulation and the potential for achieving desired discharge limits are investigated. Results indicate that *P. chrysosporium* efficiently removes COD, TOC, total nitrogen, and total phosphorus in synthetic domestic wastewater, aligning with the Urban Wastewater Treatment Regulation. The utilization of constituents within the synthetic wastewater as nutrients contributes to effective removal. While the regulation lacks a specific TOC limit, the correlation with COD suggests the importance of considering TOC as an alternative parameter. Expanding the study to real or simulated domestic wastewaters, using various fungal and bacterial consortia, holds promise for future research avenues. Interestingly, the study investigates the TOC parameter, which isn't covered by the discharge regulation, yet achieves effective removal. Proposing a TOC limit in the Urban Wastewater Treatment Regulation can complement existing BOD and COD parameters and enrich the regulatory framework. The effectiveness of *P. chrysosporium* in COD, TOC, total nitrogen, and total phosphorus removal is evident. Thus, it's recommended to cultivate, isolate, and promote the use of this fungus in biological treatment systems. Capitalizing on its capabilities can significantly enhance the removal efficiency of both organic and inorganic pollutants in domestic wastewater bioremediation. Comparing with existing literature, this study highlights a rare achievement: *P. chrysosporium* attains over 80% removal efficiency in vital environmental parameters such as COD, TOC, total nitrogen, and total phosphorus within a brief 11-day period for both synthetic and original domestic wastewater. Further exploration is encouraged to assess removal efficacy across a broader spectrum of environmental parameters.

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