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Experimental Investigation of *Chlorella vulgaris* and *Enterobacter* sp. MN17 for Decolorization and Removal of Heavy Metals from Textile Wastewater

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Abstract: The present study evaluated the performance of microalgae *Chlorella vulgaris* in an *Enterobacter* sp. MN17-assisted textile industry wastewater treatment system for decolorization, removal of heavy metals (Cu, Cr, Pb, and Cd), and chemical oxygen demand (COD). Different dilutions (5, 10, and 20%) of wastewater were prepared to decrease the pollutant toxicity for culturing microalgae and bacteria. Reduction of color, COD, and metal contents by microalgal treatment of wastewater varied greatly, while removal efficiency (RE) was significantly enhanced when endophytic bacterial strain MN17 inoculum was applied. Most notable, results were found at a 5% dilution level by *Enterobacter* sp. MN17-inoculated *C. vulgaris* medium, as chromium (Cr), cadmium (Cd), copper (Cu), and lead (Pb) concentrations were decreased from 1.32 to 0.27 mg L^{-1} (79% decrease), 0.79–0.14 mg L^{-1} (93% decrease), 1.33–0.36 mg L^{-1} (72% decrease), and 1.2–0.25 mg L^{-1} (79% decrease), respectively. The values of COD and color were also significantly decreased by 74% and 70%, respectively, by a *C. vulgaris–Enterobacter* sp. MN17 consortium. The present investigation revealed that bacterial inoculation of microalgae significantly enhanced the removal of coloring agents and heavy metals from textile wastewater by stimulating the growth of algal biomass. This study manifested the usefulness of microalgae–bacterial mutualism for the remediation of heavy metals, COD, and color in industrial effluents. Microalgae consortia with growth promoting bacteria could be a breakthrough for better bioremediation and bioprocess economy. Thus, further studies are needed for successful integration of microalgae–plant growth promoting bacterial (PGPB) consortium for wastewater treatments.

Keywords: textile effluents; wastewater treatment; algal–bacterial consortium; *Chlorella vulgaris*; *Enterobacter* sp. MN17

1. Introduction

The textile industry is the most important sector in Pakistan's economy, contributing about 9% to gross domestic product (GDP), but its impact on the environment is getting worse day by day. Recently, it has become a common practice to discharge wastewater originating from various production processes of textile and dying industrial units directly into water channels. This indiscriminate discharge of industrial effluents is posing serious threats to human and animal health [1]. The textile sector contains many units like dyeing, bleaching, printing, etc., using large quantities of water. About 3840 m^3/day of water is used in these processes of the textile industry [2]. These activities are held responsible for environmental water pollution due to the use of huge amounts of water throughout all the processing operations and release about 35 billion tons of wastewater with high values of pH, electrical conductivity (EC), and total suspended solids (TSS) [3]. More than 70 types of chemicals are present in textile effluents including 30 chemical types that are unable to be removed [4]. Textile wastewater effluents contain substantial amounts of heavy metals, high biological oxygen demand (BOD), and chemical oxygen demand (COD). Therefore, it is highly indispensable to adopt environmentally-friendly methods for the treatment of textile effluents prior to their discharge into natural water bodies, particularly in heavily industrial cities [5].

Wastewater has been characterized with high BOD, COD [6], volatile acids, proteins, lipids, inorganic compounds, i.e., potassium, calcium, chlorine, and heavy metals [7]. Water conservation and reuse have become of the utmost necessity of the textile industry due to the increasing demand of water by both industrial and residential sectors and hence water resource depletion and health risks associated with contaminated water [8].

Treatment of wastewater prior to its discharge into natural water bodies is in dire need currently, owing to its highly hazardous effects on the ecosystem and human health. Application of various physical and chemical technologies requiring high cost and energy for operation and maintenance is not feasible in poor countries like Pakistan [9]. Therefore, biological means of pollutant removal provide cost effective alternative strategies, using biological agents such as algae, fungi, bacteria, etc., and crop residues for the removal of pollutants from wastewater in an efficient, eco-friendly, and sustainable way [10]. Algae sequester atmospheric CO_2 with the assimilation of nutrients from wastewater [11]. Algal biomass production can be increased by synergism with suitable bacterial species [12]. Microalgae provide organic compounds and oxygen to the bacteria that are released as algogenic organic matter (AOM) during photosynthesis [13]. Alternatively, bacteria provide CO_2 and other growth promoting elements to microalgae, thus acting as growth enhancers for microalgae [13]. Under extreme environmental conditions, bacteria may help algae in buffering the stresses against evapotranspiration and photoinhibition, whereas microalgae in turn provide nitrogen (N) and minerals by the process of mineralization [14]. This microalgal–bacterial symbiosis may produce anti-oxidants that also help them be sustained under extreme conditions [14].

The addition of bacteria in algal culture medium not only enhances algal growth but also its photosynthetic potential by supplying additional CO_2 and organic compounds [15]. Algal–bacterial synergistic interaction helps to enhance phosphorus (P) and N assimilation, which can be recycled by using algae as a fertilizer [16].

Previous work has specifically focused on algal application for wastewater treatments [17,18]. However, little is known about algal–endophytic bacterial interactions for treating industrial wastewater. Interestingly, the role of *Enterobacter* sp. MN17 in treating and decolorization textile wastewater has not yet been explored. Therefore, the present research was designed to investigate the potential of

microalgae *C. vulgaris* co-applied with *Enterobacter* sp. MN17 to remove heavy metals and color from textile wastewater.

2. Materials and Methods

2.1. Culturing Microalgae and Bacterial Strains

Freshwater green microalgae strain *C. vulgaris* was kindly donated by Punjab Bioenergy Institute, University of Agriculture, Faisalabad, and bacterial strain *Enterobacter* sp. MN17 was obtained from the Environmental Sciences Laboratory, University of Agriculture, Faisalabad, Pakistan. Microalgae was pre-cultured in BG-11 media containing 1.5 g L^{-1} $NaNO_3$, 0.12 g L^{-1} K_2HPO_4, 0.287 g L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.075 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.036 g L^{-1} $CaCl_2{\cdot}2H_2O$, 0.02 g L^{-1} Na_2CO_3, 0.169 g L^{-1} $MnSO_2{\cdot}2H_2O$, 0.0006 g L^{-1} Ferric ammonium citrate, 0.001 g L^{-1} EDTA (disodium salt), 0.061 g L^{-1} H_3BO_3, 0.012 g L^{-1} $NaMoO_4{\cdot}2H_2O$, 0.0025 g L^{-1} $CuSO_4{\cdot}5H_2O$, 0.049 g L^{-1} $Co(NO_3)_2.6H_2O$, and 0.001 g L^{-1} $MnCl_2{\cdot}4H_2O$. Bacterium was cultured in tryptic soy broth (TSB) media (Merck, Germany) with pre-sterilization in an autoclave for 20 min at 121 °C before its use for microalgae and bacteria cultivation [19]. For seed propagation, *Chlorella vulgaris* was first cultured in flasks in a growth chamber at light intensity of 60–80 µmol m^{-2} s^{-1} and temperature of 28 ± 2 °C followed by culturing in 5 L laboratory grade plastic tubes for 7 days. The culture was then used as seed for microalgae growth on textile wastewater. The bacterial culture was prepared in 500 mL Erlenmeyer flasks in a shaking incubator (Firstek Scientific, Tokyo, Japan) at 150 rpm for 48 h at 25 ± 2 °C.

Chlorella vulgaris is widely used for the treatment of wastewater [20,21] and has been proved to efficiently assimilate nutrients from wastewater. The bacterial strain *Enterobacter* sp. MN17 was selected on the basis of its growth promoting characteristics [22–24] and involvement in improving photosynthetic activity.

Textile industry wastewater was used as a culture medium for microalgae growth, collected from the dying unit of Masood Textile Mill (MTM), Faisalabad and stored at 4 °C prior to its use. Wastewater was filtered and analyzed for various physico-chemical parameters such as COD, color, and concentration of heavy metals, including chromium (Cr), cadmium (Cd), copper (Cu), and lead (Pb).

2.2. Algal Growth Determination

The effect of the growth promoting bacterium *Enterobacter* sp. MN17 on algal biomass production was evaluated by examining the growth response of pure algal culture and the algal–bacterial consortium for a period of 5 days, and the growth was expressed in terms of biomass concentration. For comparing the growth rate, *C. vulgaris* and the *C. vulgaris–Enterobacter* sp. MN17 consortium were cultured in BG-11 in 1 L flasks with working volumes of 800 mL having a 0.2 optical density (OD) in a growth chamber at 25 ± 1 °C and 24 h light at 100–120 µmol/m^2/s intensity along with 2% CO_2 mixed with air. All treatments were performed in triplicate, and algal biomass was measured after every 24 h period using the gravity method.

2.3. Experimental Setup and Culture Conditions

For removal of pollutants from textile wastewater, the microalgae strain *C. vulgaris* was applied with bacterial strain *Enterobacter* sp. MN17 under different wastewater concentrations. The experiment was performed in 5 L laboratory grade plastic tubes in a growth chamber. Three different dilutions (5%, 10%, and 20%) of wastewater were prepared by distilled water to reduce its toxicity. For assessment of the wastewater treatment potential of microalgae with respect to controls of the same dilutions, the pH of all the dilutions was adjusted to 7.0 before inoculation of algal–bacterial strains. The wastewater dilutions were sterilized after filtering through sterile 0.22 µm pore-size filters (ShangHai XinYa Purification Equipment Co., Ltd., Shanghai, China). The microalgal strain *C. vulgaris* was grown in all these dilutions of wastewater for biomass production. Pre-cultured microalgae were centrifuged at 3700× *g* at 20 °C for 5 min. After the supernatant was discarded, the microalgal cells were washed

twice with sterile distilled water, and an OD of 0.2 was set using a spectrophotometer (Hach, Loveland, CO, USA) at 750 nm absorbance. Indoor culture conditions were maintained in a growth chamber with a temperature of 25 ± 1 °C and 14:10 h light:dark cycle at intensity of 100–120 μmol m^{-2} s^{-1} provided with cool white fluorescent lamps. Agitation in culturing flasks was maintained by using air mixed with 2% CO_2.

After culturing, *Enterobacter* sp. MN17 was placed in a shaking incubator (Firstek Scientific, Tokyo, Japan) at 150 rpm for 48 h at 25 ± 2 °C, and the culture was centrifuged (3020× *g* rpm for 3 min) followed by washing with sterile distilled water. The initial concentrations of MN17 was determined as 2.3×10^5 cells mL^{-1}, whereas the inoculum ratio of *Chlorella* to *Enterobacter* was 5:1, which was selected based on the preliminary testing of the effective consortium ratio. The higher *Chlorella* to *Enterobacter* ratio was selected based on the fact that a bacterial population does not result in an uncontrolled culture, and a good productivity and removal of heavy metals should be maintained under a higher microalgae population.

2.4. Analytical Procedures

Chemical oxygen demand was determined by following the method described by [25]. The samples were oxidized by potassium dichromate ($K_2Cr_2O_7$) in sulfuric acid. Silver sulfate ($AgSO_4$) and mercuric sulfate ($HgSO_4$) were used as catalysts. The excess dichromate concentration was measured by titration with ferrous ammonium sulfate. Decolorization was measured by using the difference between spectrophotometer absorbance readings before and after the experiment. The maximum absorbance wavelength (λ_{max}) was determined using a spectrophotometer (Hach, Loveland, CO, USA) at wavelengths ranging between 250 and 700 nm of the wastewater sample. After microalgae culturing in selected dilutions, the cells were harvested by centrifugation at 3500 rpm for 5 min. The supernatant was collected and again subjected to absorption spectrums till the disappearance of the absorbance peaks at λ_{max}. Percentage color removal was calculated as follows:

$$\% \text{ decolorization} = (\text{Initial } \lambda_{max} - \text{final } \lambda_{max}/\text{initial } \lambda_{max}) \times 100\% \quad (1)$$

The amount of heavy metals in the prepared wastewater samples was determined by using an atomic absorption spectrophotometer (AAS) (Hitachi Polarized Zeeman AAS, Z-8200, Japan), following the conditions described by [25,26]. The metal removal efficiency (RE) was calculated using the following equation:

$$\text{Removal efficiency (RE\%)} = \text{xi} - \text{xf/xi} \times 100 \quad (2)$$

where xi is the parameter value before the experiment, and xf is the parameter value after the experiment. For dry weight, cells were harvested by filtration (Whatman GF/C) after drying the pre-weighed filter at 105 °C in an oven for 24 h followed by cooling in a desiccator for 30 min; dry weight was determined by using the following formula:

$$\text{Dry weight } (g/L) = \frac{(\text{Weight of filter with algal sample} - \text{weight of filter before})}{\text{volume of sample}} \times 1000 \quad (3)$$

2.5. Statistical Data Analysis

Statistical analysis was carried out using SPSS 16.0 (Chicago, IL, USA) for windows. A completely randomized design (CRD) factorial test followed by least significant difference (LSD) was used to analyze data and to check the significance of treatments at a 5% level of significance. Data were presented as mean ± standard deviation unless otherwise stated. All the graphs were prepared on Microsoft Excel 365 version.

3. Results

3.1. Growth Comparison of C. vulgaris vs. C. vulgaris–Enterobacter sp. Mn17 Consortium

The growth of *C. vulgaris* reached its maximum level after day 4 and then levelled off, whereas for the consortium, the *C. vulgaris* response was much better even after 96 h. Maximum biomass was achieved with bacterial inoculated culture of 2.97 g L^{-1} with a maximum biomass productivity of 0.902 g $L^{-1}day^{-1}$, which was much higher than the individual algal culture, with a maximum biomass of 2.18 g L^{-1} and a maximum biomass productivity of 0.67 g $L^{-1}day^{-1}$. Growth on the first day was the same for both cases but on the second day showed the highest difference, which might be the result of more CO_2 incorporation to the biomass, which further increased nutrient uptake (Figure 1).

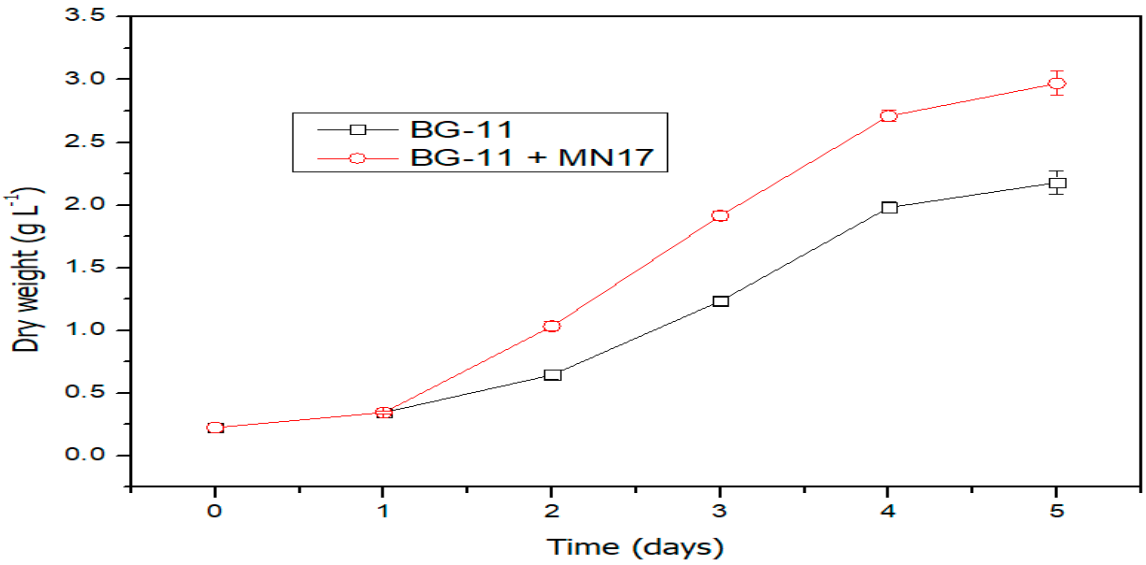

Figure 1. Biomass changes of *Chlorella vulgaris* and *C. vulgaris–Enterobacter* sp. MN17 consortium in BG-11 media.

3.2. Effects of Algal–Bacterial Consortium on Wastewater Treatment

3.2.1. Decolorization Assay

The effect of *C. vulgaris* individually and in combination with *Enterobacter* sp. MN17 on decolorization of three diluted concentrations of textile dyeing unit wastewater is shown in (Figure 2). The coloring was removed from all the dilutions to varying extents. The efficiency of *C. vulgaris* in decolorization was 43%, 37%, and 29% at wastewater dilutions of 5, 10, and 20%, respectively. However, decolorization with application of *C. vulgaris* in combination with *Enterobacter* sp. MN17 was much better as compared to the individual application of *C. vulgaris*, as decolorization RE was increased up to 71.5%, 56%, and 49% in all (5, 10, and 20%) dilutions, respectively, which was much better than with only microalgae. An increase in microalgal biomass was observed with *Enterobacter* sp. MN17 application, which resulted in more decolorization. Color removal by *C. vulgaris* decreased with the increase in initial color, especially in medium containing 20% wastewater, whereas bacteria assisted microalgae to perform better in more toxic environments. Maximum decolorization was recorded at 5% dilution while inoculation of bacteria had significant effect on decolorization (Figure 2). In general, *Enterobacter* sp. MN17 improved the growth of *C. vulgaris* in all dilutions that resulted in enhanced decolorization.

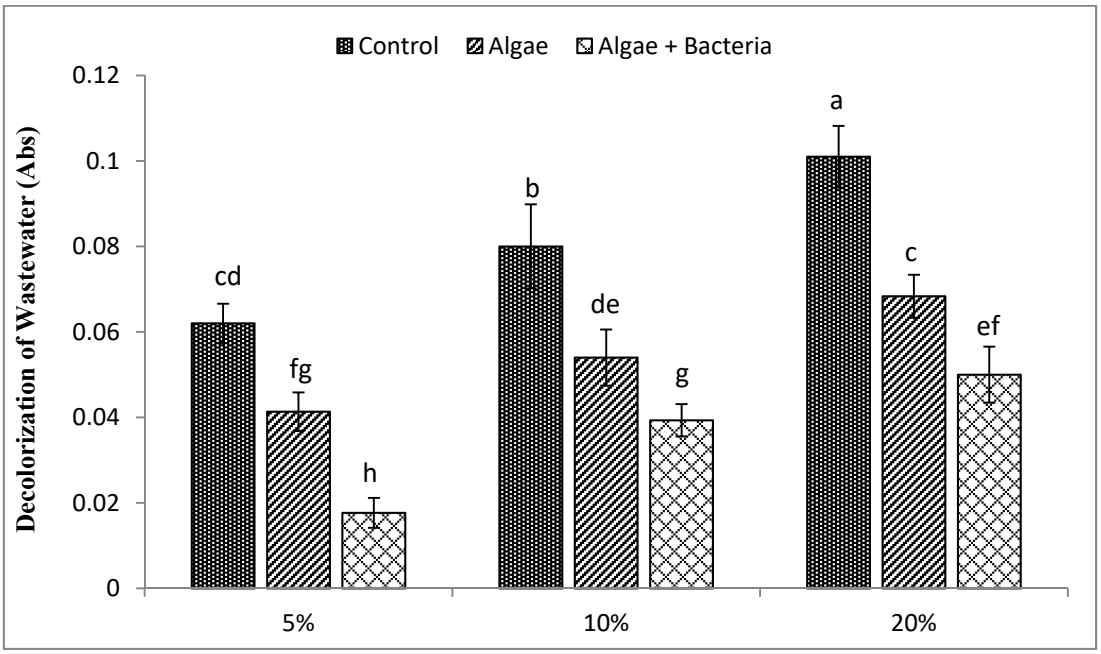

Figure 2. Efficiency of algae and algal–bacterial consortium for decolorization of textile industry wastewater with respect to their control values at different dilution levels. Columns show mean values and bars show standard deviation (SD) of means. All means followed by different letters were significantly different according to Tukey HSD (honestly significant difference) test at $p < 0.05$.

3.2.2. Removal of Heavy Metals

The dying unit wastewater contained various heavy metals of which Cr, Cd, Cu, and Pb were found above the permissible limits, whereas others were below the acceptable limits, which is why they were ignored in this study. In the present study, Cr, Cd, Cu, and Pb concentration ranges were 1.32–2.02 mg L^{-1}, 0.79–1.08 mg L^{-1}, 1.33–1.88 mg L^{-1}, and 1.21–1.58 mg L^{-1}, respectively, in all wastewater dilutions. The concentrations of these heavy metals in different dilutions are presented in (Table 1).

Table 1. Pollutant removal efficiencies of microalgae and microalgal–bacterial consortium from textile industry wastewater at different dilution levels.

Parameters	Dilution Level	Control mg L^{-1}	Removal Efficiency (%)	
			Algae	Algae + Bacteria
Chromium (Cr)	5%	1.32	58	79
	10%	1.79	56	71
	20%	2.02	44	62
Cadmium (Cd)	5%	0.79	59	93
	10%	0.91	25	53
	20%	1.08	36	37
Copper (Cu)	5%	1.33	45	72
	10%	1.49	32	62
	20%	1.88	32	60
Lead (Pb)	5%	1.21	61	79
	10%	1.41	53	70
	20%	1.58	39	63
Chemical oxygen demand (COD)	5%	755	19	74
	10%	820	41	53
	20%	871	30	38

The removal of Cr, Cd, Cu, and Pb by *C. vulgaris* was individually compared to the consortium of *C. vulgaris* and *Enterobacter* sp. MN17 from initial concentrations with their removal efficiencies in (Table 1). The highest concentrations were found for Cu followed by Cr, Pb, and Cd in all three dilutions. Removal efficiency of Cr (Figure 3) was 58%, 56%, and 44%, respectively, by algae, while RE by the algal–bacterial consortium for Cr was 79%, 71%, and 62% at 5%, 10%, and 20% dilutions, respectively. Algae removed Cd at all dilutions. Cd (Figure 4) removal by algae at 5% dilution ranged from 0.79 mg L^{-1} to 0.32 mg L^{-1} (RE 59%), at 10% dilution Cd decreased from 0.91 mg L^{-1} to 0.68 mg L^{-1} (RE 25%), and at 20% dilution level Cd decreased from 1.08 mg L^{-1} to 0.69 mg L^{-1} (RE 36%). The algal–bacterial consortium showed better results for Cd treatment with an RE of 93%, 53%, and 36% at 5%, 10%, and 20% dilutions, respectively, over other studied metals.

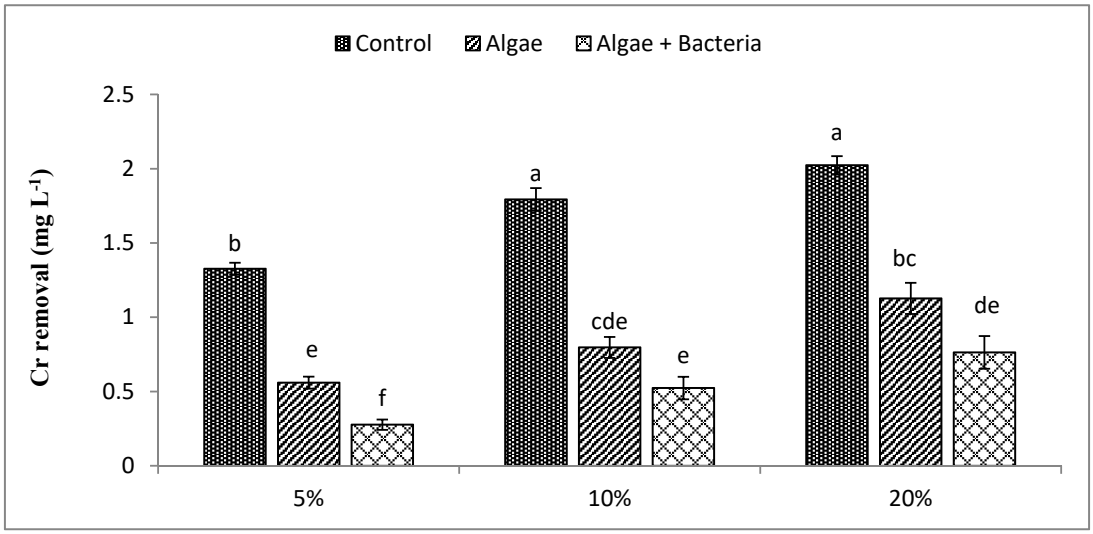

Figure 3. Efficiency of algae and algal–bacterial consortium for chromium removal from textile industry wastewater. Column shows mean values, and bars shows standard deviations (SD) of means. All means followed by different letters were significantly different according to Tukey HSD test at $p < 0.05$.

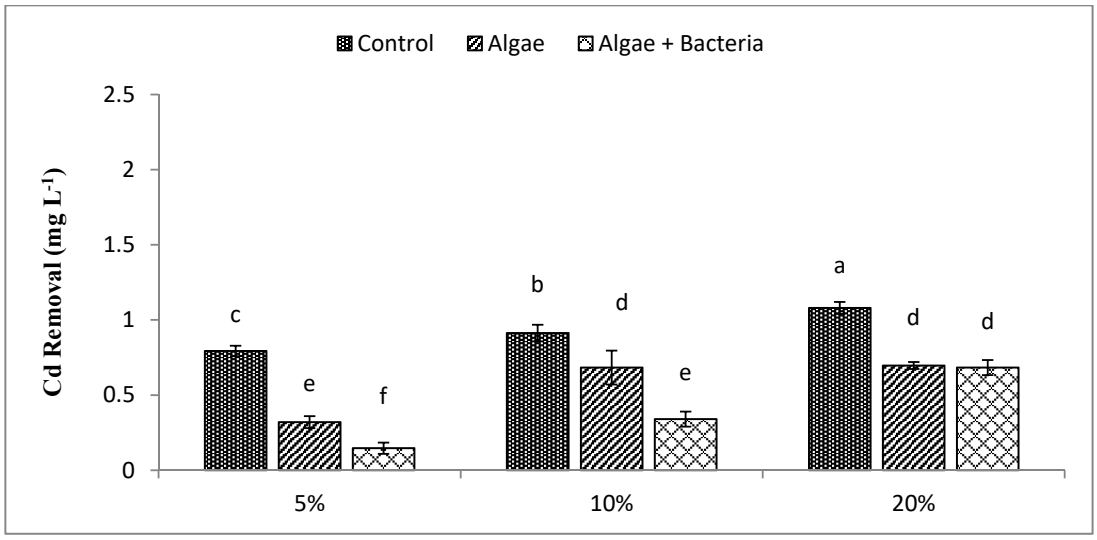

Figure 4. Efficiency of algae and algal–bacterial consortium for cadmium removal from textile industry wastewater at different dilution levels. Column shows mean values, and bars shows standard deviation (SD) of means. All means followed by different letters were significantly different according to Tukey HSD test at $p < 0.05$.

By the end of the experiment, residual quantities of Cr, Cd, Cu, and Pb were still detected, reaching concentrations of 0.56, 0.32, 0.73, and 0.47 mg L^{-1}, with algae, whereas they reached concentrations of 0.27, 0.14, 0.37, and 0.25 mg L^{-1} with the microalgal–bacterial consortium, respectively, at 5% dilution level. Results indicated that the lowest RE at 5% dilution was achieved for Cu (45%) (Figure 5) and the highest for Pb (61%) (Figure 6) by algae, while the algal–bacterial consortium showed the highest RE for Cd (93%) (Figure 4) and the lowest for Cu (72%) at 5% dilution. At all dilution levels, heavy metal removal improved with bacterial inoculation due to higher growth of *C. vulgaris*, except for Cd (37%) at 20% dilution, which is contradictory to results for other metals from the same study, which might be due to the decreased uptake efficiency of *C. vulgaris*.

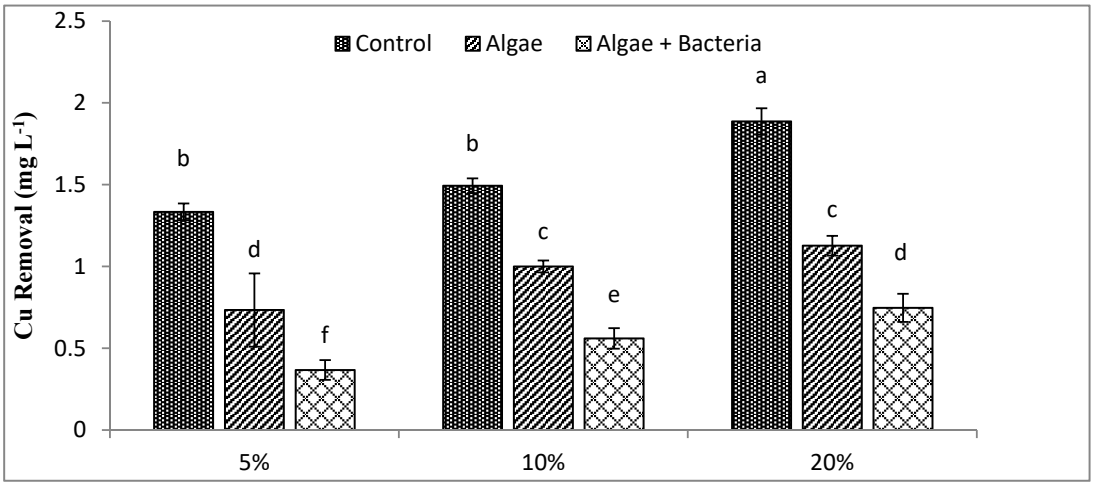

Figure 5. Efficiency of algae and algal–bacterial consortium for copper removal from textile industry wastewater at different dilution levels. Columns show mean values, and bars show standard deviation (SD) of means. All means followed by different letters were significantly different according to Tukey HSD test at $p < 0.05$.

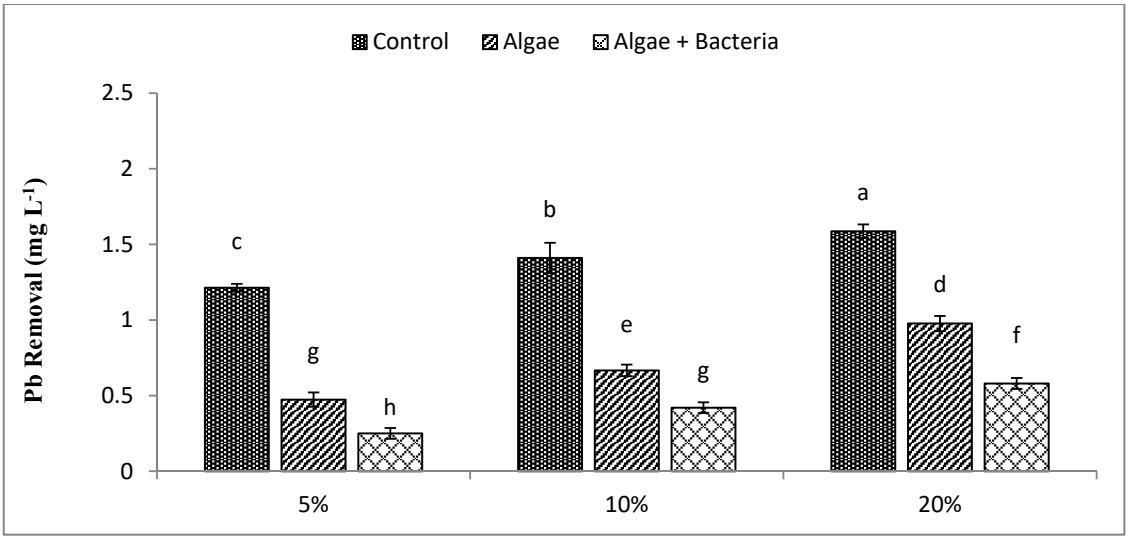

Figure 6. Efficiency of algae and algal–bacterial consortium for lead removal from textile industry wastewater at different dilution levels. Column shows mean values, and bars shows standard deviation (SD) of means. All means followed by different letters were significantly different according to Tukey HSD test at $p < 0.05$.

Removal efficiencies of *C. vulgaris* and the *C. vulgaris–Enterobacter* sp. MN17 consortium for COD in textile wastewater treatment are given in (Table 1). Removal efficiency of *C. vulgaris* at 5%, 10%,

and 20% dilutions were 49%, 41%, and 30%, respectively, for COD. The concentration of COD in the control was 755, 820, and 871 mg L^{-1} at 5%, 10%, and 20% dilutions, respectively, which decreased to 395, 486, and 601 mg L^{-1} by *C. vulgaris,* and to 200, 387, and 545 mg L^{-1} by the *C. vulgaris* and *Enterobacter* sp. MN17 consortium in the corresponding dilution levels, respectively. The highest removal efficiencies of 49% and 74% were achieved by *C. vulgaris* and the consortium, respectively, at 5% dilution level in both treatments. Thus, the results revealed that the algal–bacterial consortium had comparatively high COD removal efficiency as compared to algae.

4. Discussion

Application of growth promoting bacteria for enhancing plant growth has recently achieved much attention under normal as well as stressed environments [27–30], but utilization of these bacteria for algae culturing was scarcely evaluated, especially for wastewater treatment. Here, we evaluated the potential of *Enterobacter* sp. MN17, a potential growth promoting bacterium, for enhancing algal growth in a textile wastewater treatment system. Bacteria can assist microalgae in multiple ways to improve productivity or decrease toxicity of a medium. Microalgae in symbiotic association with bacteria may help deoxygenate the culture media and avoid oxyinhibition. Alternatively, microalgae may serve as a habitat for bacteria for protection from severe environmental conditions [15]. Bacteria also decrease the time to half, required by algae for wastewater treatment, to achieve standard limits of wastewater [31].

We found 0.8 g L^{-1} more biomass by application of *Enterobacter* sp. MN17 to *C. vulgaris* culture. Maximum biomass productivity of 0.902 g L^{-1} day^{-1} was achieved with *Enterobacter* sp. MN17, which is also higher compared to only *C. vulgaris* for the same period. In the *C. vulgaris–Enterobacter* consortium, NO_3^- concentration on the fourth day of cultivation was only 56 mg L^{-1}, which might be the reason for decreased growth after the fourth day, which at the end of the fifth day further decreased to 21 mg L^{-1}. The authors in [32] cultured *C. vulgaris* with *Bacillus pumilus* and reported enhanced growth of *C. vulgaris* after 48 h, which in the MN17 case, started after a 24 h period, which means MN17 also played a role in shortening the lag phase. The authors in [33] also cultured *Azospirillum brasilense,* a growth promoting bacteria, with *C. vulgaris* and reported enhanced nutritional capacity of *C. vulgaris* with bacterial association, which aligns with the results of our study.

Coloring agents are main components of textile industry effluents, which are highly hazardous to the environment. Microalgae play a significant role in the decolorization of these effluents in a cost-effective way by the adsorption of dyes on algal cells [34], while other studies reported that color removal from effluents using microalgae involves biosorption [35]. *Chlorella* sp. has the ability to decolorize the textile wastewater over a certain range of toxicity, but with increases in toxicity, its ability to decolorize dyed water decreases. We found decolorization efficiency of 29–43% for *C. vulgaris* and 49–71.5% for the *Chlorella–Enterobacter* consortium in corresponding dilutions. These results are in good agreement with [36], who observed that the decolorization is actually linked with active *C. vulgaris* growth, which supports our results of enhanced decolorization by *Enterobacter* sp. MN17. The present study also indicated that growth promoting bacterium *Enterobacter* sp. MN17 assisted *C. vulgaris* growth in toxic conditions, resulting in enhanced color removal efficiency.

Concentrations of all the studied heavy metal were beyond the permissible limits of 1 mg L^{-1} Cr, 1 mg L^{-1} Cu, 0.5 mg L^{-1} Pb, and 0.1 mg L^{-1} Cd given by Punjab Environmental Quality Standards for Municipal and Liquid Industrial Effluents (PEQS) [37] and also greater than the reported values from previous studies [38,39]. Abnormal growth of *C. vulgaris* occurs if heavy metal concentration increases to a certain range [40], which is why textile wastewater was subjected to different dilutions to decrease the toxicity level for microalgae, and also why *Enterobacter* sp. MN17 was selected to assist microalgae in coping with the more toxic environment.

Almost all autotrophic microorganisms have the capability to fix heavy metals by a variety of processes such as physical adsorption, chelation, chemisorption, ion exchange, precipitation, and crystallization on the cell surface or extracellular metabolites [11,41]. The removal efficiencies (Table 1) of these metals were affected by their initial concentrations in the present study. Our results

for initial concentrations were consistent with previous studies [17,42], revealing a direct link between removal efficiency and initial concentration of pollutants. In this study, removal of metals was improved by diluting the textile wastewater to varying extents. At these dilutions, removal efficiency of *C. vulgaris* varied widely in the ranges of 44–58%, 25–59%, 32–45%, and 39–61% for Cr, Cd, Cu, and Pb, respectively, whereas in the case of the algal–bacterial consortium, RE varied in the ranges of 62–79%, 37–93%, 60–72%, and 63–79%, respectively. The removal of these metals involves a combination of several processes, including metal species, initial concentration, and bacterial abundance [42].

In the present study, Cu RE ranged between 32 and 45% in their corresponding dilutions by *C. vulgaris*, which is in contrast to studies reporting RE of *C. vulgaris* as 55% and *Chlorella pyrenoidosa* as 80% [43] and [17]. The initial Cu concentrations in [43] and [17] were much lower than our three dilution levels. Less removal in our study might be due to the saturation of *C. vulgaris.* Similar results were reported by authors in [44], who studied the contact time for biosorption of Cu with *Scenedesmus obliquus* and reported high initial removal followed by a decrease in performance due to saturation. Cadmium RE at 20% dilution was very low for both microalgae and the microalgae–bacteria consortium, which might be due to the inability of *C. vulgaris* to store Cd in its biomass, but still, results in this study are much more satisfactory compared to other studies. The authors in [45] achieved only 24.1 ± 3.1% of Cd removal from simulated wastewater using *Scenedesmus incrassatulus*. Authors in [42] also reported lower RE for Cr, Cd, and Pb by *C. vulgaris.* Cadmium results of 20% dilution by both microalgae and consortia align with our study, but for Cr and Pb, our study results are contradictory to the findings of [42]. A possible reason for higher RE for some metals might be that these metals were used by microalgae for maintenance of their activities and functions [46].

Gupta et al. [47] tested COD RE (%) of *Chlorella sorokiniana* and *Scenedesmus obliquus* from raw sewage wastewater. They achieved 76.13 ± 1.59% COD removal by *S. obliquus* and 69.38 ± 1.81% by *C. sorokiniana,* which are much higher than our results found by *C. vulgaris* but closer to the *Chlorella–Enterobacter* consortium. The reason for higher RE in [47] might be that the initial COD was only 320.07 ± 3.78 mg L^{-1}, which is much lower compared to our study. Comparing the RE of *S. obliquus* and *C. sorokiniana* with the *C. vulgaris–Enterobacter* consortium results from our study revealed that the *Chlorella–Enterobacter* consortium is more efficient for COD removal. Authors in [48] studied the symbiotic relationship of wastewater borne bacteria and *C. vulgaris* and reported enhanced COD removal. Wastewater borne bacteria break down complex organic compounds into simple molecules, which make nutrients usable for algae, thus removing higher amounts of COD; but *Enterobacter* is a growth promoting endophytic bacteria, so higher pollutant removal in our study was due to the enhanced growth of *C. vulgaris* in the presence of *Enterobacter* sp. MN17.

The concentrations of all heavy metals for microalgae–bacteria treatment after harvesting were much lower than permissible limits of PEQS except for those of Cd, which were in range of only 5% dilution. Keeping in view these results, wastewater was safe to release to water bodies or to use for irrigation purposes. Although application of bacteria improved heavy metal removal efficiency in diluted samples, culturing microalgae in real textile wastewater still needs a breakthrough in research. For higher degradation and removal of toxic pollutants, attention should be given to developing an effective and efficient consortium where either genetically engineered microalgae or bacteria should be involved for higher efficiencies [31].

For microalgae growth, the dilutions of textile wastewater are limited due to the high cost of clean water, increased volume, artificial nutrients for improving the efficiency, and other inconvenient factors regarding operation and maintenance of the treatment system at a large scale. Therefore, development of an efficient system that allows microalgae to grow well in undiluted textile wastewater is very important. For the same reason, authors in [49] introduced a biofilm-attached culture system for swine wastewater for making the application of microalgae-based technology environmentally and economically feasible for large scale wastewater treatment operations that reduced the pollutant toxicity with improved microalgal growth. Some researchers also proposed mixed microalgae cultures for enhancing nutrient and heavy metal uptake from wastewater and proposed that one microalga can

remove N, whereas another can remove metals [50–52] from wastewater. Application of bacteria to microalgae-based treatment systems is also a novel approach for reducing the toxicity of wastewater and improving algal growth, but this field still needs much attention to be able to replace chemical-based costly technologies with no or little resource recovery.

5. Conclusions

The present investigation revealed considerable growth of the microalgae strain *C. vulgaris* in different dilution levels of textile wastewater. Addition of bacterial inoculation (*Enterobacter* sp. MN17) to the treatment system further enhanced the biomass production and remediation potential of *C. vulgaris*, resulting in the effective removal of color, metals, and other contaminants from wastewater. Removal of COD from wastewater by *C. vulgaris* was 49% at 5% dilution, which increased to 74% with bacterial inoculation. Similarly, significant removal efficiencies were also achieved for all the studied heavy metals (Pb, Cd, Cr, and Cu). The maximum removal achieved was 59% by microalgae and 93% by the microalgal–bacterial consortium for Cd at a 5% dilution level of wastewater. The resulting algal biomass after wastewater treatment can be profitably converted into bioenergy, thus making this biological process more feasible, reliable, and appealing with the dual benefits of waste reduction and bioenergy production in an economical and environmentally-friendly and sustainable way.

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