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ORIGINAL RESEARCH

The efficiency and antioxidant response of microalgae biofilm in the phycoremediation of wastewater resulting from tannery, textile, and dyeing activities

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Abstract The tanning, textile, and dye industries consume a large amount of water during production, leading to a large quantity of wastewater. This study aims to provide insight into the phycoremediation of the tannery, textile, and dye wastewater microalgae biofilm. Microalgae biofilm was grown in a photobioreactor with BG-11 as the culture medium, used to treat tannery, textile, and dye wastewater. The antioxidant response of the microalgae biofilm before and after treatment of the wastewater was determined. The results obtained show the microalgae biofilm best reduced biochemical oxygen demand (BOD) (68%), chemical oxygen demand (COD) (83%), chloride (52%), phenol (39%), and alkalinity (47%), from textile wastewater, Total dissolve solids (TDS) (88%), total suspended solids (TSS) (94%), sulfate (56%), phosphate (45%), total nitrogen (63%), oil and grease (42%), and total chromium (64%) were all reduced in dyeing wastewater, while electrical conductivity (EC) (64%) and total chromium were reduced the best in tannery wastewater. The result also shows an increase in peroxidase activity, catalase activity, superoxide dismutase activity (SOD), and glutathione reductase activity, indicating the role of enzymatic antioxidants in pollutant degradation and counteracting the effect of reactive oxygen species (ROS). The decrease in flavonoid, anthocyanin, and carotenoid indicates non-enzymatic antioxidants involvement in the degradation and role of the pollutant in osmoregulatory balance maintenance. Simultaneously, the increase in TOC and lipid content signifies the strong role of antioxidants in radical scavenging. The increase in phosphomolybdate capacity, hydrogen peroxide scavenging, and DPPH scavenging ability is also a strong indication of high ROS production and prevention of its effect. The microalgae biofilm's ability to remove pollutants from wastewater is by the activities of enzyme release by the microalgae or adsorption of the pollutants to the wall of the participant microalgae in the biofilm.

Keywords Antioxidant response . Pollutant degradation . Wastewater treatment . Reactive oxygen species . Catalase . Superoxide dismutase activity (SOD) . Flavonoid . Anthocyanin

Introduction

The worldwide increase in population has led to a tremendous increase in production and the use of chemicals (Aktar et al. 2009). The incessant production is attributed partly to the increased population and partly to the development of new products for the sake of progress (Ugya et al. 2019a; Wang et al. 2012). The chemical's environmental impact during production is a cause for concern because most of the substance resists decay and are biologically non-degradable (Jain et al. 2016; Brooks et al. 2020). The tanning, textile, and dye industries consume a large amount of water during production, leading to a large quantity of wastewater (Roy et al. 2015). Studies have shown that the wastewater generated by these industries is associated with high COD, BOS, TDS, acidity, chloride, sulphate, phenolic compounds, oil, grease, and heavy metals

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(chromium, lead, copper, mercury, and nickel) (Yaseem and Scholz 2019). Pollution control is recognized as a crucial part of the production, but the inefficiency in its application has led to the presence of a heavy backlog of pollutants in the aquatic environment (Yaseem and Scholz 2018). Different physical, chemical, and biological methods have been used to treat this wastewater to reduce the wastewater pollutants and render it less dangerous and repulsive to man before discharge (Li and Zhao 1999). However, these methods are inefficient and not easily accessible, thereby exposing many people in developing countries to different diseases due to the pollution of surface and groundwater (Redfern and Gunsch 2016).

Phycoremediation is a method of remediation that involves the use of cyanobacteria, microalgae, or macro algae in the treatment of polluted water (Dayana et al. 2021). The method involves the use of biosorption, bioconcentration, assimilation, sequestration, and biotransformation in the removal of pollutants from wastewater (Koul et al. 2022). Phycoremediation is a novel process of remediation that is a better alternative to other bioremediation methods because it is sustainable, feasible, and economically cost-effective (Stauffer et al. 2019). The difficulty in the harvesting of microalgae for phytoremediation is reason why microalgae biofilm reactor is develop (Buettner 2011). Microalgae biofilm is a novel algae-prokaryotic system which leads to the production of less suspended solid during phycoremediation over the suspended growth system (Xuemei et al. 2010). This is due to the fact that wastewater enters the system when the microalgae biofilm is bound on the substrate of the bioreactor (Wang et al. 2020). Microalgae tend to reduce the organic load, maintain PH, remove phosphorus, nitrogen, sludge, and forage heavy metals from wastewater (Han et al. 2017). Microalgae also tend to generate more biomass in wastewater in the presence of sunlight using inorganic carbon. This biomass is utilized in the production of biofuel and other bioresources (Ugya et al. 2020a). Many studies have been conducted, including immobilization techniques, pre-treatment methods, genetic engineering, and combined technologies, in order to completely utilize the ability of microalgae for phycoremediation and bioresource production (Abdel-Raouf et al. 2012).

Many factors influence the uptake, bioconcentration, and degradation of chemical contaminants in wastewater (Ugya et al. 2019b). These factors include pH, temperature, and nutrient availability in the medium, cell size, chemical composition and physiological capacity of the microalgae (Puyol et al. 2017). A previous study by Ugya et al (2021a) showed the role of microalgae biofilm in the removal of pollutants from petroleum-contaminated water. The study shows the role of phytochemicals in pollutant removal and response to prevent oxidative damage resulting from the exposure of the microalgae to contaminated water. Another study by Ugya et al. (2021b) shows the role of microalgae biofilm in the treatment and response to oxidative stress caused by the overproduction of reactive oxygen species by the microalgae biofilm. There is a need to study the efficacy of microalgae biofilm in the treatment of tannery, textile and dyeing wastewater. Therefore, this paper aims to provide insight into the efficiency and antioxidant response of microalgae biofilm in the phycoremediation of industrial wastewater.

Materials and methods

Photo-bioreactor construction and freshwater biofilm cultivation

The experimental setup for each microalgae biofilm consists of three photo-bioreactors; each of the bioreactor comprises 3 glass containing slides representing the substrate and a water heater, as shown in Fig. 1. The photo-bioreactor is a glass container that measures 21.1 cm in length, 31.0 cm in height, and 18.1 cm in width, and its capacity is about 11.8 L. Transparent polymethylmethacrylate was employed to make the racks which act as the substrates. The length of a rack is 8.4 cm, the width is 6.2 cm, and the height is 25.0 cm. In a rack, there are 30 grooves of 3 mm in width to place and hold the glass slides, and the grooves are spaced at 5 mm. The glass slides used as the substrate for biofilm are 4.9 cm in length, 7.6 cm in width, and 1 mm in thickness. The light intensity was within the range of 150-170 Klux (Ugya et al. 2021c). A total of 86 mL of BG 11 solution was added to the photo-bioreactors to support the biofilm formation. The BG 11 medium is made up of 1.5 g $NaNO_3$, 0.040 g K_2HPO_4, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl \cdot 2H_2O$, 0.006 g citric acid, 0.006 g ferric citrate green, 0.001 g $EDTANa_2$, 0.020 g Na_2CO_3, and 1 ml A5 trace mental solution in 1 L deionized water. The A5 trace mental solution contains 2.860 g H_3BO_3, 1.810 g $MnCl \cdot 4H_2O$, 0.222 g $ZnSO_4 \cdot 7H_2O$, 0.390 g Na_2MoO_4, 0.079 g $CuSO_4 \cdot 5H_2O$, 0.49 g $Co(NO_3)_2 \cdot 6H_2O$ in per liter deionized water (Ugya et al. 2021b).

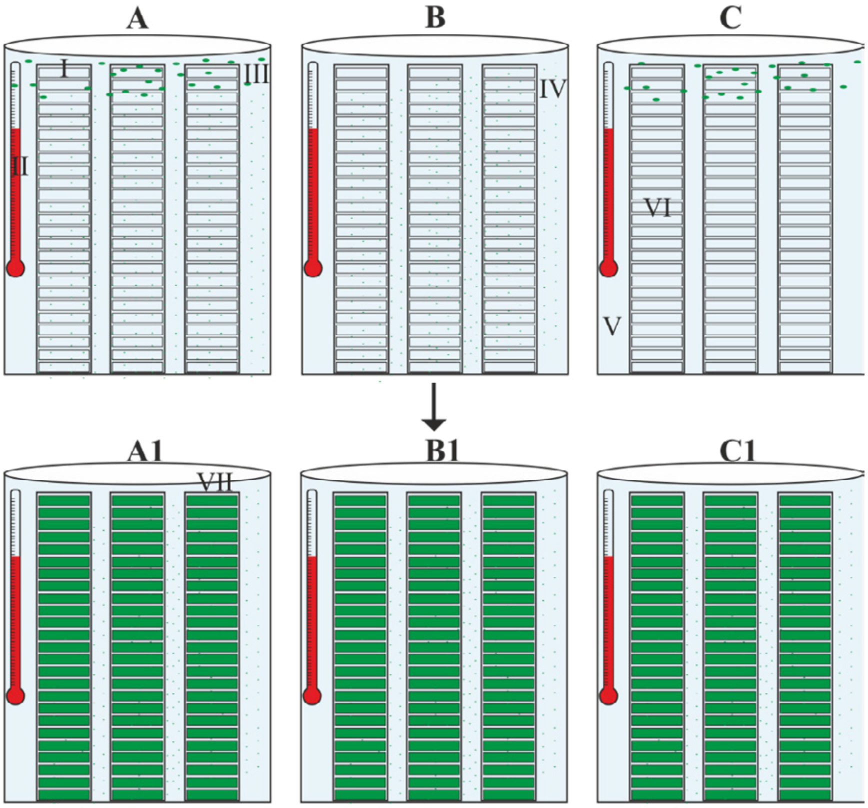

Fig. 1 Schematic representation of the process of microalgae biofilm cultivation. A-C-Early stage of microalgae biofilm cultivation, A1-C1-Late stage of microalgae biofilm cultivation, I- Substrate containing microscopic slide, II-Water heater to regulate temperature at 30°C, III and IV- Freshwater microalgae, V- Freshwater microalgae, V-Freshwater containing culture solution, VI-Microscopic slide embedded in the substrate, VII-Freshwater microalgae biofilm form on the surface of the microscopic slide.

Treatment of the wastewater using microalgae biofilm

Tannery and Textile wastewater was collected from the industrial effluent point of Unique Leather Finishing Company Ltd, Kano and United Textile Limited, Kaduna respectively. Dyeing wastewater was collected in dyeing point of Faskari Road, Ungwar Sanusi Tudun Wada Kaduna using Grap method from January-June, 2020. The wastewater was poured into three different photo-bioreactors so that the treatment of the three collected wastewaters was done differently (Ugya et al. 2021a). The substrate containing microalgae biofilm was inserted into three photo-bioreactors containing tannery, dyeing, and textile wastewater for treatment for three weeks. During the treatment period, water samples and microalgae biofilms were collected weekly for parameter determination and the determination of the antioxidant response of microalgae biofilms. The physicochemical parameter (electrical conductivity, sulphate, alkalinity, chloride, TDS, TSS, phosphate, total chromium, COD, and BOD of the wastewater was determined according to standard method. EC by 2510-laboratory method, TDS 2540-gravimetric method, TSS by 2540-gravimetric method, alkalinity by 2320- titrimetric method, chloride by 4500-Cl-calorimetric method, sulphate in water was measured by 4500-SO$_4^{2-}$ - gravimetric method, COD by 5220-colorimetric method, and BOD by 5210-calorimetric method according to the standard method of APHA (2005). Concentrations of Cr were determined by digesting the wastewater sample and analysing using an Atomic Absorption Spectrophotometer (Varian AA 240 FS, USA). Phenol, total nitrogen, oil and grease of the wastewater were determined using spectrophotometric method. The microalgae biofilm composition was investigated using a compound light microscope and the dominate microalgae were identified (Ramakrishnan et al. 2010; Lares et al. 2018).

The antioxidant responses associated with the microalgae biofilm

To investigate the antioxidant response associated with the freshwater microalgae biofilm during the treatment, the peroxidase activity, catalase activity, SOD, glutathione reductase activity, phosphomolybdate capacity, anthocyanin content, flavonoid content, chlorophyll, carotenoid, hydrogen peroxide scavenging and DPPH scavenging ability of the microalgae biofilms were determined using the spectrophotometric method (Patias et al. 2017; Haida and Hakiman 2019). TOC of the biofilm was determined using a Shimadzu total organic carbon analyser (TOC-91105-34). Lipid content of the biofilm was determined using spectrophotometric method (El-Sheekh et al. 2013), by grinding 100 mg of dry biomass of the freshwater microalgae biofilm.

Microalgae biofilm composition and characterization

The microalgae biofilm composition was investigated using a compound light microscope and the dominate microalgae were identified (Lares et al. 2018).

Statistical analysis

The determination of reduction efficiency and antioxidants was conducted in triplicate and data were presented statistically as mean and standard deviation. One-way analysis of variance at 0.05% was used to assess the significant differences in microalgae biofilm responses. The statistical analysis was done using SPSS version 23.

Results and discussion

Biofilm formation

Fig. 1 shows the efficacy of the photo-bioreactor and growth conditions in the production of high biomass of microalgae biofilm. This is the reason why bioreactors A, B, and C contain a substrate that is transparent without a mass of biofilm. In contrast, the substrate of the bioreactors A1, B1, and C1 is greenish, signifying the presence of a thick mass of microalgae biofilm. The thick mass of microalgae biofilm formed at the substrate of bioreactor A1, B1, and C1 signifies the successful attachment of the free-floating freshwater microalgae in the bioreactor to the substrate **(**Lares et al. 2018**)**. According to the Fig. 2, the family of freshwater microalgae after the formation of the biofilm includes *Desmidiaceae*, *Baciclariaceace*, *Klebsormidiaceace*, *Oocystaceae*, *Micractiniaceae*, *Chlorollaceae*, *Xenococcaceae*, *Microcystaceae*, *Hydrococcaceae*, *Merismopediaceae*, *Chroococcaceae*, *Chrysocapsaceae.* The presence of these microalgae family after treatment of the wastewater is due to the increase in species diversity and richness (Axelsson and Gentili 2014; Khan and Malik 2014; Durotoye et al. 2018; Roostaei et al. 2018; Mustapha et al. 2020).

Treatment of the wastewater using microalgae biofilm

Fig. 3 shows the efficacy of microalgae biofilm in the treatment of tannery, textile and dyeing wastewater. Fig. 3a shows how microalgae biofilm effectively reduces alkalinity, BOD, COD, EC, chloride, TDS, TSS sulfides, and total chromium of tannery wastewater. The ability of microalgae biofilm to reduce these pollutants from tannery wastewater increases with retention time (table SM1). Fig. 3b shows the efficiency of microalgae biofilm in the removal of TDS, TSS, alkalinity, phenol, BOD, COD, chlorides, sulfates, oil and grease from textile wastewater. The ability of microalgae biofilm to remove these parameters from textile wastewater tends to also increase with retention time (table SM2). Fig. 3c shows that there are significant differences between the reduction efficiency of TDS, TSS, BOD, COD, chlorides, sulfates, phosphates, total nitrogen, phenol, oil and grease before and after treatment of dyeing wastewater. This result signifies the efficacy of microalgae biofilm in the treatment of dyeing wastewater.

The ability of the microalgae biofilm to remove chromium from tannery wastewater is due to adsorption of the metal to the extracellular matrix of the biofilm or to the cell surface of the microalgae forming the biofilm, as evident from the study of Ugya et al. (2021c). The microalgae biofilm's ability to reduce COD and BOD in the wastewater is attributed to the ability of microalgae biofilm to facilitate the degradation

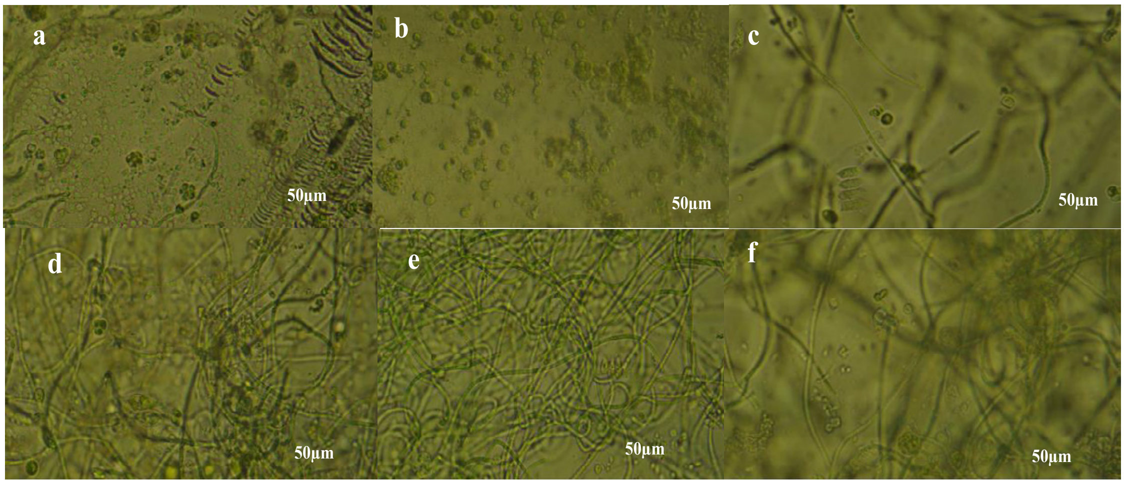

Fig. 2 Microscopic analysis of the microalgae constituent of biofilm formation

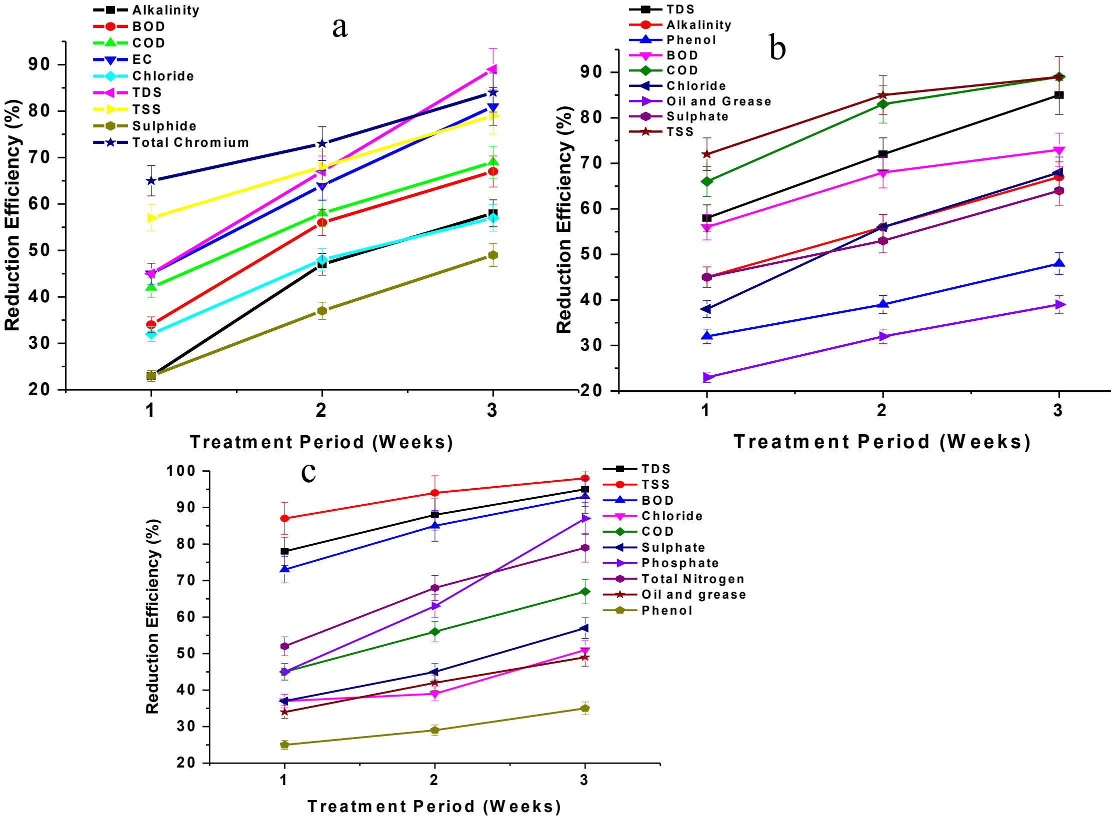

Fig. 3 Phytoremediation efficacy of freshwater microalgae biofilm. (a) tannery wastewater, (b) textile wastewater, and (c) dyein wastewater

of organic compounds present in the wastewater, which in turn led to the reduction of the oxygen demand and consumption in the wastewater (Delgadillo-Mirquez et al. 2016; Lellis et al. 2019; Zahmatkesh and Pirouzi 2020). The reduction in TSS and TDS in the tannery, textile, and dyeing wastewater signifies the microalgae biofilm's efficacy to attract both TSS and TDS to accumulate on the biofilm substrate, thereby becoming part of the biofilm (Ugya 2015; Decho and Gutierrez 2017). The process is similar to the particle sedimentation process but differs because, in this case, the extracellular matrix of the biofilm is responsible

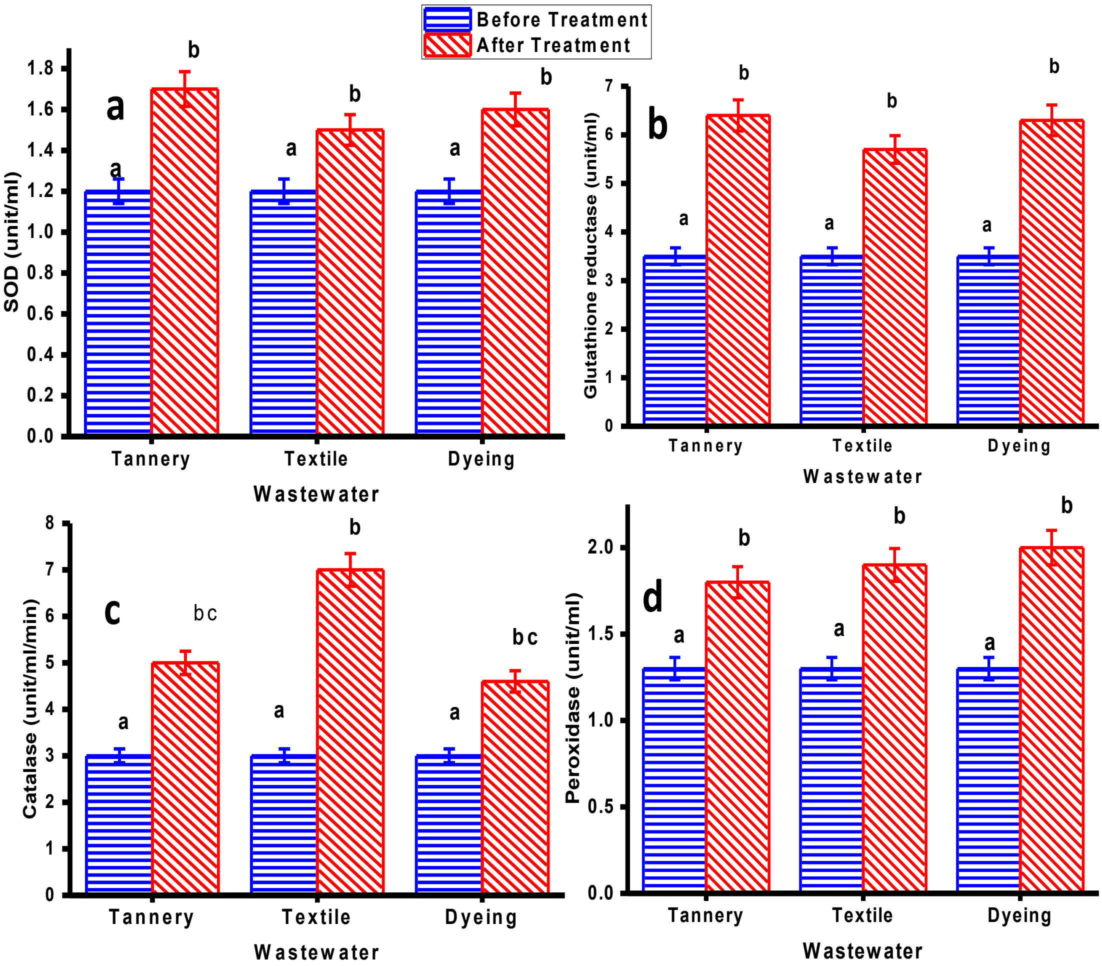

Fig. 4 Microalgae biofilm enzymatic antioxidant response in wastewater. (a) SOD, (b) glutathione reductase, (c) catalase, and (d) peroxidase The error bar in the represents the mean and standard deviation of triplicated experiment. Different letters on top of error bar represent significant difference ($P < 0.05$) with ANOVA analysis.

for the trapping of TDS and TSS and keeping the particles in close relationship with the participant's cells (Stoodley et al. 2002). The microalgae biofilm's ability to reduce chlorides from the tannery, textile, and dyeing wastewater is attributed to the participant microalgae cells' ability in the biofilm to utilize the chlorides for osmoregulation and prevention of osmotic stress and dehydration (Singh et al. 2019). The reduction efficiency of chloride in the present study is higher than the reported cases in literature because microalgae biofilm contain more cells playing different metabolic functions (Barros et al. 2019; Ugya et al. 2020b). The high reduction of alkalinity in the tannery, textile, and dyeing wastewater depends on the high chloride removal efficiency (Brossia 2018). The high removal efficiency of total nitrogen and phosphate from dyeing wastewater is attributed to the microalgae biofilm's ability to utilize this substance for biomass production (Whitton et al. 2015). The assimilation and utilization of nitrogen compounds and phosphate prevent this substance's accumulation to a toxic level (Nagi et al. 2020).

The ability of the microalgae biofilm to successfully reduce sulfur-containing compounds (sulfide and sulfate). It is because the participant microalgae cells in the biofilm tend to donate an electron. This, in turn, favors desulfurization of compounds and eventual removal of sulfur and sulfide from the wastewater (Ayala-Parra et al. 2016). The desulfurization of sulfur-containing could also be due to the production and release of reactive oxygen species by the microalgae biofilm (Xu et al. 2006). The removal of oil and grease from textile and dyeing wastewater is a clear indication of the microalgae biofilm's efficacy in pollutant removal. This is because the microalgae biofilm can interact with oil and grease present in the wastewater leading to the degradation of oil and grease (Effendi et al. 2017; Ugya et al. 2019). The reduction of phenol from textile and dyeing wastewater is due to the interaction of the microalgae biofilm with the phenol and

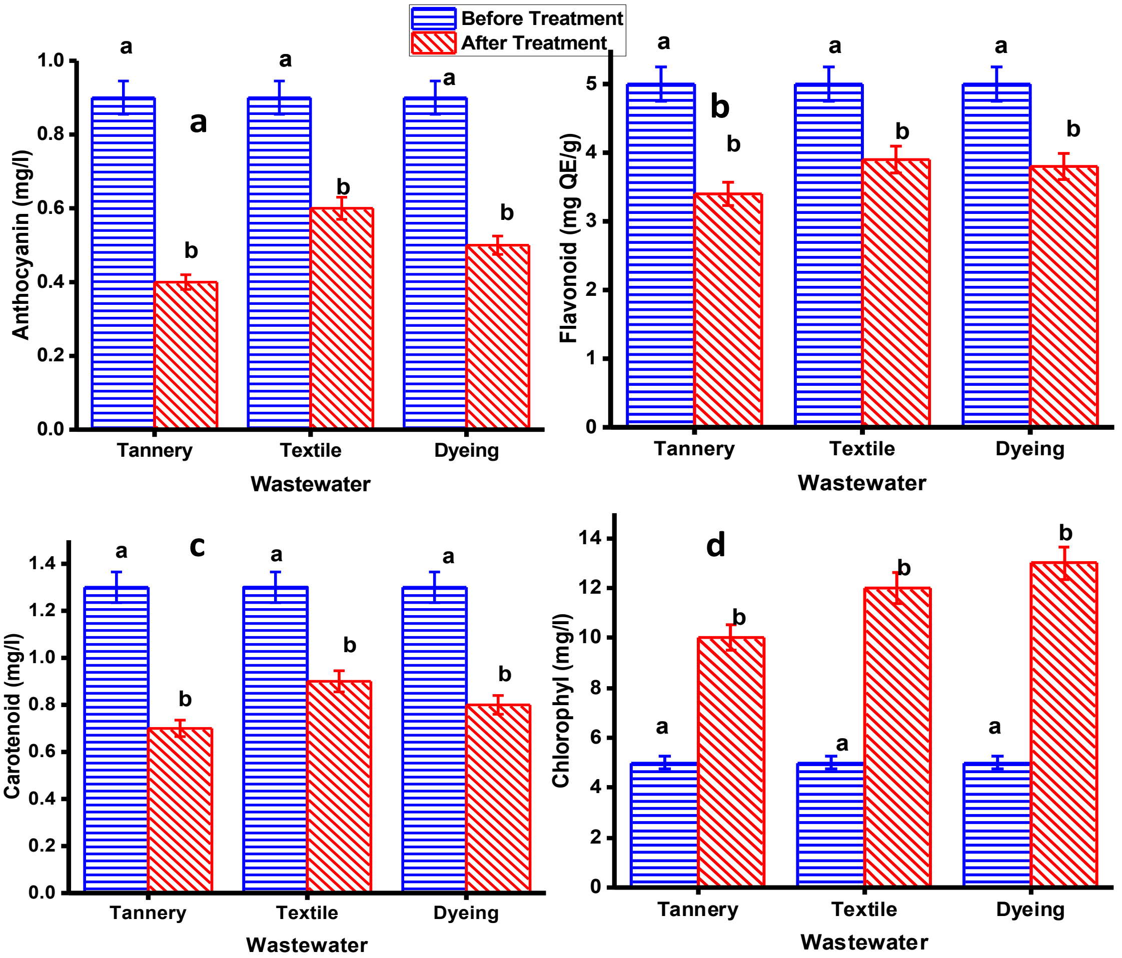

Fig. 5 Microalgae biofilm non- enzymatic antioxidant response in wastewater phycoremediation. (a) anthocyanin, (b) flavonoid, (c) carotenoid, and (d) chlorophyll. The error bar in the represents the mean and standard deviation of triplicated experiment. Different letters on top of error bar represent significant difference (P <0.05) with ANOVA analysis.

the presence of bacteria that oxidize phenol, thereby facilitating phenol degradation in the wastewater (Hirooka et al. 2003).

Enzymatic antioxidant response of the microalgae biofilm

The result displayed in Fig. 4 shows significant differences between enzymatic antioxidant produce before and after treatment of tannery, textile, and dyeing wastewater. The increase in enzymatic antioxidants in microalgae biofilm after treatment of tannery, textile, and dyeing wastewater. The result shows significant differences at 0.05 in the ability of the microalgae biofilm to produce SOD, GR, catalase and peroxidase.

The general increase in enzymatic antioxidants is to counteract the oxidative stress resulting from the exposure of the microalgae biofilm to the wastewater (Gauthier et al. 2020). The increase in the enzyme SOD validates a high level of ROS, which plays a role in the degradation of organic and inorganic pollutants (Subashchandrabose et al. 2013). The high level of SOD is also the reason why chromium was efficiently reduced in tannery wastewater. This is because SOD provides the points of interaction that are critical for the binding of heavy metals by microalgae biofilm (Ragsdale 2009). The increase in the level of catalase and peroxidase of the microalgae biofilm is a clear indication of the increase of hydrogen peroxide production, which in turn contributes to the degradation of organic and inorganic pollutants in the wastewater. The increase in peroxidase and catalase also influences the degradation of phenol in textile and dyeing wastewater. The reduction in COD and BOD results from the degradation action of catalase and peroxidase on organic pollutants present in the wastewater (Ojuederie and Babalola 2017). The increase in glutathione reductase activities in the microalgae biofilm signifies the scavenging of hydrogen peroxide to prevent oxidative stress on the microalgae biofilm as a result of exposure to the wastewater. The increased level of glutathione reductase is also why the freshwater microalgae can reduce sulfur-containing compounds in the tannery, textile, and dyeing wastewater (Couto et al. 2016).

No-enzymatic antioxidant response of the microalgae biofilm

The result displayed in Fig. 5b shows significant differences in the reduction of microalgae biofilm's flavonoid content after treatment with a tannery, textile, and dyeing wastewater. This decrease in flavonoid content after treatment with the wastewater is a clear indication of the microalgae biofilm involvement in the prevention of oxidative stress resulting from exposure to the wastewater pollutants (Bansal and Kanwar 2013). The ability of flavonoids to inhibit the generation of ROS by the microalgae biofilm is by forming complexes with Cu^{2+} and Fe^{2+} because of the decrease of flavonoid content in the microalgae biofilm after exposure to the wastewater (Al-Rashed et al. 2016). The result presented in Fig. 5c shows the significant difference in the decrease in carotenoid content of microalgae biofilm after exposure to tannery, textile, and dyeing wastewater. This decrease in microalgae biofilm carotenoid content shows the carotenoid content's involvement in counteracting the effect resulting from ROS's overproduction (Rezayian et al. 2019). Hence, the cell of the microalgae biofilm was not affected despite the efficiency of the microalgae biofilm in pollutants' degradation of the wastewater (Haoujar et al. 2019). Carotenoid content of the microalgae biofilm can annul any effect resulting from ROS overproduction by donating an electron to the ROS during the triplet-triplet transfer, thereby preventing any adverse effect that may result from the exposure (Sharma et al. 2012; Sansone and Brunet 2019). The Fig. 5d shows significant difference in the increases of chlorophyll content after the treatment of the wastewater. The slight increase in chlorophyll content of the microalgae biofilm after treatment of tannery, textile, and dyeing wastewater shows that during the treatment of the wastewater, the cell participating in the biofilm was not adversely affected due to the antioxidant defense mechanism shown by the microalgae biofilm and this is the reason why microalgae biofilm is effective for the treatment of tannery, textile, and dyeing wastewater (Chen et al. 2020). The Fig. 5a shows significant difference in the reduction of anthocyanin content after treatment of the wastewater. The decrease in anthocyanin content of the microalgae biofilm signifies anthocyanin's role in the defense system of the microalgae biofilm high-level ROS production (Yong et al. 2018). The high pH and the presence of metal ions in the wastewater are responsible for decreasing the anthocyanin content of the microalgae biofilm (Yong et al. 2020).

The result presented in Fig. 6 shows significant difference in the increase of the radical scavenging ability of the microalgae biofilm after treatment of the wastewater. The increase in DPPH reducing capacity, phosphomolybdate reducing capacity, and hydrogen peroxide scavenging potential after treatment of tannery, textile, and dyeing wastewater signifies the scavenging potential of the microalgae biofilm to counteract the effect of any radical that is produced during the treatment of the wastewater (Manivannan et al. 2012). This increase in the non-enzymatic antioxidant defense system is a strong indication of the microalgae biofilm's affectivity to treat tannery, textile, and dyeing wastewater because the ability of microalgae to survive after treatment of wastewater is critical in phycoremediation (Manivannan et al. 2012).

Effect of wastewater on lipid and TOC of the microalgae biofilm

The Fig. 7a shows significant difference in the increase in lipid and TOC content of the microalgae biofilm after treatment of the wastewater. The increase in the lipid and TOC of the microalgae biofilm after treatment of tannery, textile, and dyeing wastewater validates the fact that there is increasing ROS production. This increase in ROS turn causes oxidative stress in the wastewater environment capable of causing the degradation of the pollutants present in the wastewater (Mohsenpour et al. 2021). The decrease in carotenoid content after treatment supports the fact that the increase in lipid is due to carotenoids' ability to counteract the effect of ROS produced during the wastewater treatment. Studies by Shi et al. (2020) reveal that oxidative stress tends to facilitate the accumulation of lipids and TOC in microalgae (Ruenwai et al. 2011). Microalgae biofilm lipid will help to eradicate the problems associated with the rising oil price and concern over climatic changes (Anderson et al. 2015). The current study is also a disruption technique that can be used to increase the efficacy of microalgae lipid production (Lee et al. 2014; Yang et

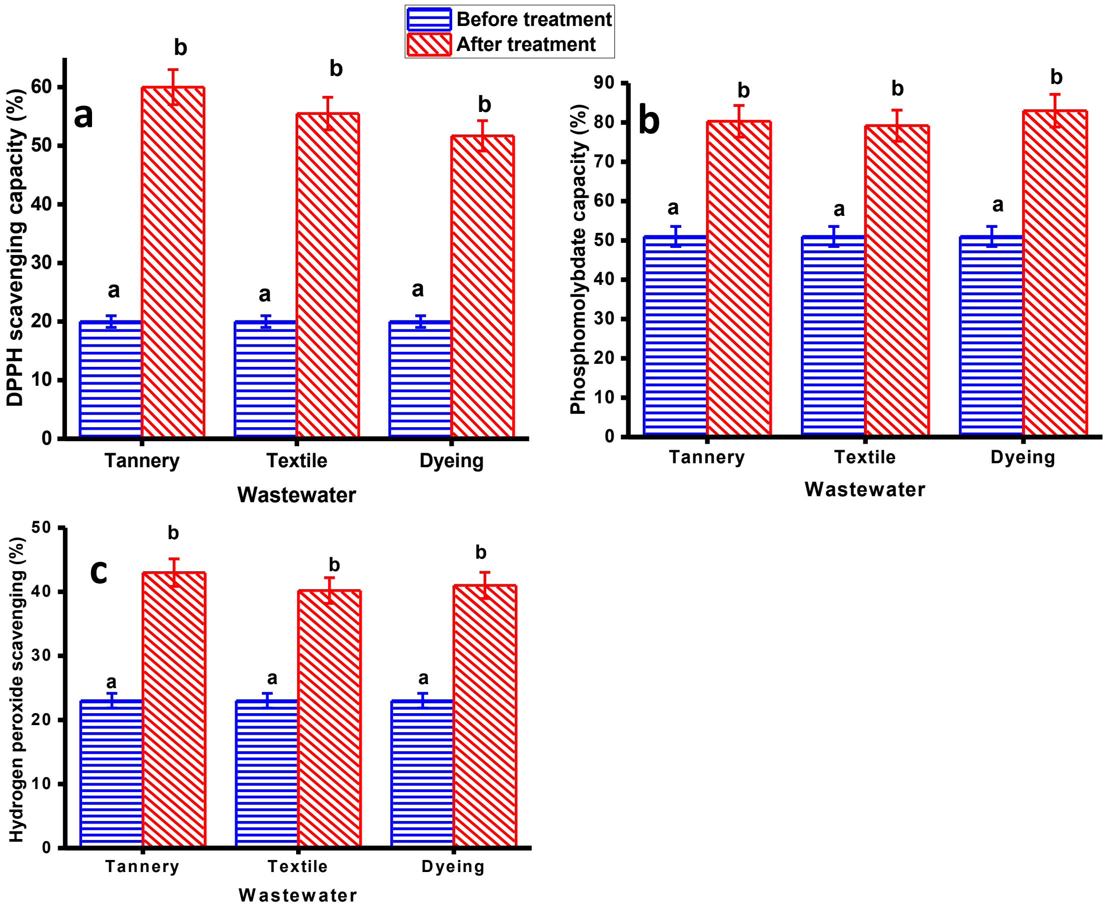

Fig. 6 Ability of microalgae biofilm to scavenge radicals. (a) DPPH, (b) phosphomolybdate, and (c) H_2O_2 scavenging activity. The error bar in the represents the mean and standard deviation of triplicated experiment. Different letters on top of error bar represent significant difference (P <0.05) with ANOVA analysis.

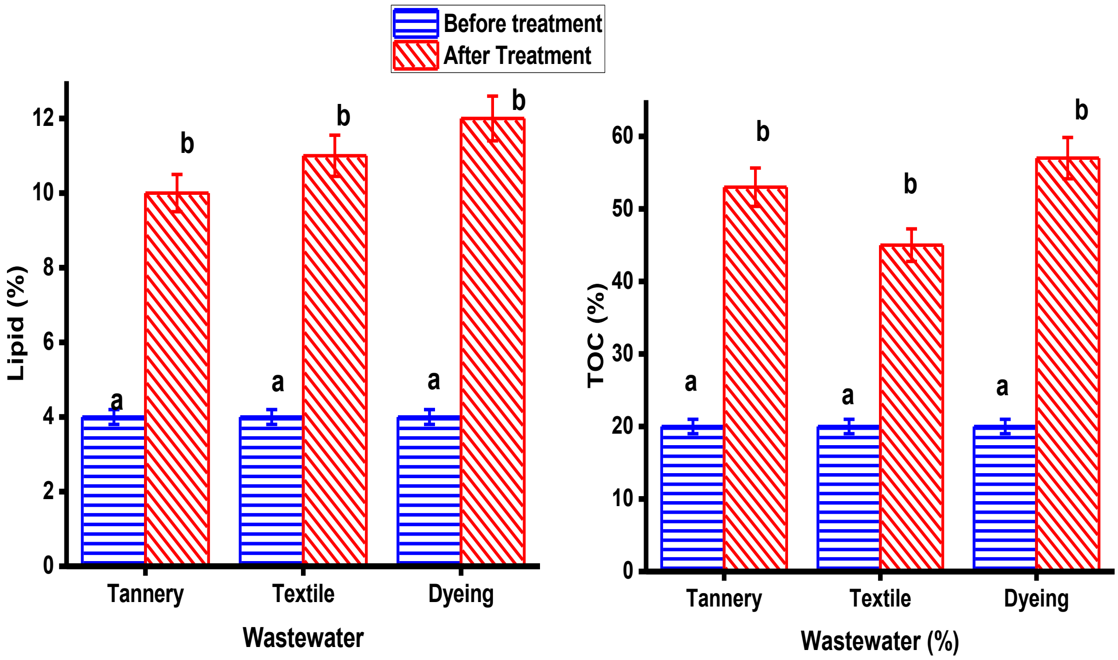

Fig. 7 Effect of wastewater on lipid and TOC of microalgae biofilm. The error bar in the represents the mean and standard deviation of triplicated experiment. Different letters on top of error bar represent significant difference (P <0.05) with ANOVA analysis.

al. 2018). The increase in TOC signifies that the microalgae is able to counteract the effect that result from the oxidative stress, this is because both TOC and biomass carbon (BC) are the most significant component of microalgae that shows the state of the microalgae (Ndikubwimana et al. 2016; Ma et al. 2020).

Conclusion

The current study shows the role of microalgae biofilm as an alternative for the treatment of tannery, textile and dyeing wastewater. The reduction efficiency shows biochemical oxygen demand (BOD) (68%), chemical oxygen demand (COD) (83%), chloride (52%), phenol (39%), and alkalinity (47%), from textile wastewater, Total dissolve solids (TDS) (88%), total suspended solids (TSS) (94%), sulfate (56%), phosphate (45%), total nitrogen (63%), oil and grease (42%), and total chromium (64%). The study also shows that the wastewater induces oxidative stress to the microalgae biofilm. But the microalgae biofilm can counteract the oxidative effect resulting from the exposure to the wastewater using enzymatic and non-enzymatic antioxidants. The study also shows that microalgae biofilm's ability to degraded organic pollutants largely depends on the type of antioxidant produce, while the ability to remediate inorganic pollutants is either by assimilation or adsorption. It is a well-established fact that microalgae can utilize inorganic contaminants such as nitrogen and phosphate for metabolic activities, but scanty literature exists for the mechanism of adsorption of pollutants microalgae film. It is recommended that more studies be done to clearly show the kinetic model that suits the process of pollutant adsorption by microalgae biofilm.

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