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Efficiency of Azo dyes Biodegradation by *Nostoc carneum*

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ABSTRACT

In this study, *Nostoc carneum* was taken as a model microorganism (cyanobacterium) for studying the biodegradation efficiency of azo dyes. The degradation of methyl orange (MO) and one of the commercial textile azo dyes (YA) were investigated. Decolorization efficiency (DE) increased progressively with contact time (12 days) for all concentrations of the two dyes (10, 30, 50 and 70 mg L^{-1}). The highest DE was observed with 30 mg L^{-1} for MO (69.8%) and 50 mg L^{-1} YA (84.9%). There was a progressive increment in biomass with both MO and YA dye supplementation. The maximum recorded biomass was 911.20 mg L^{-1} for 30 mg L^{-1} MO and 746.57 mg L^{-1} for50 mg L^{-1} YA. The tow azo dyes enhanced chlorophyll a and carotenoids production. 30 mg L^{-1} MO addition increased protein content while 50 mg L^{-1} YA supplementation promoted protein accumulation. *N. carneum* potentiality of producing laccase enzyme for the two azo dyes degradation was investigated. There was exponential relationship between dye concentration and laccase activity until reached maximum laccase activity (14.848 U ml^{-1}) for 70 mg L^{-1} MO and (22.592 U ml^{-1}) for 70 mg L^{-1}. According to the UV-Vis. spectral analysis profile of culture filtrates, the mechanism by which *N. carneum* could decolorize the two investigated azo dyes may be due to biosorption of dye molecules onto cell surface as well as biodegradation by laccase. It is concluded that *N. carneum* could be an efficient cyanobacterium for decolorization of dye effluents via adsorption and/or biodegradation.

Keywords: *Nostoc carneum*, Azo dyes, Methyl orange, Decolorization, Biodegradation, Laccase

INTRODUCTION

Nowadays the aquatic ecosystem worldwide is subjected to many environmental challenges as a result of the anthropogenic activities. Jin et al. [1] reported that wastewater of the textile industry represented one of the principle sources of serious pollution issues all over the world, since the textile industrial effluents received many tons of textile dyes annually which causes severe risks according to the dyes and their metabolites [2]. Azo dyes as xenobiotic pollutants are members of the aromatic heterocyclic compounds possessing azo bond (–N=N–) which compose most of the textile dyestuff discharged in many industries demonstrating their impact in stability, toxicity and carcinogenicity [3]. Consequently, different physico-chemical methods of dyes remediation from polluted wastewater were recommended such as coagulation, adsorption, photo-degradation, ozonation and flocculation which varied in decolonization efficiency as well as mechanistic capability of treatment. Though, the most important criticism of these remediation protocols is that they only transfer dyes between the partitioning phases of the remediation process without any degradation [4]. In addition, these methods are highly cost, effective in the low effluent volume and produced carcinogenic byproducts as documented by [5]. Bioremediation, the ecofriendly alternative process, is a promising method of detoxification and recycling of industrial wastewater [6]. Dyedecolonization potentiality was indicated for different microorganisms such as fungi, bacteria, algae as well as their metabolic machinery for biodegradation [7]. As reported by Jinqi and Houtian [8], biodegradation of azo dyes by microalgae are influenced by the availability of dyes to be degraded depending on dye chemical structure as well as the algal capability of dye removal in response to the physiological characteristics of the microalga. Moreover, the

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environmental conditions as temperature, pH, salinity and the oxidation reduction potential have influences as well. Cyanobacteria, the prokaryotic microalgae, have ubiquitous distribution in various habitats involving several polluted ecosystems and taking an effective role in managing industrial wastewater detoxification [9]. Concerning the ability of cyanobacteria to decolorize and mineralize textile azo dyes, Parikh and Madamwar [10] found that Gloeocapsapleurocapsoides removed 90% of FF Sky Blue dye and 83% of Acid Red 97 dye. Moreover, treatment of Amido Black 10B by *Chroococcus minutus* resulted in 55% color reduction at the end of 26 days incubation. Ali et al. [11] considered *Oscillatoria formosa* NTDM02 as effective cyanobacteria for bioremediation of textile dyes wastewater. Pourbabaei et al. [12] who studied the decolorization of MO using *Bacillus cereus*, found that the bacterial isolate could grow and remove MO after 24 h incubations with no need to use additional carbon source. Shah et al. [13] revealed the ability of *Pseudomonas* spp. to tolerate relatively high concentrations of MO (up to 500 mg L^{-1}) as well as Subasioglu et al. [14] who found that dry biomass of *Humicola fuscoatra* could remove methyl orange.

Enzymes having protein containing cupper atoms are co-factors for some metabolic pathways of fungi which incorporated in the catabolic pathways of some xenobiotic pollutants. As indicated by Kiiskinen et al. [15], laccase enzyme is a member of this cupper containing enzymes (polyphenol oxidases) represented the oxidoreductases. Where the oxidation effect of laccase resulted in oxidation of the substrate by losing an electron and the formation of a free radical mostly takes place without production of toxic aromatic amines where cross linking or depolymerisation reaction occurred. Some substrates may function as redox mediators, permitting an indirect oxidation of some substances like poly-aromatic hydrocarbons [16]. Currently, applications of laccase may be seen as the start of an environmental versatile green catalyst in bioremediation processes [4]. In the enzymatic pathway of synthetic dyes remediation, laccases are the most effective enzymes where they are commonly extracellular and catalyzes the oxidation of numerous phenolic compounds, thiols, aromatic amines through an electron acceptor (molecular oxygen)as reported by Faraco et al. [17]. These enzymes are preferred in biotechnological applications in numerous industries and bioremediation of industrial pollutants due to their low substrate specificity and their potentiality to act on chromophore group compounds.

The current study focused on studying the potential of *Nostoc carneum* to decolorize and/or degrade methyl orange (MO) as a model azo dye for investigating the mode of action of decolorization process and the enzymes that may be involved for MO metabolism. Therefore, the main objectives of this study are; to evaluate the potentiality of *N. carneum* to decolorize MO; to follow growth of *N. carneum* in presence of MO and to monitor dye degradation via assessing laccase activity.

MATERIALS AND METHODS

Biosorbent

Fresh biomass of the Cyanobacterium, *Nostoc carneum*, that grown in BG11 medium, was used as a biosorbent for studying methyl orange decolorization. Algal cells were harvested at the beginning of stationary phase (18^{th} day).

Adsorbate

Methyl orange, 4-[4-(dimethyl amino) phenyl azo] benzene sulfonic acid, is a sulfonated mono-azo dye having molecular formula $C_{14}H_{14}N_3NaO_3S$ was selected as an adsorbate (model dye). The yellow azo (YA) were one of the commercial textile dyes used in laundries. The dye stock solution was adjusted to the concentration 100 mg/L.

Dye biodegradation study

Batch scale decolorization experiments were performed using axenic cultures of the cyanobacteria *N. carneum* in glass bottles of 0.5 L capacity as experimental cultivation units. In preparation for the inoculation, *N. carneum* was grown previously in BG11 medium (pH 7) for 14 days under static-incubation condition and continuous illumination (3000 Lux) at 28 ± 2°C. The total volume of BG11 medium used was 240 ml and was inoculated with 10 ml of *N. carneum* culture (in the early stationary growth phase) and the following concentrations of MO and YA dye were supplemented (10, 30, 50 and 70 mg L^{-1}). Control treatment contained only growth medium and dye. This control was used to ensure the photo-stability of dye solutions and/or no adsorption occurred in the cultures vessels. Another control was used contained only growth medium and inoculum to identify the changes in growth behavior throughout the experiment [18]. A known volume of algal culture was filtered using Millipore filter paper; then dried in an oven at about 70°C till a constant weight was obtained.

Aliquots of cell free culture filtrate were taken at time intervals of 2 days under sterile conditions for decolorization assay. The samples were centrifuged at 4000 rpm for 10 min residual dye concentration was calculated following a calibration curve using Unico UV-2000 Spectrophotometer. The absorbance was measured at the maximum wavelength of both MO dye (λ_{max}=470 nm) and YA dye (λ_{max}=362 nm) using scan spectrophotometer Unican UV/Vis **(Figure 1)**. The samples were discarded with time course intervals every two days up to 12 days. Dye biodegradation was evaluated spectrophotometry (Unican UV/Vis, England) and the decolorization efficiency was calculated as previously explained in **Eq. 1**. UV-scan profile was performed for culture filtrates before and after biodegradation within 12 days incubation.

$$DE=\frac{Ci-Ce}{Ce} \times 100 \quad (1)$$

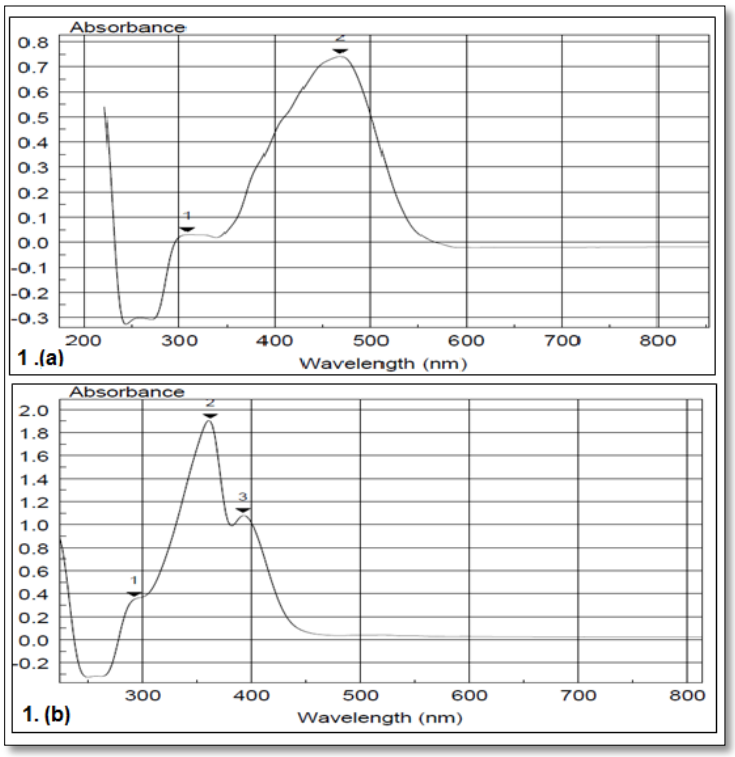

Figure 1. UV-vis absorption spectral profile of (a) MO and (b) YA.

Determination of photosynthetic pigments

A known volume of *N. carneum* culture was centrifuged at 4000 rpm for 10 min; algal pellet was extracted with acetone. Chlorophyll a and carotenoids were estimated according to Metzner et al. [19], using UNICO UV-2000 spectrophotometer at wavelengths 470, 645 and 662 nm. Pigments were calculated according to the following equations:

Chlorophyll a=11.75 λ_{662}-2.350 λ_{645}

Chlorophyll b=18.6 λ_{645}-3.960 λ_{662}

Carotenoids=(1000 λ_{470}-2.270 chl.a – 81.4 chl.b)/227

A known volume of *N. carneum* culture was centrifuged at 4000 rpm for 10 min, 5 ml buffer pH=6.7 were added to the algal pellet, extraction was performed using ultrasonication (Sinacator: Col-Pormer instrument Co Chicago, Illinois 60648, USA), finally the extract was centrifuged to get rid of algal depresses. Phycobilli proteins were calculated according to the following equations:

Phycocyanin (PC)=λ_{615} – (0.474 × λ_{652}/5.34)

Allophytocyanin (APC)=λ_{652} – (0.208 × λ_{615}/5.09)

Phycoerithrin (PE)=λ_{562} – (0.241 × PC) – (0.849 × APC) ÷ 9.62

Determination of protein content

Protein content was estimated according to Lowry et al. [20] using crystalline bovine serum albumin as standard. Culture aliquot of 50 ml was centrifuged and the biomass pellet was extracted, using phosphate buffer (pH 7). Protein is estimated using phenol Folin reagent.

Determination of laccase activity

Fresh *N. carneum* biomass was collected by centrifugation of certain aliquot of algal culture at 4000 rpm for 15 min. Laccase crude preparation was extracted using 0.2 mM sodium phosphate buffer pH 7.0. To estimate laccase activity, guaiacol used as a substrate, the following assay procedures were achieved. 2 ml of 100 mM acetate buffer (pH 4.5), 1 ml, 50 mM sodium malonate, in addition to 1 ml of 10 mM guaiacol and the reaction was initiated by supplying 1 ml enzyme preparation then incubated at room temperature for 10 min to ensure the developing of brown color. The brown color development was attributed to the oxidative polymerization of substrate (guaiacol) which verifying the presence of laccase. The phenolic product formation was determined by measuring the absorbance at 530 nm with extension coefficient (ε=6740 $M^{-1}cm^{-1}$) as documented by Jhadav et al. [21]. The absorbance for the negative control was estimated at 470 nm. The enzyme activity was calculated as U ml^{-1} which represented the

amount of enzyme needed to produce 1 μM product min^{-1} at 30°C.

RESULTS

UV-vis spectral analysis of MO and YA decolorization by *N. carneum*

The present data indicated that *N. carneum* had the ability to decolorize the two azo dyes in BG11 medium. Data **(Figures 2 and 3)** indicated that the decolorization efficiency (DE) increased progressively with contact time for all concentrations of the two dyes. The highest DE was noticed with 30 mg L^{-1} MO (69.8%) and 50 mg L^{-1} YA (84.9%).The decolorization process was verified by UV-scan spectroscopy presented in **Figures 4 and 5** for MO and **Figure 5** for YA. Generally, λ_{max} of all concentrations exhibit small move in the direction of lower wavelength **(Table 1)**. In case of MO (10 mg L^{-1}), the spectral beak at 306 nm (A=0.083 nm) disappeared. For 30 mg L^{-1} MO, the two beaks that could be concerned at λ_{294} nm (A=0.556 nm) and λ_{368} nm (A=0.455 nm), disappeared. With respect to 50 mg L^{-1} MO supplementation, two spectral beaks were recognized at λ_{292} nm (A=0.0854 nm) and at λ_{368} nm (A=0.065 nm) disappeared. In case of 70 mg L^{-1} MO addition, there were two beaks detected at λ_{292} nm (A=0.099 nm) was shifted to λ_{298} nm (A=0.062 nm) and the other was detected at λ_{366} nm (A=0.073 nm) appeared with reduced absorbance (A=0.018 nm) concerning YA, a comparable behavior was attained where beaks (λ_{312} nm with A=0.095 nm as well as λ_{390} nm with A=0.261 nm) were disappeared in case of 10 mg L^{-1}. The same pattern of response, in case of 30 mg L^{-1} YA addition, the two beaks λ_{294} nm (A=0.286 nm) and λ_{400} nm (A=0.645 nm) were dissipated addition. The same trend was repeated in case of 50 mg L^{-1} YA supplementation where beaks at λ_{290} nm (A=0.412 nm) as well as λ_{406} nm (A=0.650 nm) could not be detected. Finally, 70 mg L^{-1} YA addition, spectral beak at λ_{292} nm (A=0.693 nm) exhibited decreased absorption (A=0.224 nm) with shifting in wavelength to λ_{296} nm. **Photos 1a and 1b** indicated deep colored biomass in cultures of either MO or YA in all performed concentrations.

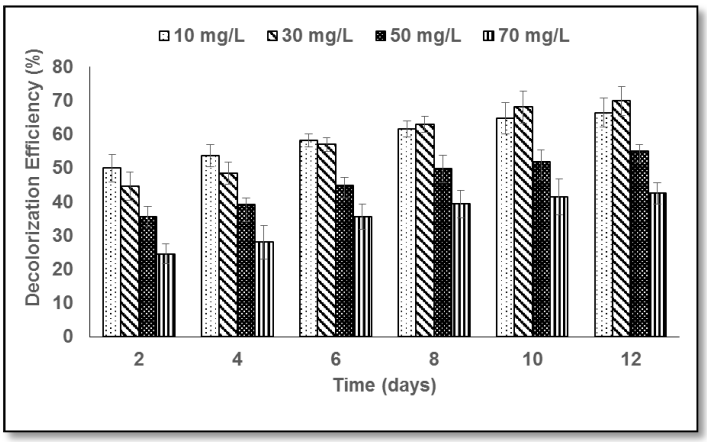

Figure 1. Effect of contact time on MO decolorization efficiency of different initial concentrations (10, 30, 50 and 70 mg L^{-1}) by *N. carneum* fresh biomass.
Values are means (n=3), error bars indicate standard deviation

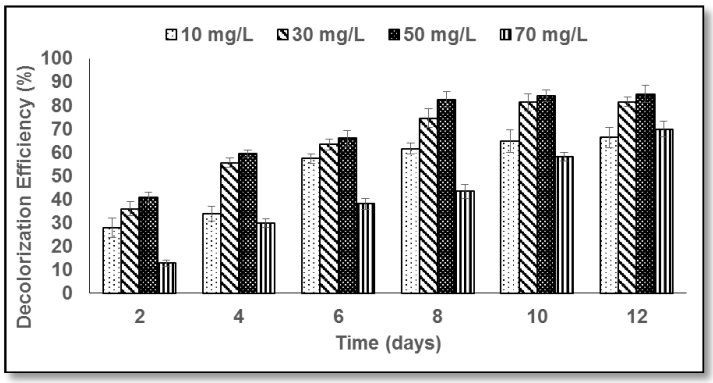

Figure 2. Effect of contact time on YA decolorization efficiency of different initial concentrations (10, 30, 50 and 70 mg L^{-1}) by *N. carneum* fresh biomass.
Values are means (n=3), error bars indicate standard deviation

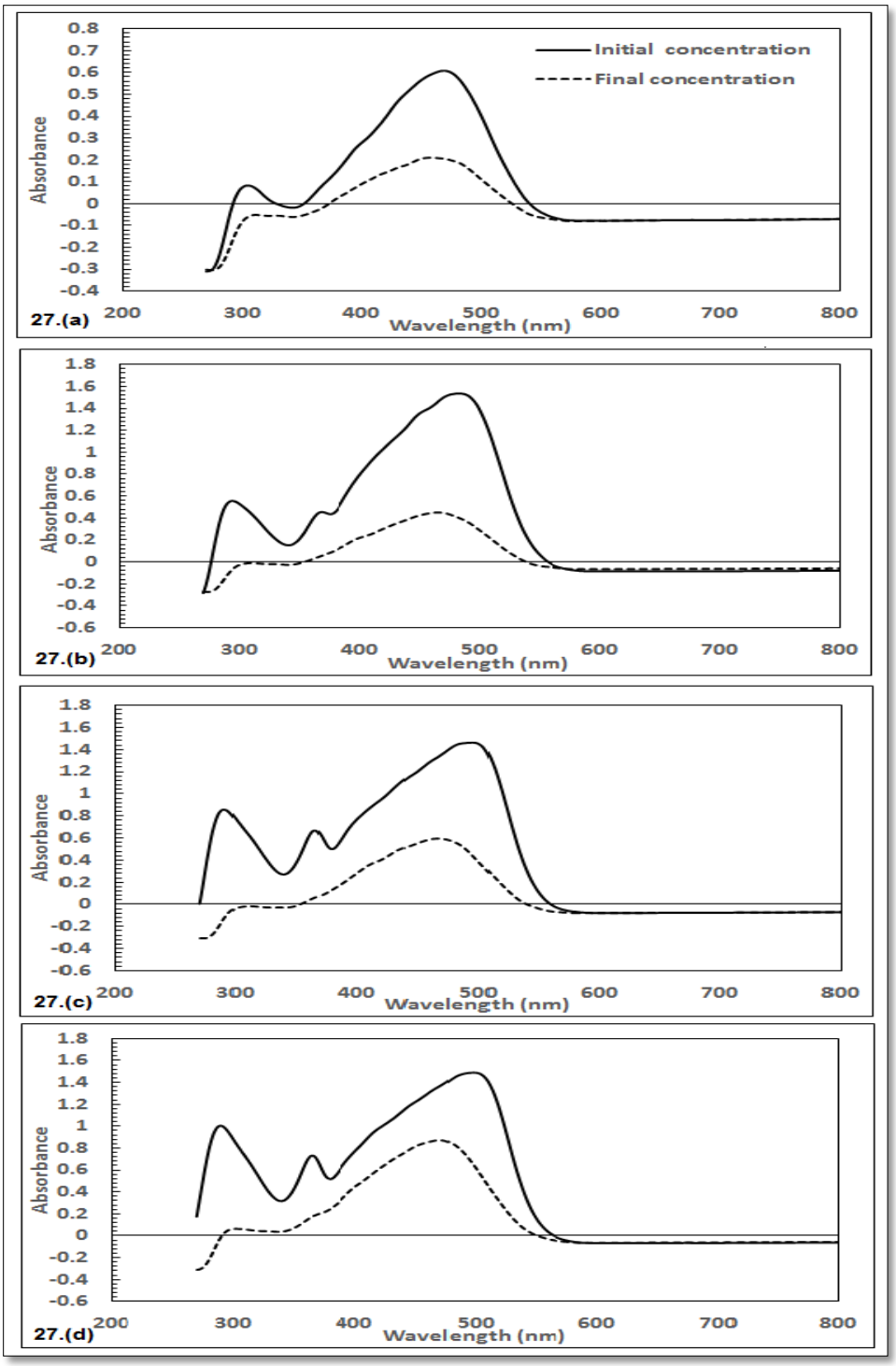

Figure 3. UV-visible spectral profile of culture filtrates before and after biodegradation within 12 days incubation of MO dye concentrations (a) 10 mg L^{-1} (b) 30 mg L^{-1} (c) 50 mg L^{-1} (d) 70 mg L^{-1}. Data of these curves represent the mean of three independent measurements.

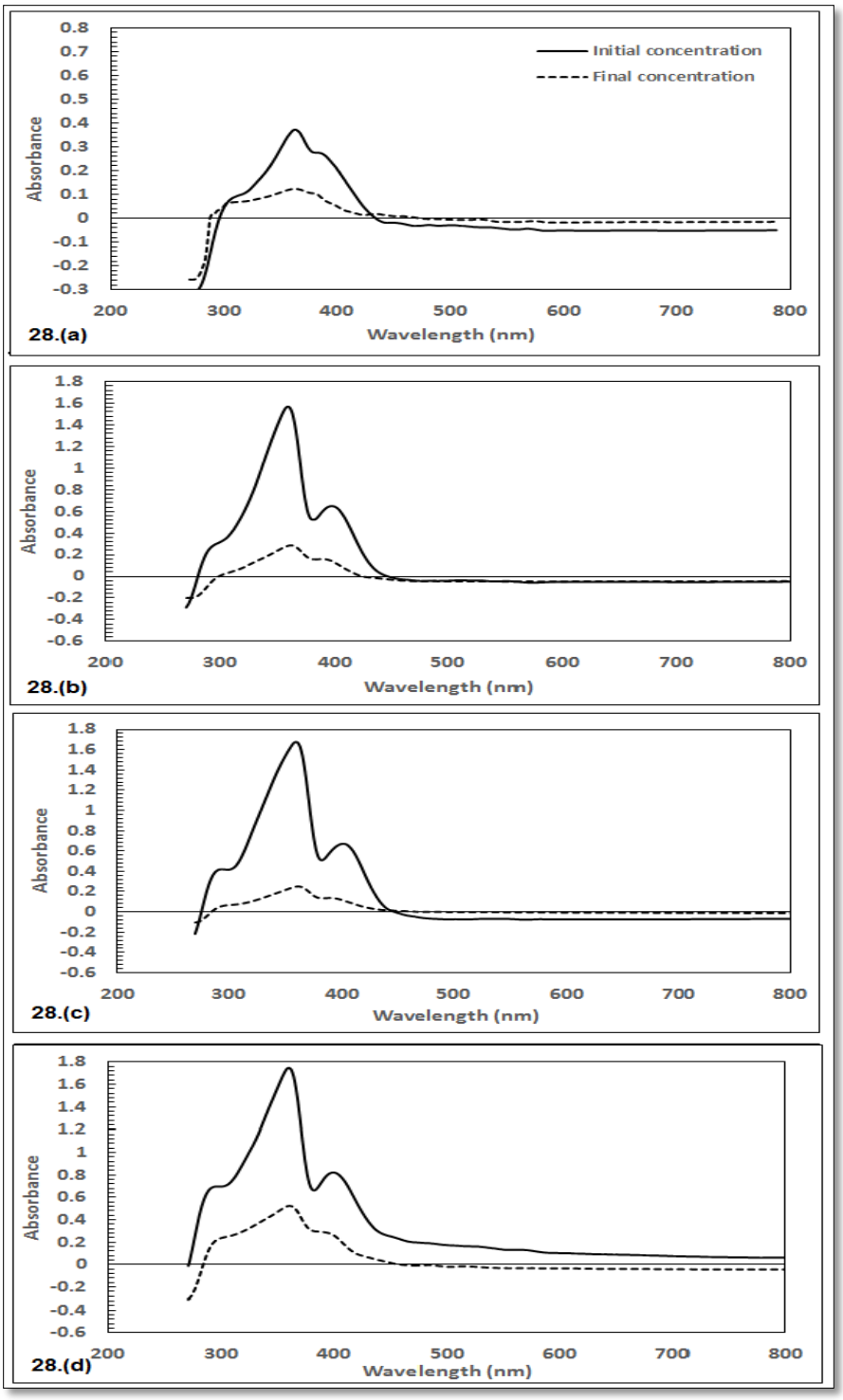

Figure 4. UV-visible spectral profile of culture filtrates before and after biodegradation within 12 days incubation of YA dye concentrations (a) 10 mg L^{-1} (b) 30 mg L^{-1} (c) 50 mg L^{-1} (d) 70 mg L^{-1}. Data of these curves represent the mean of three independent measurements.

Table 1. UV-visible absorption spectra values of culture filtrates before and after biodegradation within 12 days incubation of MO dye concentrations (10, 30, 50 and 70 mg L^{-1}).

Dyes	Dye concentrations (mg L^{-1})	λ_{max} (nm)		Absorbance (nm)	
		Before	After	Before	After
MO	10	470	458	0.680	0.209
	30	484	466	1.535	0.455
	50	498	468	1.461	0.599
	70	498	472	1.489	0.631
YA	10	362	356	0.372	0.118
	30	360	362	1.571	0.286
	50	360	361	1.675	0.252
	70	360	359	1.749	0.523

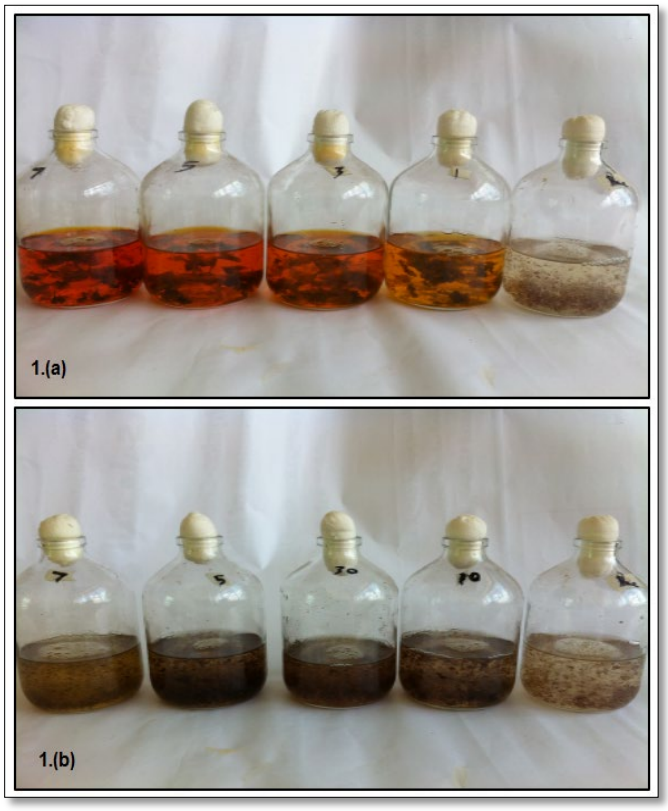

Photo 1. Cultures after 12 days incubation in BG11 supplemented with different concentrations (10, 30, 50 and 70 mg L-1) of (a) MO and (b) YA.

Physiological study

Stimulatory growth pattern was illustrated in **Figures 6 and 7** showing a progressive increments in biomass with both MO and YA dye supplementation throughout the experimental period. With respect to MO supplementation the following descending order was attained 30>10>50>70 mg L^{-1}, while the following descending order was allowed to YA addition 50>30>10>70 mg L^{-1}. The maximum biomass was recorded to 30 mg L^{-1} for MO (911.20 mg L^{-1} dry tissue) and 50 mg L^{-1} YA (746.57 mg L^{-1} dry tissue).

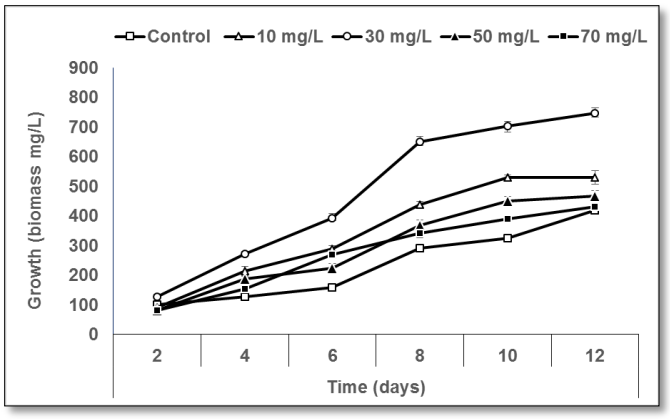

Figure 5. Effect of different MO concentrations on the growth of *N. carneum* measured as biomass (mg L^{-1}). Values are means (n=3), error bars indicate standard deviation.

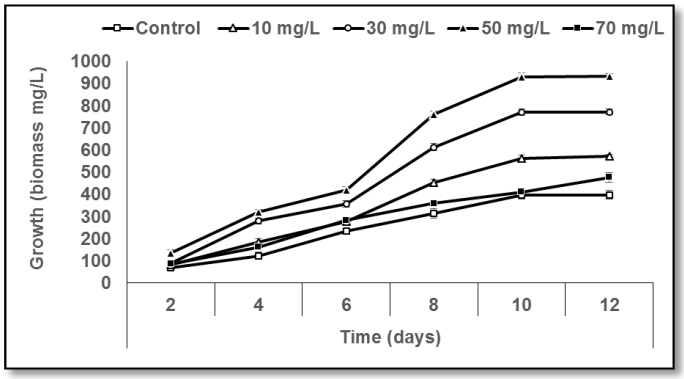

Figure 6. Effect of different YA concentrations on the growth of *N. carneum* measured as dry biomass (mg L^{-1}). Values are means (n=3), error bars indicate standard deviation.

Chlorophyll a and carotenoids are significant parameters for growth assessment which increased progressively throughout time course of experiment. The present results showed significant increments in these parameters with all dye supplementations as well as total photosynthetic pigments which gradually increased throughout the incubation period. MO induced maximum chlorophyll a content at the 10th day (0.0328 mg g^{-1} dry biomass) for 30 mg L^{-1} addition, while carotenoids content reached its maximum value at 12th day (6.6972 mg g^{-1} dry biomass) for 70 mg L^{-1} as indicated in **Figures 8 and 9**. Data presented in **Figures 10 and 11** indicated that chlorophyll a and carotenoids contents as affected by YA supplementations, exhibited the same pattern of response with maximum chlorophyll a content at the 10th day (0.0382 mg g^{-1} dry biomass) for 50 mg L^{-1} addition and carotenoids content reached its maximum value at 12th day (6.7142 mg g^{-1} dry biomass) for 70 mg L^{-1} supplementation.

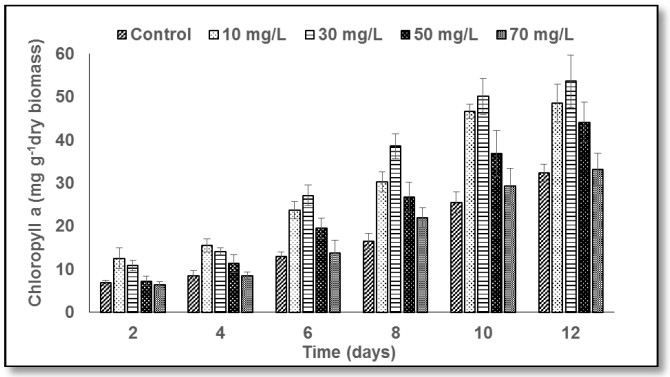

Figure 7. Effect of different MO concentrations on chlorophyll a content of *N. carneum* measured as mg g^{-1} dry biomass. Values are means (n=3), error bars indicate standard deviation.

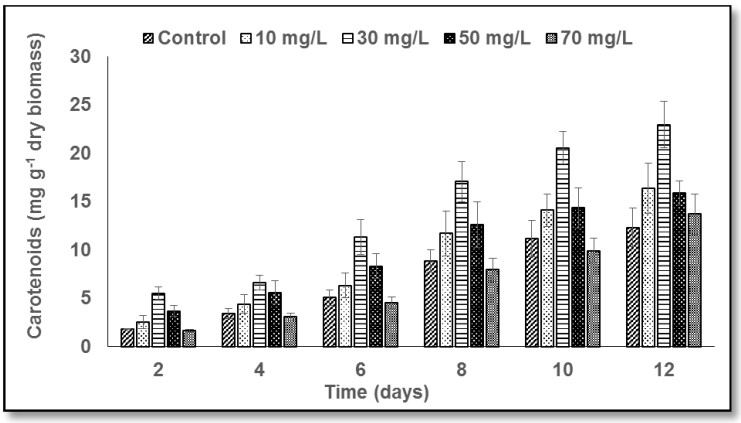

Figure 8. Effect of different MO concentrations of on the carotenoids content of *N. carneum* measured as mg g^{-1} dry biomass. Values are means (n=3), error bars indicate standard deviation.

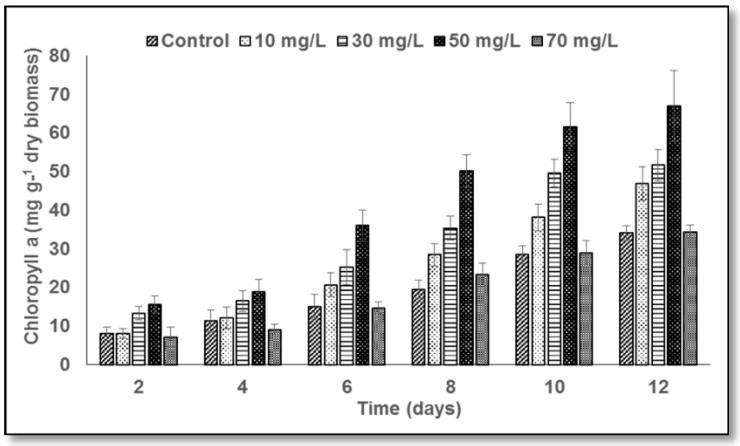

Figure 9. Effect of different YA concentrations on the chlorophyll a content of *N. carneum* measured as mg g^{-1} dry biomass. Values are means (n=3), error bars indicate standard deviation.

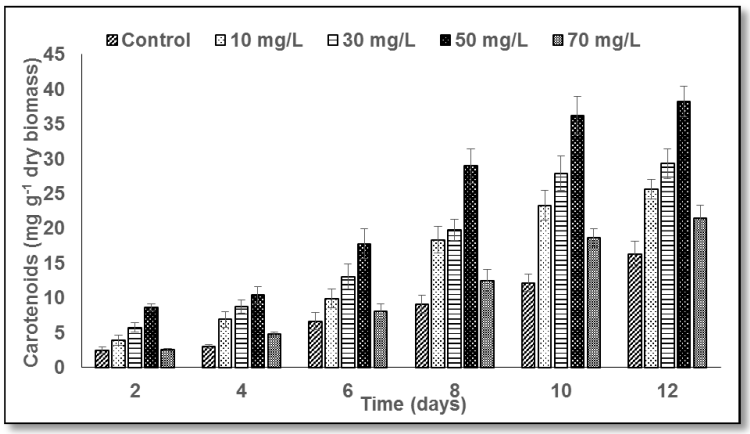

Figure 10. Effect of different YA concentrations on carotenoids content of *N. carneum* measured as mg g^{-1} dry biomass. Values are means (n=3), error bars indicate standard deviation.

In the current study, 30 mg L^{-1} MO addition induced the highest content of PC, APC and PE during the experimental period where the magnitude of response was proportional to MO concentrations giving the highest value at the end of the incubation period (29.09, 21.95 and 137.01 mg g^{-1} dry biomass, respectively) as illustrated in **Figures 12-17**. The same pattern of response was recorded, with respect to the effect of YA on PC, APC and PE contents, 35.91, 29.83 and 140.50 mg g^{-1} dry biomass as the maximum values of phycobilli proteins, respectively **(Figures 14 and 16)**. In

general, PE occupied the first level within the phycobilli YA. protein pigments in all treated cultures with either MO or

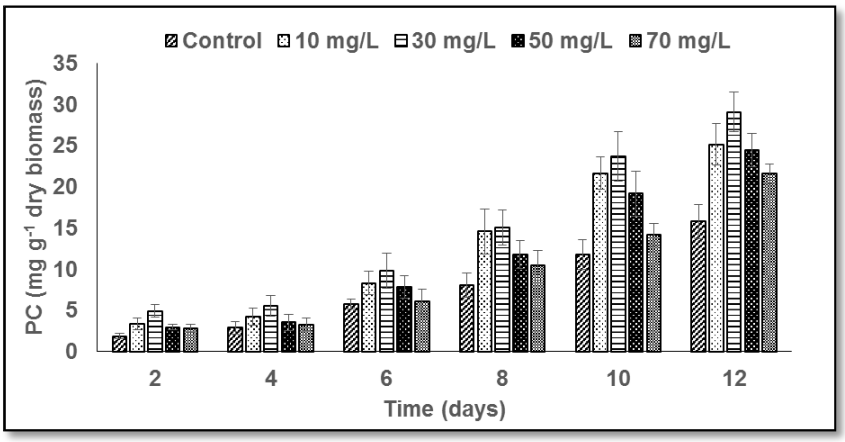

Figure 11. Effect of different MO concentration sonphycocyanin (PC) content of *N. carneum* (mg g^{-1} dry biomass). *Values are means (n=3), error bars indicate standard deviation.*

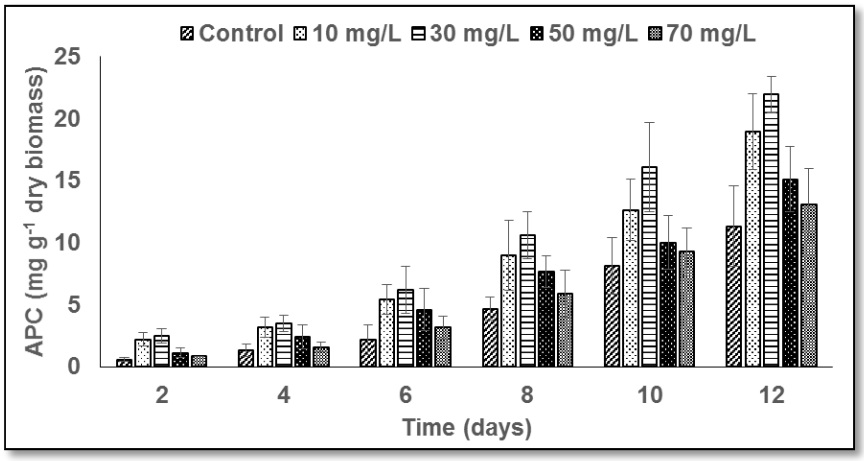

Figure 12. Effect of different MO concentrations of on allophycocyanin (APC) content of *N. carneum* (mg g^{-1} dry biomass). *Values are means (n=3), error bars indicate standard deviation.*

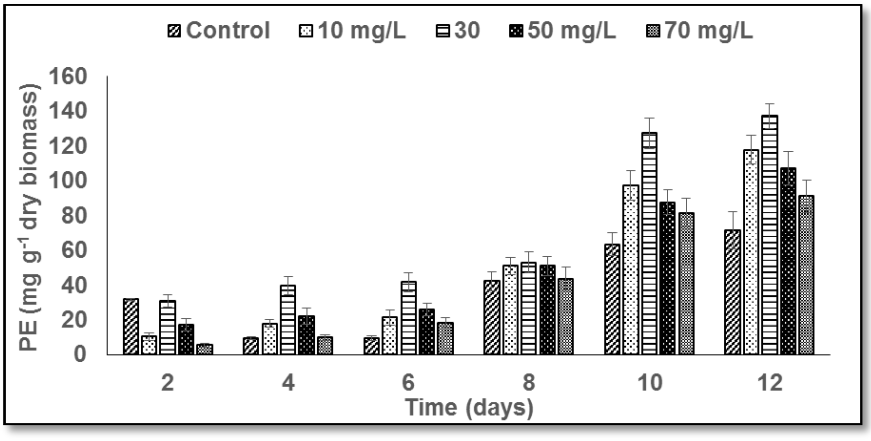

Figure 13. Effect of different MO concentrations on phycoerythrin (PE) content of *N. carneum* (mg g^{-1} dry biomass). *Values are means (n=3), error bars indicate standard deviation.*

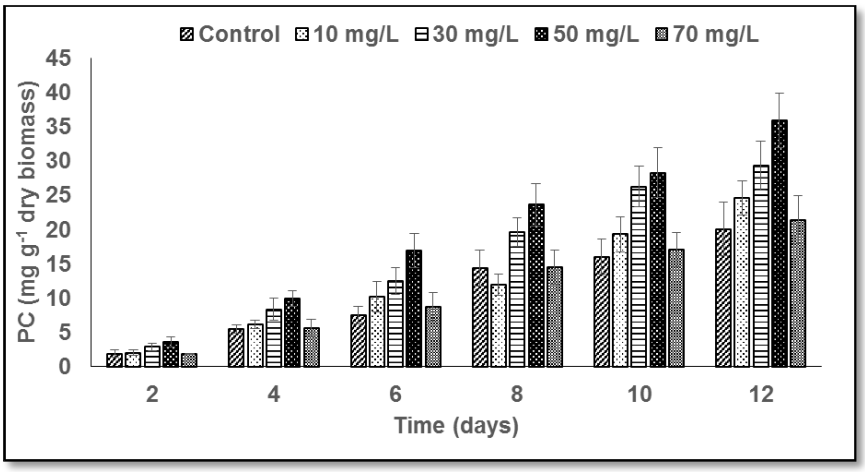

Figure 14. Effect of different YA concentrations on the phycocyanin (PC) content of *N. carneum* (mg g^{-1} dry biomass). *Values are means (n=3), error bars indicate standard deviation.*

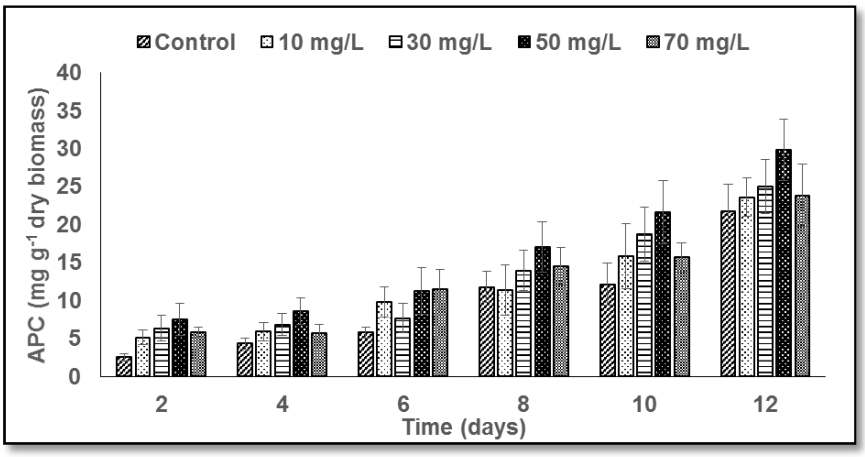

Figure 15. Effect of different YA concentrations on the allophycocyanin (APC) content of *N. carneum* (mg g^{-1} dry biomass). *Values are means (n=3), error bars indicate standard deviation.*

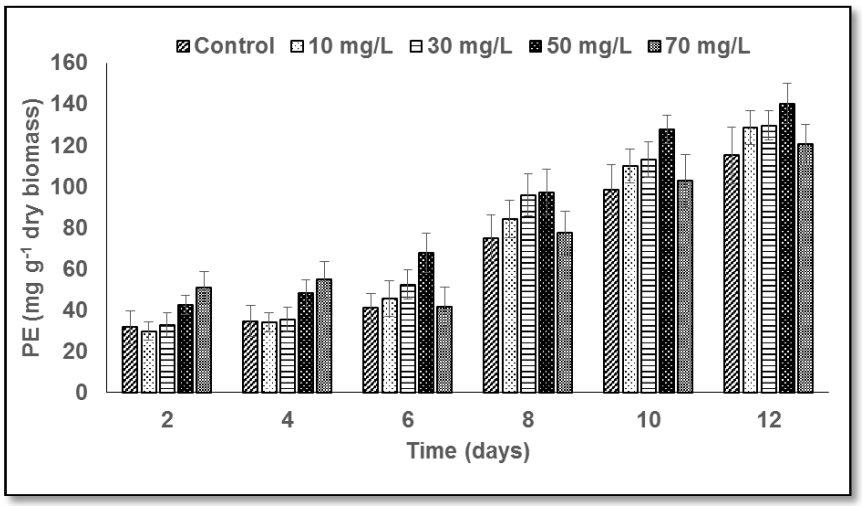

Figure 16. Effect of different YA concentrations on the phycoerithrin (PE) content of *N. carneum* (mg g^{-1} dry biomass). *Values are means (n=3), error bars indicate standard deviation.*

Figure 18 illustrated that 30 mg L^{-1} MO supplementation enhanced the protein production corresponding to 306.248 mg L^{-1} while 50 mg L^{-1} YA supplementation increased protein content reaching 368.710 mg L^{-1} as the maximum value.

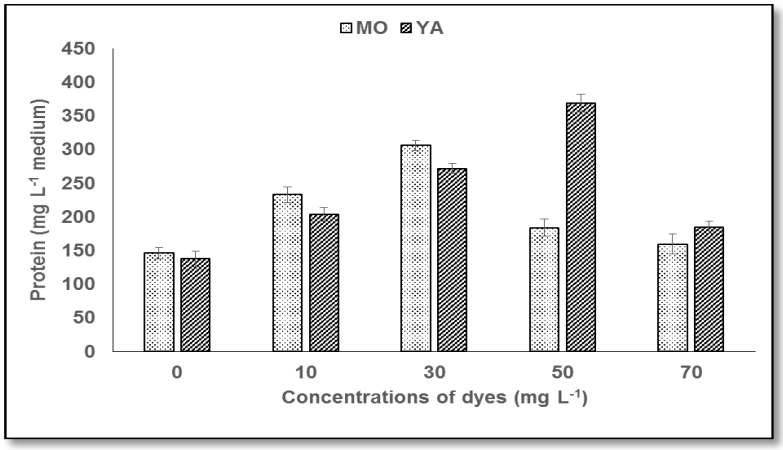

Figure 17. Effect of MO and YA concentrations on protein content (mg L^{-1}) of *N. carneum* after 12 days incubation. *Values are means (n=3), error bars indicate standard deviation.*

The obtained data **(Figure 19)** demonstrated that laccase activity exhibited higher activity in case of YA supplementation than MO at the beginning of stationary phase (12^{th} day). It was shown that laccase activity was in accordance with *N. carneum* growth (biomass production) which interpreted the decolorization pattern of MO and YA (**Figures 3 and 4**, respectively) on basis of dyes oxidation which illustrated an exponential relationship between dye concentration and laccase activity until reached maximum laccase activity (14.85 U ml^{-1}) for 70 mg L^{-1} MO and (22.59 U ml^{-1}) for 70 mg L^{-1} YA

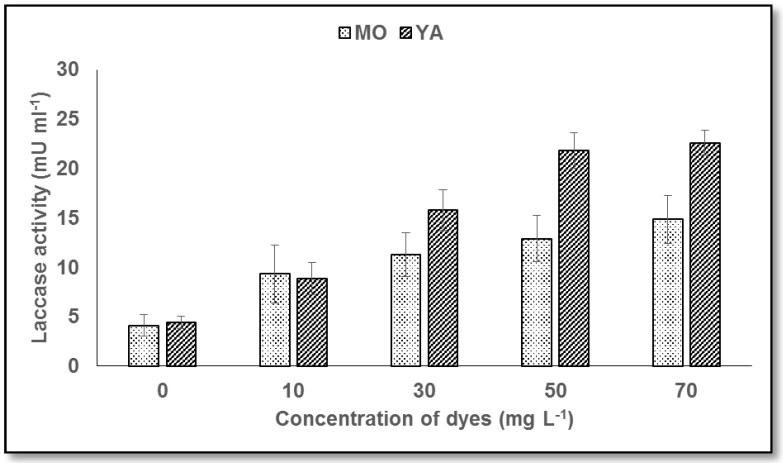

Figure 18. Effect of MO and YA concentrations on the laccase activity (mU/ml) of *N. carneum*12 days incubation. *Values are means (n=3), error bars indicate standard deviation.*

DISCUSSION

Bioremediation activity of algae varied with different dyes and this may be interpreted according to adsorption or/and biodegradation by algae. Therefore, Acuner and Dilek [22] recommended that dye characters such as chemical formula, electric charge as well as adaptation period were significant factors affecting dye bioremediation. The current study exhibit cyanobacteria-induced decoloration and degradation of MO and YA dyes by *N. carneum*. Some azo dyes (methyl red, orange II and G-Red) represented carbon source for some algae as reported by Kulla et al. [23]. Algae could reduce azo dyes by cleavage the azo bridges by the effect of azo reductase where aromatic amine formed as resulted metabolite. Bacteria had a similar mechanism in degrading azo dyes resulted in formation of aromatic amine [24]. According to Legerska et al. [25] suggestion, the metabolites formation and the shifting of recorded λ_{max} of UV-visible scan could be pointed to dye degradation. Decreases in absorbance might be due to the broken down of the dye chromophores ($-NO_2$, $-N=N-$, $-NH_2$) and merged aromatic rings with formation of UV absorbing intermediates as

indicated by Parikh and Madamwar [10]. Likewise Kulla et al. [23] reported that Pseudomonas strain could fully metabolize some C14-labeled aromatic amines to carbon dioxide, in which many of these compounds could be utilized by some algae as documented by Wang et al. [26]. According to Shah et al. [27], decolorization of azo dye solution using Pseudomonas spp. ETL-M might be attributed to either adsorption onto bacterial cell wall or to biodegradation. In accordance of the present results, Pourbabaei et al. [28] documented that MO was decolorized by *Bacillus cereus* within 2 days of incubation in aerobic cultures. These findings are in agreement with the current data which demonstrated that *N. carneum* could degrade 69.8% of MO and 84.9% of YA. There were disappearance of some spectral beaks as well as darkening in biomass in case of the two used dyes; this indicated that the resulted dye removal could be attributed to biosorption activity and/or biodegradation. The present data indicated the tolerance of *N. carneum* to high concentration (70 mg L^{-1}) of both dyes. The mechanism by which *N. carneum* could decolorize the two investigated azo dyes might be due to biosorption of dye molecules onto cell surface and biodegradation as suggested by Ayed et al. [29]. Dellamatrice et al. [30] studied the degradation of the following azo dyes; Indigo, Remazol Brilliant Blue R and Sulphur Black using *Anabeanaflos aquae* UTCC64, *Phormidium autumnale* UTEX1580 and *Synechococcus* sp. PCC7942 as biosorbents. They found that the investigated cyanobacteria exhibited color bioremoval potentiality and biodegradation activity as well as they concluded that the degree of either decolorization or dye degradation was influenced by both the cyanobacterial species used and dye structure. El-Sheekh et al. [31] studied the capability of *Pseudoanabaena* sp. and *Microcystis aeruginosa* for biodegradation of the following dyes; Disp. orange (2RL), Reactive yellow (3RN), Reactive Black (NN) and Tracid Red (BS) and found that the biodegradation activity were reliant to the kind of dyes and the species of algae used. Moreover, De Philippis et al. [32] documented that large number of cyanobacteria had an outer membrane with supplementary outer substance such as polysaccharide that were capable of pollutant elimination from aquatic system. The ability of microorganisms to remove dyes might be in cause of adsorption onto algal biomass or algal biodegradation [33]. Decolorization of azo dye might be due to partial reduction or complete breaking down the azo bond as noticed by Chang et al. [34].

As supplementing *N. carneum* culture with different concentrations of the investigated azo dyes, the intracellular nutrients could be transported across the cell membrane which forcing cells to metabolize the molecules of azo dyes as external nutrient sources earlier in order to initiate both decolorization as well as algal biomass production. Moreover, on the basis of the passive diffusion and the influence of higher concentration gradient as a driving forces from transporting azo dyes molecules from the exterior of bacterial cells to their interior across plasma membrane. Mohanty et al. [35] interpreted the enhancement of MO decolorization as well as *Pseudomonase luteola* growth in the MO supplemented cultures. Owing to the spontaneous passive diffusion, relatively higher azo dye concentrations were more preferred to penetrate to cell via the plasma membrane. El-Sheekh et al. [24] who studied the effect of different dyes concentrations (methyl red, orange II, G-Red (FN-3G), basic cationic and basic fuchsin) on growth of microalgae (*Chlorella vulgaris*, *Lyngbya lagerlerimi*, *Nostoc lincki*, *Oscillatoria rubescens*, *Elkatothrix viridis* and *Volvox aureus*). They found significant decreases in dry weight production of the tested algae as compared with control by increasing incubation time. Whereas, El-Sheekh et al. [31] found that the growth of *Microcystis aeruginosa* and *Pseudoanabaena* sp. was decreased as a result of the effect of different dyes supplementations compared to control where, there was little progressive increase in growth during incubation period that was in accordance with dyes degradation. In the same context, Yadav et al. [36] demonstrated that the indigo dye exhibited a negative growth response as well as biomass production of Chlorella vulgaris at different concentrations where, they deduced this pattern of response to the ability of indigo to deal with microalgae by three strategies: additive, synergistic or antagonistic to affect growth as well as chemical constituent of biomass.

Mahalakshmi et al. [37] reported that either Congo red or textile dye effluent increased chlorophyll a content in Chlorella sp. with different dye concentrations (2, 5, 10, 12 and 15 ppm). On investigating tolerance of *Spirulina* spp., *Lyngbya* sp., *Phormedium* sp. and *Synechocyctis* sp. to methyl red (MR) supplementation (25-100 mg L^{-1}) for 18 days incubation period, Ansari et al. [4] found photosynthetic pigments reduction in some cultures. Moreover, presence of MR induced some increments in pigments, whereas Vajpayee et al. [38] explained the evolution of adaptive mechanism for tolerance, where there was an enhancement of photosynthetic pigment production in Spirulina – C11 cultures supplemented with MR recording high degree of adaptation. On the other hand, Ali et al. [11] studied the decolorization of Amido Black dye using marine *Oscillatoria formosa*, chlorophyll a content decreased in all experimental treatments compared to control which attributed to decline in the biosynthesis of pigments. The same pattern of response was reported by Parikh and Madamwar [10] where they found that *Gloeocapsa pleurocapsoides* and *Phormidium ceylanicum* could degrade about 80% of both Acid Red 97 and FF Sky Blue dyes after 26 days incubation. This response could attribute to the harmful impact of the dye addition of the photosynthetic apparatus (thylakoid bands) and the biosynthesis of pigments as explained by Vajpayee et al. [38].

The obtained results for phycobilli proteins were agreed with that of Mona and Kaushik [9] who studied the phycobilli

protein contents of the cyanobacterial species (*Nostoc linckia* HA-46, *Myxosarcina spectabilis* HP-43 and *Gloeocapsa calcarea* HP-45) as affected by different concentrations of reactive dye red 198. They documented that phycobilli protein pigments responded positively with the increase of dye concentration as compared to control. The biomass of algal periphyton decreased to about 40% of the initial mass after culture exposed to high concentration of crystal violet (CV), where decolorization of CV was found to be a synergistic process in combination with adsorption one ending with biodegradation mechanism resulting in non-toxic aliphatic molecules as documented by Shabbir et al. [39]. It is concluded that *N. carneum* might be try to safe its photosynthetic pigment system from the adverse effects of the studied dyes as well as to evolve of defense mechanism for tolerating the toxic effects of dyes as suggested by Stratton and Corke [40] and Vajpayee et al. [38], where dyes addition improved biomass production, chlorophyll a and phycobilli proteins as well. These results may suggest the presence of some tolerance mechanism, since the high content of phycobilli proteins in case of MO and YA *N. carneum* supplemented cultures referred to an effective photosynthesis process via effectual energy transference. Many studies demonstrated inhibitory consequences in response to dye addition on microalgal thylakoid membranes and biosynthesis of chlorophyll. Moreover, the current results indicated high level of carotenoids and billi proteins with dye addition which may reflect the capability of *N. carneum* to tolerate presence of azo dyes [38].

According to Mahalakshmi et al. [37], the protein content was not much affected by different concentrations of Congo red where increased in *Chlorella* sp., Arthrospira as well as Hematococcus with dye supplementations even more than control as detected in *Chlorella* sp. with the same response pattern. Owing to Prabhakar and Krishna [41], the aromatic amines were the principle metabolite resulted from the degradation of azo dye. The observed dark colored biomass in the present study might be attributed to the sensitivity of aromatic amines to auto oxidation resulted in the production of dimers as suggested by Kudlich et al. [42]. Increasing protein content in MR supplemented Spirulina-C11 could be referred to de novo biosynthesis of some phenol-decomposing enzymes as well as presence of some stress-associated proteins as a result of the presence of aromatic compounds [43].

According to Telke et al. [44] and Legerská et al. [45], azo dyes could be broken either symmetrically or asymmetrically in the enzymatic degradation pathway by a highly non-specific free radical mechanism producing phenolic compounds with hydroxy-, carboxy-, methoxy-, amino- or sulpho- functional groups and might be applied as laccase activity for bioremediation. Chivukula and Renganathan [46] previously proposed that laccases could only transform a limited spectrum of azo dyes, specially dyes that possess a phenolic substituent in para-position to the azo bond and further methyl- or methoxy-substituents in 2- or 2,6-position relatively to the hydroxy-group. In try to interpret the laccase function on azo dye degradation and detoxification, Telke et al. [47] suggested that azo dye degradation according to laccase action beginning with asymmetrically broken down of the azo bond, then oxidative broken down, deamination, demethylation, desulfonation and dihydroxylation depending on dye chemical formula. On the other hand, Chen [48] explained azo dyes degradation without breaking down of the azo bond and attributed the production of phenolic compound to the action of highly non-specific free radical mechanism. An explanation of the mechanism by which azo dye (MO) could be degraded by laccases produced by *Aspergillus ochraceus* NCIM-1146 was illustrated by Telke et al. [49] in **Figure 20**. Firstly, laccases degraded MO (mono azo dye) by carbocation, followed by production of an electron-deficient reaction centre and consequently, extremely reactive intermediates according to the presence of nucleophilic attack by -OH, -SO_3 or halogen nucleophiles produced in asymmetric azo bond breakdown producing p-N,N'-dimethylamine phenyldiazine and p-hydroxybenzene sulfonic acid. In spite of toxicity of these products [50], the biological degradation of MO in aqueous solution by *Aeromonas* sp. strain DH-6 possessing reduced phytotoxicty when as documented by Du et al. [51].

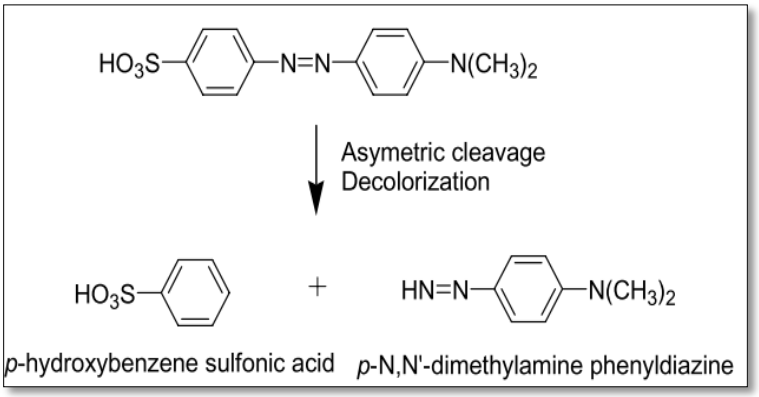

Figure 20. Methyl Orange degradation mechanism by *Aspergillus ochraceus* laccase [49].

The potential of laccase induction by different cyanoacterial species had been investigated by Ansari et al. [4] using azo dye (MR). They found an enhanced induction in laccase activity attributed to the addition of MR. Afreen and Fatma [52] reported that laccase activity of Synechocystis NCCU-370 characterized by low activity in absence of aromatic inducer giving its maximum activity (25.37 mU/ml) after 7 days of incubation. Laccase activity was investigated in many fungi and bacteria where, Patel et al. [53] found that 1 mM guaiacol addition improved laccase production in many fungi as *Phlebia* spp. and *Pleurotus ostreatus*, whereas 8 mM guaiacol had been suggested to be good laccase inducer in case of *Rhodococcus* sp. and *Enterobacter* sp. as demonstrated by Mongkolthanaruk et al. [54]. Laccase activity exhibited increment with increasing growth (biomass) that recommended the necessity of appropriate growth for laccase induction, since reduction in laccase activity and/or growth of *Spirulina plantesis* might be according to nutrients depletion as a consequent result of aging where the maximum laccase activity was recorded on the 10th day of incubation as indicated by Afreen et al. [55].

It is concluded that *N. carneum* exhibited decolorization activity via degradation mechanism rather than adsorption one according to its adaptive nature as well as degrading ability of azo dyes contaminants in addition to its tolerance and decolorizing potentiality to relatively high concentration of MO which recommended this cyanobacterium for bioremediation process of textile wastewater.

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