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Article

Comparative Assessment of Treatment of Mushroom Farm Wastewater Using Plant (*Ceratophyllum demersum* L.) and Algae (*Chlorella vulgaris*): Experimental and Kinetic Studies

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Abstract: Mushroom cultivation produces a significant amount of wastewater containing high levels of both organic and inorganic contaminants. In this study, mushroom farm wastewater (MFW) was treated separately by aquatic macrophytes (*Ceratophyllum demersum* L.) and algae (*Chlorella vulgaris*). The laboratory experiments consisted of a constructed reactor planted with selected aquatic plants and a microalgal culture and operated for 16 days. The pollutant removal efficiency was evaluated using different experimental combinations such as control 1 (*C. demersum* using borewell water), control 2 (*C. vulgaris* using borewell water), T1 (*C. demersum* using MFW), and T2 (*C. vulgaris* using MFW), respectively. The results showed that the T1 treatment had the highest significant ($p < 0.05$) removal efficiency of selected pollutant parameters (total dissolved solids: 86.00%; biochemical oxygen demand: 83.10%; chemical oxygen demand: 86.60%; total nitrogen: 84.30%; total phosphorus: 75.60%). The kinetic studies using the first-order reaction model showed a good fit ($R^2 > 0.8317$) and the maximum rate constant (k) of pollutant reduction in T1 treatment. In addition, the growth, biochemical, and proximate parameters of both *C. demersum* and *C. vulgaris* were highest in the same treatment. Therefore, the proposed experiment offers a promising approach for the efficient and environmentally friendly treatment of MFW.

Keywords: agro-industrial waste; bioremediation; growth performance; horticulture wastewater; pollutants

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1. Introduction

Mushroom farm wastewater (MFW) can be defined as the result of various mushroom cultivation processes, including composting, pasteurization, substrate moistening, and cultivation. Mushrooms are increasingly grown on substrates containing high concentrations of potentially toxic elements (PTE) due to their bioremediation potential [1,2]. However, the resulting effluents from such substrates can pose serious environmental concerns when disposed of. First, the agro-industrial wastes used for mushroom cultivation retain significant amounts of pesticides (e.g., diazinon, linuron, and myclobutanil), which have deleterious effects on soil, irrigation water, and subsequently on plants and humans [3]. Second, the incorporation of chemical fertilizers in cereal crops (whose straw is used for mushroom production) leads to the eutrophication and hypoxia of water bodies [4], as these compounds are retained in MFW. Third, MFW is characterized by high levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), unpleasant color (black) and odor, and high levels of total phenols, total solids, carbohydrates, reducing sugars, ammoniacal nitrogen, and phosphorus [5]. Disposal of these effluents leads to a reduction in dissolved oxygen levels, resulting in oxygen-depleted water zones, toxic accumulation in water bodies, and the growth of undesirable algae that destroy the aquatic ecosystem [6–9]. Although some countries, such as Canada, have begun to fine mushroom farms for releasing their effluent into fish-bearing waters without any pre-treatment, there is still much to be done in this area. Such small government actions are still critically lacking in developing countries. On the other hand, only a few studies have attempted to decontaminate or treat MFW. For example, Rodríguez Pérez et al. [10] used *Pleurotus ostreatus* to decolorize and decontaminate MFW. They reported reductions of 50 and 92% in COD and phenols, respectively, and a 50% reduction in MFW color intensity. Chong et al. [11] used MFW for the fertilization of several flowering shrubs with no visible signs of toxicity. However, their study did not aim to treat such wastewater or assess its environmental risk but rather use it for agricultural purposes.

Phytoremediation is the use of plants to remove contaminants from contaminated sites. This process is very effective in the removal of PTEs, pesticide residues, organic contaminants, oil, municipal, domestic, agricultural, and industrial effluents, and many others. Phytoremediation is based on a variety of mechanisms to remove pollutants, i.e., immobilization, uptake, stabilization, and toxicity reduction of pollutants and degradation of pollutant compounds [12]. On the other hand, phytoremediation faces some limitations, mainly related to low yields. In this regard, many solutions have been proposed, including organic and chemical amendments, microbial stimulation, and genetic engineering [12]. However, such proposals are difficult to implement in developing countries as they are not economically feasible due to high costs. Although several plants have been used for wastewater remediation, only one study has evaluated the potential of phytoremediation management of MFW. In this study, Kumar et al. [5] grew two *Azolla* spp. (*A. pinnata* and *A. filiculoides*), floating aquatic ferns, on MFW. They observed significant reductions in BOD, COD, EC, pH, total dissolved solids, and total Kjeldahl nitrogen of MFW. The latter also improved the growth and biochemical characteristics of Azolla spp. This highlights the high potential of plants in the phytoremediation of MFW.

Coontail (*Ceratophyllum demersum* L.) is a submerged and free-floating aquatic plant that grows in almost all regions of the world, especially in calm, moderately to highly nutrient-rich waters. Regarding the phytoremediation concept, *C. demersum* has been reported to take up large amounts of nitrogen and phosphorus from water (35 and 73%, respectively) [13]. Other researchers reported removal efficiencies of approximately 82 and 50% for cadmium (Cd) and nickel (Ni), respectively, from synthetic aqueous wastewater using *C. demersum* [14]. Al-Nabhan and Al-Abbawy [15] reported removal efficiencies of 89.5, 90.0, 47.4, and 50.6% for BOD, COD, nitrate, and phosphate from wastewater using *C. demersum*. The reduction of calcium, magnesium, sodium, and chloride ions (55.9, 13.3, 16.3, and 40.2%, respectively) by *C. demersum* was also outlined in this study. Fawzy et al. [16] stated that *C. demersum* had the highest ability to absorb cadmium (Cd) from polluted water, followed by lead (Pb), copper (Cu), and zinc (Zn). Furthermore, the pH of the polluted water can influence the uptake capacity of arsenic (As) by *C. demersum*. Specifically, the highest bioaccumulation capacity of arsenic (As) was possible at pH 5 and decreased with increasing pH [17]. Therefore, the *C. demersum* plant can be considered an important phytoremediation agent for polluted wastewater treatment. It is also worth mentioning that the present study is the first to test the potentiality of *C. demersum* in the treatment of MFW.

Phycoremediation is the use of algae to treat wastewater. It is considered one of the most cost-effective tools to achieve carbon neutrality in a closed-loop process. Phycoremediation relies on a combination of mechanisms, i.e., biosorption, which allows the immobilization of organic pollutants, bioaccumulation of organic and inorganic compounds, thus removing them from the pollution source, and biodegradation, which converts pollutant molecules into intermediates or mineralizes them [18,19]. On the other hand, phycoremediation faces several challenges and limitations, such as the need for large spaces to grow algae, the inconsistent remediation rate depending on the type and concentration of pollutant to be removed, high operational costs, and potential risk of secondary pollution [20,21]. Although many studies have reported the use of macro- and microalgae in the remediation of wastewater, only one recent investigation has been conducted on the use of algae for the phycoremediation of MFW. In a previous study, Širić et al. [22] used the famous *Chlorella vulgaris*, a green microalga, for the treatment of MFW. They reported removal rates of 90.2, 91.5, 84.0, 86.3, and 94.2% for BOD, COD, total dissolved solids, total nitrogen, and total phosphorus, respectively. In conclusion, *C. vulgaris* was found to be very efficient for MFW treatment because it contributed to the reduction in released CO_2 and generated high amounts of oxygen through photosynthesis.

Kinetic studies allow us to understand the processes that promote the degradation of wastewater contaminants by providing evidence related to previously simulated biochemical processes. Several studies have outlined the use of kinetics to evaluate phytoremediation and phycoremediation performance. For example, Emiliani et al. [23] used first- and second-order kinetic models to evaluate the phytoremediation efficiency of heavy metal-polluted water using *Salvinia biloba*, a common water fern. High R^2 values ($\geq$0.89 and $\geq$0.83) were reported for the first- and second-order kinetic models, respectively. Kumar et al. [5] used logistic and modified Gompertz growth kinetic models to evaluate the growth performance of *Azolla* spp. on MFW. They reported very high R^2 values ($\geq$0.98 and $\geq$0.97) for both logistic and modified Gompertz models, respectively. Mahajan and Kaushal [24] found that the pseudo-second-order kinetic model was well suited to describe the phycoremediation of the azo dye methyl red using *Chara vulgaris* L. (green algae species) with $R^2 \geq 0.99$. The use of logistic and modified Gompertz kinetic models by Širić et al. [22] to evaluate the growth of *C. vulgaris* on MFW shed light on the higher efficiency of the former ($R^2 \geq 0.9938$) over the latter ($R^2 \geq 0.9876$).

Although several plants and algae have been used to treat agricultural, domestic, and industrial wastewater, there are still many gaps in this field. This study aimed to investigate the potential of a plant and algae (*C. demersum* and *C. vulgaris*) in the treatment of MFW. In the current investigation, the growth kinetic performance of *C. demersum* and *C. vulgaris* was evaluated using a first-order reaction-based model. Our results showed high pollutant removal efficiencies using plants and algae.

2. Materials and Methods

2.1. Wastewater, Plant, and Algae Collection

For the present experiment, mushroom farm wastewater (MFW) was collected from the disposal site of Kashyap Mushroom Farm located in Roorkee, Haridwar, India (29°47′14.8′′ N and 77°47′18.4′′ E). For this purpose, MFW samples were collected on a single occasion in polyvinyl chloride (PVC) cans of 25 L capacity and immediately transported to the experimental facility of the Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to be University), Haridwar, India. Borewell water (BW) samples were collected from the submersible water pump of the experimental facility. For the phytoremediation experiments, juvenile plants of *C. demersum* were collected from the Missarpur wetland, located near the Ganges bank in Haridwar, India (29°53′23.7′′ N and 78°08′22.9′′ E). In addition, the viable culture of *C. vulgaris* (NIES-220) was used for phycoremediation experiments as explained in our previous study [22].

2.2. Experimental Design and Reactor Operation

Two types of experimental reactor configurations were used to achieve phyto- and phycoremediation experiments, as shown in Figure 1. Here, MFW was filled into 15 L glass aquariums used as reactors and operated for 16 days for both plant and algae cultivation, separately. A total of four experimental treatments were tested including, control: CD (*C. demersum* using borewell water; BW), control: CV (*C. vulgaris* using borewell water), T1: CD (*C. demersum* using MFW), and T2: CV (*C. vulgaris* using MFW), respectively. For the phytoremediation experiments, a total of 50 g of fresh and healthy whorled plant leaves were added to the reactor filled with a 10 L working volume of MFW. The plants were grown under natural environmental conditions (8 h light vs. 16 h dark). For phycoremediation experiments, a 100 mL culture of *C. vulgaris* prepared in BG-11 medium containing 0.125 g/L viable cell biomass was added to the reactor containing 10 L MFW. For *C. vulgaris* cultivation, a 3% CO_2 supply, 4000 lx light intensity (8 h light vs. 16 h dark), and a 25 °C reactor temperature were maintained as previously described by Širić et al. [22].

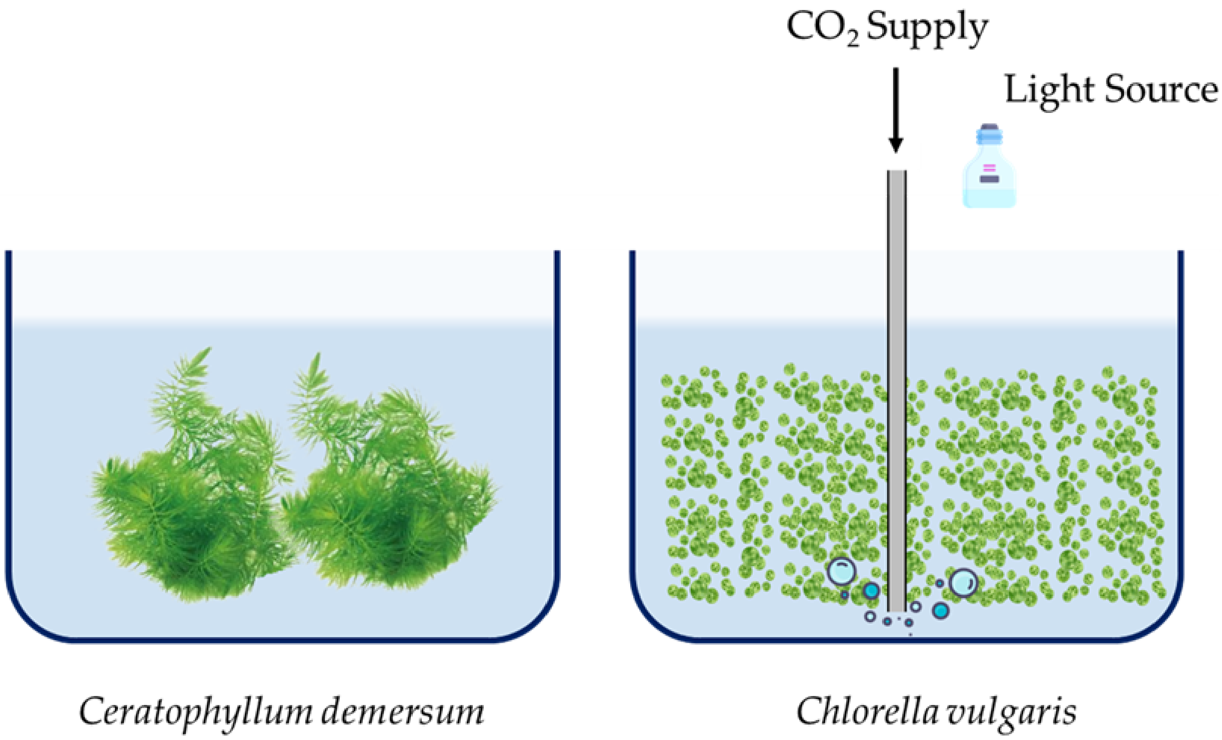

Figure 1. Experiment setup and layout of reactors used for mushroom farm wastewater treatment.

2.3. Chemical and Analytical Methods

In this study, standard methods of water and wastewater analysis were used as recommended by AOAC [25] and APHA [26]. The BW and MFW used in this study were analyzed for selected physicochemical properties. In particular, pH and electrical conductivity (EC: dS/m) were determined using a 1615 multimeter (ESICO International, Parwanoo, India). Total dissolved solids (TDS: mg/L) were estimated using a microprocessor-based 1611 m (ESICO, India). In addition, the biochemical oxygen demand (BOD: mg/L) and chemical oxygen demand (COD: mg/L) parameters were determined according to the Winkler and open-reflux digestion methods [27], respectively. Total nitrogen (TN: mg/L) was determined by the Kjeldahl acid digestion and distillation method [28], while total phosphorus (TP: mg/L) was determined by UV-vis spectrophotometer using ammonium molybdate [29].

On the other hand, the harvested plant and algal biomass were also analyzed for their growth, biochemical, and proximate parameters. For *C. vulgaris*, the fresh weight was determined using the optical density (680 nm) method as previously described by Pathak et al. [30], while the biomass of *C. demersum* was measured directly using a calibrated electronic balance (Samson India Pvt. Ltd., Pune, India). Chlorophyll and carotenoid contents (mg/g) were determined using 80% acetone as an extraction method followed by spectrophotometric determination at selected wavelengths [31]. Carbohydrate content (%) was estimated as previously described by Mansfield [32], while protein content was estimated using the bicinchoninic acid (BCA) method [33]. Total ash content (%) was estimated by combustion in a muffle furnace at 550 °C for 1 h. Finally, lipid content (%) was determined using the n-hexane-assisted Soxhlet extraction method [30].

2.4. Data Analysis and Software

The pollutant reduction potential was calculated using the removal efficiency index (%) [34] as given in Equation (1):

$$\text{Removal efficiency (\%)} = [(\text{Ci} - \text{Cf})/\text{Ci}] \times 100 \quad (1)$$

where Ci and Cf correspond to the initial and final levels of pollutants before and after phyto and phycoremediation experiments, respectively. Moreover, a first-order reaction-based kinetic model was used to simulate the rate constant of pollutant removal [35]. In this, the model indicates that the pollutant removal rate is proportional to the initial concentration of pollutant present [22]. The form of the model is given in Equations (2) and (3):

$$\text{-d[C]/dt} = k\text{[C]} \quad (2)$$

$$-k\text{t} = \text{Log[Ci]/Log[Cf]} \quad (3)$$

where d[C]/dt is the rate of change of pollutant concentration over time, k indicates the rate constant (units: pH: dimensionless; EC: dS/m/day; TDS, BOD, COD, TN, and TP: mg/L/day), and t is the experimental time (days). A plot of Log[C] vs. t was drawn to obtain a linear trendline and critical kinetic parameters such as the model equation (y = bx + c), rate constant (b), intercept (c), and coefficient of determination (R^2).

In addition, the data generated in this study were analyzed using an unpaired Student's *t*-test and one-way analysis of variance (ANOVA) based on Tukey's multiple comparisons of means to derive significant differences ($p < 0.05$) between different treatment groups. The software packages Microsoft Excel (version 2019, Microsoft Corp., Redmond, WA, USA) and OriginPro (version 2023a, OriginLab, Northampton, MA, USA) were used for this purpose.

3. Results and Discussion

3.1. Physicochemical and Pollutant Load of MFW

The physicochemical properties of the borewell water (BW) and MFW are highlighted in Table 1. The results of the Student's *t*-test showed that the overall physicochemical properties of MFW were highly significantly different ($p < 0.01$) from those of BW. pH, EC, TDS, BOD, COD, TN, and TP were significantly higher in MFW than in BW. In particular, BW had an almost neutral pH (7.20 ± 0.05), while the pH of MFW (8.25 ± 0.13) was slightly alkaline. However, both values were within the standard safe limits set by the Bureau of Indian Standards (BIS). It has been reported that *C. vulgaris* grows best in a water pH range of 6.5–7.0, while better lipid accumulation occurs in a water pH range of 7.0–8.5 [36]. On the other hand, *C. demersum* showed optimal growth in a water pH range of 7.0–8.5 and can tolerate even higher pH values (around 8.5–10.0) [37]. The EC of MFW (3.80 ± 0.09 dS/m) was around 21-fold higher than that of BW (0.18 ± 0.03 dS/m). Barahoei et al. [38] showed that *C. vulgaris* was able to grow in highly saline brackish water (EC: 11,000 ppm), whereas *C. demersum* showed optimal growth in 200–1000 dS/m water EC with potential tolerance in even more saline conditions (EC: 2000 dS/m) [37]. In the present study, MFW had a relatively high TDS content (1503.60 ± 21.38 mg/L) compared to that of BW (86.08 ± 1.52 mg/L). However, TDS content was below the safe limit (1900 mg/L). Increased TDS in water sources is a direct result of human activities such as mining, agricultural practices, and industrial wastewater disposal. Such an increase has a detrimental effect on aquatic fauna and can be expressed, for example, as a reduction in the turgidity of salmonid embryos [39].

BOD and COD are among the major contaminants found in agricultural wastewater, and for this reason, MFW has always been considered an environmental threat [5].

Table 1. Properties of borewell water (BW) and mushroom farm wastewater (MFW) used in this study.

Properties	Borewell Water	Mushroom Farm Wastewater	Student's *t*-Test *		Standard Limits (BIS) ^
			p-Value	*t*-Statistics	
pH	7.20 ± 0.05	8.25 ± 0.13	<0.01	13.05	5.50–9.00
EC (dS/m)	0.18 ± 0.03	3.80 ± 0.09	<0.01	66.09	-
TDS (mg/L)	86.08 ± 1.52	1503.60 ± 21.38	<0.01	114.54	1900
BOD (mg/L)	3.15 ± 0.10	1240.26 ± 18.70	<0.01	114.58	100
COD (mg/L)	9.32 ± 0.26	2813.05 ± 45.64	<0.01	106.40	250
TN (mg/L)	1.12 ± 0.02	355.86 ± 4.89	<0.01	125.64	100
TP (mg/L)	2.07 ± 0.05	160.18 ± 8.12	<0.01	33.72	-

Values are mean ± of three analyses; *: significantly different from the borewell water at $p < 0.05$ based on unpaired *t*-test; ^: surface discharge limits of Bureau of Indian Standard (BIS); -: not available; EC: electrical conductivity; TDS: total dissolved solids; BOD: biochemical oxygen demand; COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus.

Table 1 shows that the BOD and COD contents in MFW (1240.26 ± 18.70 and 2813.05 ± 45.64 mg/L, respectively) exceeded the safe limits (100 and 250 mg/L, respectively) by more than 11-fold, indicating the associated environmental risks. The MFW used in this study contained significantly higher levels of BOD and COD than previously reported by Rodríguez Pérez et al. [10] (20.7 and 93.8 times higher). This could be attributed to the excessive use of pesticides, organic fertilizers, and chemical amendments in mushroom cultivation. Elevated levels of TN and TP in wastewater are largely associated with high risks of eutrophication in aquatic ecosystems [40]. Our results showed approximately 3.5 times higher TN content in MFW (355.86 ± 4.89 mg/L) than the safe standard limits (100 mg/L) [41]. The USEPA specifies 1 mg/L as the limit for TP in discharged wastewater [6]. Thus, both irrigation sources in the present study exceed such values by very different proportions (2.07 ± 0.05 and 160.18 ± 8.12 mg/L).

3.2. Removal of Wastewater Pollutants by C. demersum and C. vulgaris

In this study, *C. demersum* and *C. vulgaris* were used separately for phyto and phycoremediation of MFW. The results showed a significant decrease ($p < 0.05$) in pH and EC after 16 days of plant/algae cultivation (Table 2). In particular, the T1: CD treatment showed the highest decrease in pH and EC, followed by T2: CV as compared to those in their control treatments. Such a high decrease in pH may be related to the release of CO_2 by *C. vulgaris* in the growing tanks. The most pronounced decrease in EC by T1: CD can shed light on the effectiveness of phytoremediation processes for the remediation of wastewater in general and MFW in particular. Furthermore, significant removal efficiencies of TDS, BOD, and COD from MFW by T1: CD and T2: CV (72–95 and 68–94%, respectively) (T1: CD > T2: CV) were outlined (Figure 2). In this regard, TDS, BOD, and COD removal efficiencies of 76.95–84.0%, 78.89–90.17%, and 86.39–91.53% from MFW, respectively, were previously achieved by *C. vulgaris* [22]. Furthermore, our results outlined 80–84% and 66–75% removal efficiencies of TN and TP from T1: CD and T2: CV, respectively. Also, Širić et al. [22] found 80.58–86.27% and 91.21–94.19% removal efficiencies of TN and TP, respectively, from MFW by *C. vulgaris*.

C. demersum is one of the ideal candidates for phytoremediation of polluted wastewater due to its ability to remove nitrogen and phosphorus. However, *C. demersum* can become a real threat in fishponds if it is widely propagated, as cutting and raking become relatively inefficient [42]. Previous studies have reported the successful use of *C. demersum* for the treatment of sewage [15], landfill leachate [43,44], and synthetic wastewater [14]. However, this is the first study on the phytoremediation of MFW using *C. demersum*. This plant was able to absorb significant amounts of nitrogen and phosphorus from eutrophic fish ponds

(448–842 kg and 30.5–31.9 kg, respectively) [45]. Furthermore, the study by Szy-mańska-Pulikowska and Wdowczyk [43] showed that *C. demersum* reduced TP in landfill leachate by approximately 60%. Although a 93–95% reduction in contaminants is a very promising result, it may show a high remediation potential (high efficiency) and may be considered a novel cost-effective tool that may replace wastewater treatment plants in the future.

Table 2. Removal of MFW pollutants parameters by *C. demersum* and *C. vulgaris* under different treatment configurations.

Properties	**Variable**	**Treatments**			
		Control: CD	**Control: CV**	**T1: CD**	**T2: CV**
pH	Initial	7.20 ± 0.05 a		8.25 ± 0.13 a	
	Final	6.16 ± 0.08 c	6.34 ± 0.06 b	6.30 ± 0.07 c	6.65 ± 0.04 b
EC (dS/m)	Initial	0.18 ± 0.03 a		3.80 ± 0.09 a	
	Final	0.11 ± 0.02 ab	0.13 ± 0.05 a	1.05 ± 0.06 c	1.30 ± 0.04 b
TDS (mg/L)	Initial	86.08 ± 1.52 a		1503.60 ± 21.38 a	
	Final	30.80 ± 0.94 c	38.83 ± 0.66 b	210.60 ± 7.81 c	397.02 ± 14.04 b
BOD (mg/L)	Initial	3.15 ± 0.10 a		1240.26 ± 18.70 a	
	Final	1.30 ± 0.06 c	1.50 ± 0.08 b	210.14 ± 7.81 c	387.02 ± 10.30 b
COD (mg/L)	Initial	9.32 ± 0.26 a		2813.05 ± 45.64 a	
	Final	3.71 ± 0.10 c	4.10 ± 0.17 b	376.30 ± 10.79 c	502.90 ± 25.11 b
TN (mg/L)	Initial	1.12 ± 0.02 a		355.86 ± 4.89 a	
	Final	0.68 ± 0.05 b	0.72 ± 0.03 b	55.70 ± 5.53 c	68.10 ± 6.07 b
TP (mg/L)	Initial	2.07 ± 0.05 a		160.18 ± 8.12 a	
	Final	0.94 ± 0.04 bc	1.04 ± 0.07 b	39.08 ± 3.94 c	54.17 ± 4.33 b

Values are mean ± of three replicates; the same letters (a–c) indicate no significant difference between initial and final values at $p < 0.05$; CD: *C. demersum*; CV: *C. vulgaris*; EC: electrical conductivity; TDS: total dissolved solids; BOD: biochemical oxygen demand; COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus.

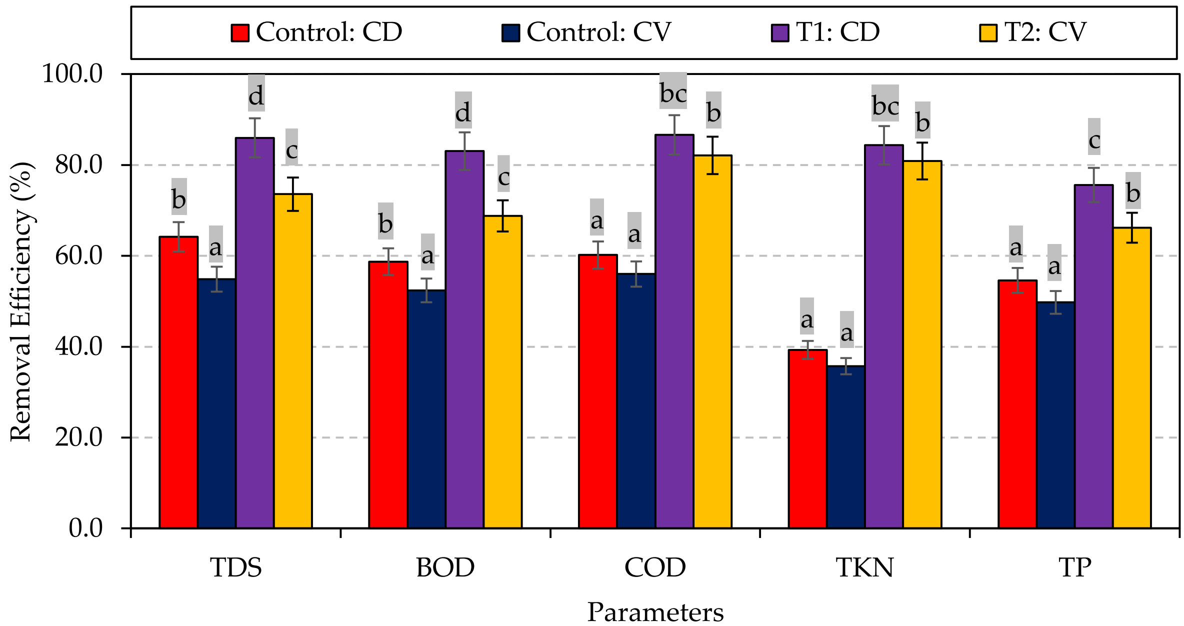

Figure 2. The pollutant removal efficiency of *C. demersum* and *C. vulgaris* from MFW. EC: electrical conductivity; TDS: total dissolved solids; BOD: biochemical oxygen demand; COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus.

3.3. Kinetics of Pollutant Removal by *C. demersum* and *C. vulgaris*

Kinetic studies are helpful in understanding the patterns of pollutant removal by bioremediation systems. In this study, the kinetic results of pollutant removal by *C. demersum* and *C. vulgaris* demonstrated their effectiveness in treating MFW. As shown by the results in Table 3, it was observed that the first-order reaction-based model showed good fitness in the experimental data to derive critical kinetic parameters. The plot of Log[C] vs. t showed a linear trend fitness as shown in Figure 3. The time course trend was used to derive the model equation in terms of $y = ax + b$. In particular, the coefficient of determination (R^2) of the kinetic models for the removal of selected pollutant parameters ranged from 0.8317 to 0.9872, indicating an acceptable goodness of fit. Moreover, the phytoremediation experiments using *C. demersum* showed higher values of rate constant (k) as compared to those recorded in the phycoremediation experiments for all parameters. However, the T1: CD treatment showed significantly higher ($p < 0.05$) removal rate constants (pH: 0.0085; EC: 0.0411; TDS: 0.0604; BOD: 0.0564; COD: 0.0622; TN: 0.0589; TP: 0.0438), which are relatively higher than those observed in other treatments. Overall, the maximum rate constant was observed for the T2: CD treatment, while the minimum was reported for the control (CV) treatment. This indicates that both *C. demersum* and *C. vulgaris* individually provide good removal rates while treating. The design and configuration of the experiment, including factors such as hydraulic retention time (HRT), may affect overall removal rates. Longer HRTs can increase the amount of time contaminants are exposed to plants, thereby increasing removal rates [46]. However, sometimes longer HRTs may not be economically feasible for wastewater treatment due to additional maintenance and operating costs. Therefore, the kinetic results could help to identify the critical point of termination of the experiments, which are more related to the lifetime and stationary phase of biomass growth [47].

Table 3. First-order reaction-based kinetic parameters of pollutant reduction from MFW using *C. demersum* and *C. vulgaris*.

Properties	Variable	Treatments			
		Control: CD	**Control: CV**	**T1: CD**	**T2: CV**
pH	y	$-0.0048x + 0.8583$	$-0.0039x + 0.8557$	$-0.0085x + 0.9259$	$-0.0068x + 0.924$
	R^2	0.9297	0.8317	0.9297	0.9275
	k	0.0048	0.0039	0.0085	0.0068
EC (dS/m)	y	$-0.0124x - 0.7739$	$-0.0092x - 0.7422$	$-0.0411x + 0.6474$	$-0.0339x + 0.6334$
	R^2	0.9199	0.9872	0.9016	0.9173
	k (dS/m/day)	0.0124	0.0092	0.0411	0.0339
TDS (mg/L)	y	$-0.0314x + 1.9793$	$-0.0248x + 1.9685$	$-0.0604x + 3.2505$	$-0.0432x + 3.2143$
	R^2	0.9418	0.9280	0.9334	0.8846
	k (mg/L/day)	0.0314	0.0248	0.0604	0.0432
BOD (mg/L)	y	$-0.0279x + 0.5488$	$-0.0235x + 0.5491$	$-0.0564x + 3.1630$	$-0.0371x + 3.1292$
	R^2	0.9015	0.8647	0.9208	0.9059
	k (mg/L/day)	0.0279	0.0235	0.0564	0.0371
COD (mg/L)	y	$-0.0292x + 0.9919$	$-0.0261x + 1.0027$	$-0.0622x + 3.5108$	$-0.0519x + 3.5144$
	R^2	0.8997	0.9212	0.9354	0.9452
	k (mg/L/day)	0.0292	0.0261	0.0622	0.0519
TN (mg/L)	y	$-0.0154x + 0.0692$	$-0.0133x + 0.0692$	$-0.0589\text{x} + 2.6234$	$-0.0530x + 2.6367$
	R^2	0.9364	0.9512	0.9215	0.9090
	k (mg/L/day)	0.0154	0.0133	0.0589	0.0530
TP (mg/L)	y	$-0.0231x + 0.3366$	$-0.0200x + 0.3329$	$-0.0438x + 2.2708$	$-0.0318x + 2.2649$
	R^2	0.9752	0.9804	0.9304	0.9456
	k (mg/L/day)	0.0231	0.0200	0.0438	0.0318

CD: *C. demersum*; CV: *C. vulgaris*; EC: electrical conductivity; TDS: total dissolved solids; BOD: biochemical oxygen demand; COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus.

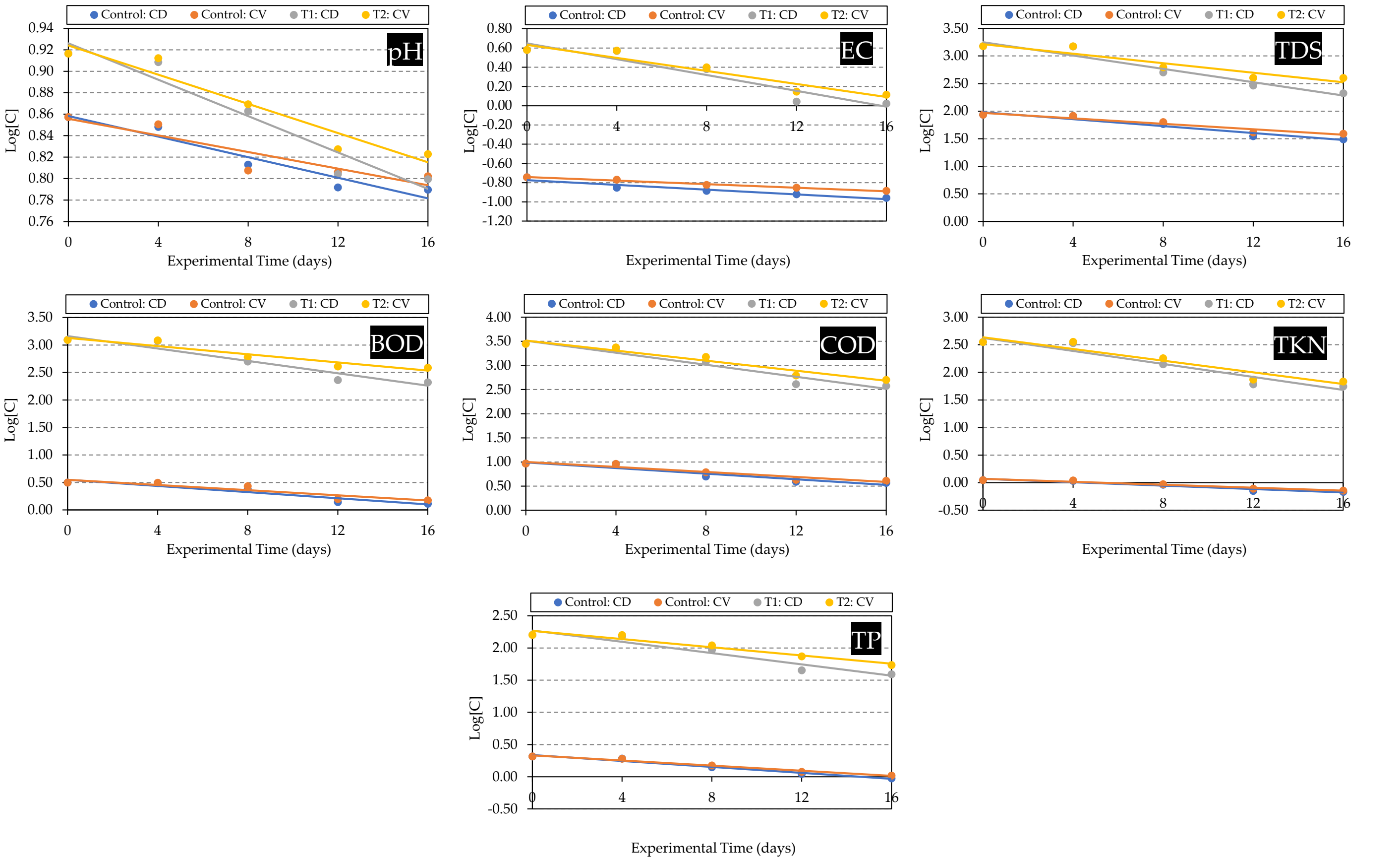

Figure 3. First-order reaction-based time course Log[C] vs. t plot (points are average of three replicates) of pollutant reduction from MFW using *C. demersum* and *C. vulgaris*. EC: electrical conductivity; TDS: total dissolved solids; BOD: biochemical oxygen demand; COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus.

Numerous studies have demonstrated the usefulness of kinetic models in wastewater bioremediation [22,48,49]. Recently, Širić et al. [22] investigated the kinetics of pollutant removal from MFW using *C. vulgaris*. They found that the first-order reaction-based kinetic model was useful for predicting the pollutant removal rate (*k*: dimensionless unit) from MFW, while the growth of *C. vulgaris* was estimated using two growth models (logistic and modified Gompertz). Ntakiyiruta et al. [48] determined the kinetic removal rate of pollutants (PO_4^{3-}, NO_3^-, and NH_4^+) from wastewater using two aquatic macrophytes (*Eichhornia crassipes* and *Pistia stratiotes*). The first-order-based reaction model was used to determine the kinetic rate constant, which showed good fit results as indicated by R^2 (>0.68). Similarly, Singh et al. [49] also evaluated the kinetics of heavy metal removal from glass industry wastewater using *E. crassipes*. They found that the first-order model best fit the data in terms of R^2 (> 0.82) and the rate constant (*k*).

3.4. Effect of MFW on Plant and Algae Biomass

Table 4 shows the effect of MFW and different experimental combinations on the growth, biochemical, and proximate composition of *C. demersum* and *C. vulgaris* used in the study. It was observed from the results that selected growth, biochemical, and proximate constituents of both *C. demersum* and *C. vulgaris* were significantly ($p < 0.05$) increased when MFW was used as a culture medium compared to control treatments. Specifically, the fresh weight of *C. vulgaris* was increased by 37.89% in MFW treatments, respectively. For *C. demersum*, the fresh biomass was increased by 30.03% when using MFW. The dry weight yield also followed the same pattern of increase for both *C. vulgaris* and *C. demersum*. The contents of chlorophyll (6.40 and 5.82 mg/g), carotenoids (2.14 and 0.24 mg/g), and carbohydrates (31.61 and 39.17%) of *C. vulgaris* and *C. demersum* were found to be maximum in MFW-based treatments. This suggests that both *C. vulgaris* and *C. demersum* can grow efficiently in MFW while producing good-quality biomass. Similarly, the protein, lipid, and ash contents were also significantly improved by using MFW. However, the protein and lipid contents of *C. demersum* were comparatively lower than those of *C. vulgaris*.

C. vulgaris is known for its great ability to fix CO_2 and convert it to O_2, which later improves water quality [50], while *C. demersum* could absorb pollutants [15]. Figure 4 shows the hypothetical mechanisms involved in the phytoremediation and phycoremediation of wastewaters. A study by Baviskar et al. [51] evaluated the combined bioremediation efficiency of *Klebsiella pneumonae* and *Lysinibacillus fusiformis* isolates for arsenic (As) removal from industrial wastewater. In their study, Wei and Pan [52] developed a multi-process phytoremediation system for the treatment of creosote. In their study, a combination of physical, photochemical, microbial, and phytoremediation techniques was employed in a series of treatments. The developed method was found to be effective in treating creosote. Similarly, Huang et al. [53] also investigated the treatment of persistent total petroleum hydrocarbons (TPHs) from contaminated soils using bacteria, plant growth-promoting rhizobacteria (PGPR), and tall fescue (*Festuca arundinacea*). It was observed that the growth parameters of *F. arundinacea* were significantly improved under the combined bacteria-PGPR-plant treatment as compared to the individual treatments.

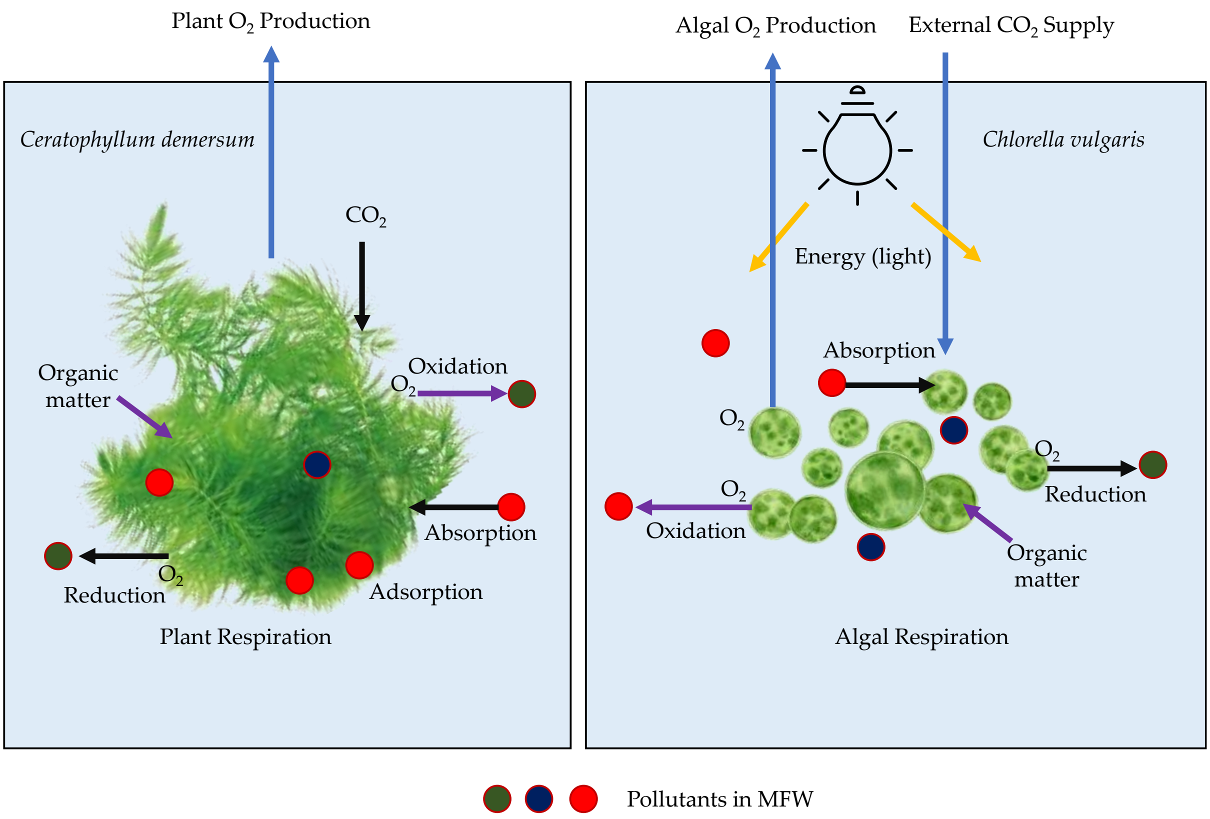

Figure 4. Processes involved in phytoremediation and phycoremediation of wastewater using plant and algal systems.

Table 4. Growth, biochemical, and proximate composition of *C. demersum* and *C. vulgaris* grown in MFW.

Parameters	Control: CD	Control: CV	T1: CD	T2: CV
Fresh Weight (g)	92.50 ± 2.25 c	13.38 ± 0.40 a	120.28 ± 6.46 d	18.45 ± 1.02 b
Dry Weight (g)	14.87 ± 0.31 c	2.01 ± 0.05 a	19.04 ± 0.74 d	2.66 ± 0.12 b
Chlorophyll content (mg/g)	3.50 ± 0.07 a	5.10 ± 0.06 b	5.82 ± 0.12 c	6.40 ± 0.14 d

Table 4. *Cont.*

Parameters	Control: CD	Control: CV	T1: CD	T2: CV
Carotenoids (mg/g)	0.21 ± 0.01 a	1.90 ± 0.03 b	0.24 ± 0.03 a	2.14 ± 0.07 c
Carbohydrates (%)	35.50 ± 1.13 b	28.47 ± 2.16 a	39.17 ± 2.68 c	31.61 ± 1.97 ab
Proteins (%)	9.70 ± 0.48 a	34.10 ± 0.29 c	13.84 ± 1.63 b	37.49 ± 1.02 d
Lipid (%)	1.10 ± 0.08 a	13.92 ± 1.03 c	2.67 ± 0.11 b	14.91 ± 0.59 c
Total ash (%)	7.06 ± 0.12 c	6.16 ± 0.07 a	8.25 ± 0.09 d	6.70 ± 0.14 b

Values are mean ± of three replicates; the same letters (a–d) indicate no significant difference between initial and final values at $p < 0.05$.

4. Conclusions

This study concluded that the plant (*C. demersum*) and algae (*C. vulgaris*) were effective in the treatment of MFW. The results showed that selected MFW pollutants were significantly ($p < 0.05$) reduced compared to the control treatments. The kinetic studies of pollutant reduction also indicated that the rate constant (k) was maximum for *C. demersum*. The growth, biochemical, and proximate composition parameters of plants and algae were also significantly improved. This study suggests that both *C. demersum* and *C. vulgaris* can be used for the efficient treatment of agro-industrial wastewater. The selected plants and algae have the potential to make a significant contribution to the conservation of water resources and sustainable water management. Further studies are needed to consider a wider range of combination ratios that would result in maximized pollutant removal from MFW and other types of wastewater.

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