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ORIGINAL PAPER

Decolorization of dyestuffs by some species of green algae and cyanobacteria and its consortium

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Abstract

Synthetic dyes are scattered in untreated or inappropriately treated effluents, and their dangerous items created during the halfway corruption are released into the water bodies that cause a horrendous smell, which prompts anomalous changes in the nature of water. In the present study, green algae and cyanobacteria are considered as a significant hotspot for decolorizing color and material gushing. *Chlorococcum* sp., *Scenedesmus obliquus*, *Chlorella vulgaris,* and *Oscillatoria* sp. were investigated for degradation and removal of some azo dyes [Reactive Orange 122 (Orange 2RL) and Reactive Red 194 (Reactive Red M-2BF)]. The results showed that the maximum decolorization was spotted at 20 ppm Reactive Orange 122 with *Oscillatoria* sp. mixed with *S. obliquus* (98.54%). 20 ppm Reactive Red 194 was decolorized by *Oscillatoria* sp. mixed with *S. obliquus* (97.58%) after 7 days of incubation. The decolorization was detected by spectroscopic analysis and Fourier transformed infrared (FTIR) spectroscopy. The suitable factors that accelerated the azo dye decolorization and enhanced the biological treatment methods to be more effective and speedier in decolorization were investigated. At 25 °C and continuous lighting, the highest percentage of the azo dye decolorization was obtained; BG11 was the suitable medium that gives a high percentage of the azo dye decolorization. However, relative to the effect pH on azo dye decolorization, results show pH 11 and pH 9 more effective on azo dye decolorization for Reactive Orange 122 and Reactive Red 194, respectively. A total of 6% of thiamine and ascorbic acid recorded maximum degradation activity at Reactive Orange 122 when treated with *Oscillatoria* sp. mixed with *S. obliquus* 79.13% and 77.18%, respectively.

Keywords Biodegradation · Decolorization · Reactive azo dyes · Green algae · Cyanophyta · Spectroscopic analysis · FTIR spectroscopy

Introduction

Dyes are classes of natural poisons in which separate items are exceptionally dangerous and mutagenic to living life forms. Azo dyes are xenobiotic natural mixes, which cannot be effectively debased through the chemical, light or even by the offensive of microbial (Zeenat et al. 2014); furthermore, the utilization of microbial or enzymatic treatment technique for the total decolorization and debasement of such colors from material gushing has the accompanying points of interest: (1) being naturally familiar (2) the existence cost-serious, (3) yielding finished results that are non-poisonous or have total mineralization; and (4) requiring less water utilization contrasted with physicochemical methods (Rahman et al. 2019; Indumathy and Kannan 2020; Abd Ellatif et al. 2020). Electrocoagulation, adsorption, ion exchange, irradiation, ultrasound, and membrane filtration were effectively used to remove the dyes from wastewaters (Xu et al. 2007), different types of chemical treatment methods have also been in practice to remove dyes with techniques such as ozone, sodium hypochlorite, oxidative active metals, photochemical treatment, and electrochemical destruction (Cao et al. 2019). Adsorption on microbial biomass (bio-absorption) or biodegradation of dyes by living cells is two methods that are used as biological methods for decolorization and decomposition of azo dyes (Varjani and Upasani 2019). The most effective mechanisms for algal utilization to decolorization

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of azo dyes were production of algal biomass by assimilation, production of carbon dioxide and H_2O while converting color to an uncolored molecule, and absorption of chromophores by algal biomass. Mechanisms of algal decolorization can involve enzymatic degradation, adsorption, or both. Synthetic dyes are broadly utilized in the material industry due to their vast size, which brings about a high proclivity to tie with cellulosic fiber. Reactive azo dyes are exceptionally hardheaded to traditional strategies (natural techniques) utilized in remediation of wastewater in view of the nearness of solid electron-pulling back gatherings that allow them strength against bacterial decolorization (Lucas et al. 2007; Gregorio et al. 2010). Losing of dye in manufacture implementation based on the type of dye used ranges from 2% loss of basic pigments to the loss of 50% for some dyes and interactive sulfonated when used with cellulosic fabrics because of the relatively low levels to install dye fibers (Shore and Shore 1995; McMullan et al. 2001; Pearce et al. 2003; Hai et al. 2007). Connections among colors and biosorbent rely upon the idea of color, explicit the feature surface of biomass, and natural conditions (e.g., pH, azo dye concentration). Degradation of reactive dyes is extremely troublesome on the grounds that they are intended to balance out and to oppose blurring against perspiring, light, water, and oxidizing agents. Accordingly, plucking out of reactive dyes from wastewaters has been a fundamental objective in numerous looks into over the most recent couple of years, because of their poisonous quality, yet in addition on account of its visibility (Radwan et al. 2020). The dry weight of bacteria and algae has been utilized effectively to expel dangerous colors by biosorption (Bhatnagar and Sillanpaa 2010). This feature of microorganism is because of the cell wall ingredients such as hetero-polysaccharides and lipids, that consists of many active groups including, amino, hydroxyl, carboxyl, phosphate, and other charged groups, making solid, appealing powers between the synthetic dye and cell wall (Srinivasan and Viraraghavan 2010; Das and Charumathi 2012). Microorganisms have the ability to playing the main role in taking off azo dyes and aromatic amines in stabilization blessing (Banat et al. 1996). A few microorganisms are recognized to metabolize/transform naphthalene, phenanthrene, anthracene, (BaP) benzo[a]pyrene, and other (PAHs) polycyclic aromatic hydrocarbons (El-Sheekh et al. 2012). Different microbes can be used for the decomposition of many types of dyes as they have many pathways and mechanisms for the degradation of dyes (Cao et al. 2019; Ebrahimi et al. 2019). Azo dyes are a useful class of dyes with the highest diversity of colors. Under anaerobic conditions and with the help of azo reductase, microorganisms degrade azo dyes, and as final product, they form colorless aromatic amines (Ali 2010; Ajaz et al. 2020; Dong et al. 2019).

As opposed to some species of microorganism, which rely upon such sources (Omar 2008), Algae get vitality from daylight and carbon from the air, and some rummage atmospheric nitrogen in this manner, the mass development of algae is more affordable (Saha et al. 2010). Algae use three unique inherent systems for removing of dyestuffs, including the use of chromophore for production of algal biomass via assimilation, creation of CO_2, and H_2O during the change of color to noncolor molecule and chromophore adsorption by algal biomass.

The research aims to investigate the ability of some species of green algae and cyanobacteria to purify water that has contaminants from reactive dyes through studying the effect of physicochemical conditions on decolorization and degradation of azo dyes. It also aims to study the effect of the consortium of organisms on the degradation efficiency of azo dyes.

Materials and methods

Organisms and growth conditions

Chlorococcum sp., *Scenedesmus obliquus*, *Chlorella vulgaris,* and *Oscillatoria* sp. were isolated from the Damietta Nile branch in January 2011. Microorganisms in axenic cultures are based on serial dilution culture techniques and agar plate methods, as described by (Jhala et al. 2017). Studied species were cultivated in 250 ml Erlenmeyer flasks containing 100 ml Kuhl medium (Kuhl 1962), and all glassware were kept at room temperature (25 ± 1 °C) under natural daylight.

Dyestuffs

The structures of the dyestuffs used are given in Fig. 1. Reactive Orange 122 (Orange 2RL) and Reactive Red 194 (Reactive Red M-2BF) were used for decolorization and biodegradation study. They were obtained from Textile Factory at Mahalla El Kobra, Gharbiya Governorate, Egypt.

Decolorizing ability

The studied species were cultivated in 250 ml Erlenmeyer flask containing 120 ml of the sterile medium. A total of 30 ml of the microbial culture and the dyestuffs at different initial concentrations (20, 40, and 60 ppm) were added to the cultures and incubated at 25 °C for 7 days. Samples with a volume of 3 ml are taken for different measurements at different periods, under sterile conditions. Samples were centrifuged at 6000 rpm for 10 min, supernatant was evaluated, and the rate of decomposition was determined in assay with an appropriate control device (El-Sheekh et al. 2009). The ratio of dye decolorization was measured after 3, 5, and 7 days of incubation by measuring the absorbance of the

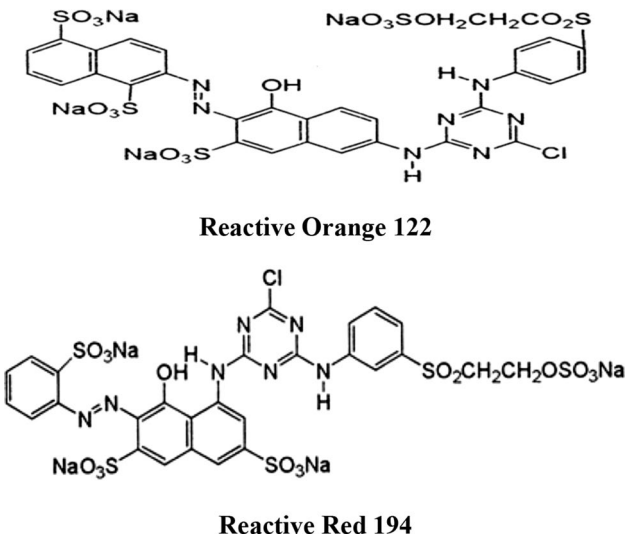

Fig. 1 Chemical structure of the dyes and azo dyes use

cell-free supernatant of the sample at wavelengths 421 nm (Nawshin Farzana et al. 2018) and 541 nm (Atul et al. 2013) for Reactive Orange 122 and Reactive Red 194, respectively, against control. The ratio of decolorization was calculated following the equation of Telke et al. (2010):

$$\text{Decolorization}\,(\%) = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}}$$

Spectroscopic analysis

The spectroscopic investigation was examined on the suspension by the (UV–visible–Invisible spectrum method) using ultraviolet (UV) Perkin-Elmer Lambda 4 B, accessory interface UV visible spectrophotometer. The analysis was done at wavelengths (421 and 541 nm) of Reactive Orange 122 and Reactive Red 194, respectively.

Effect of some environmental factors on decolorization rate

In order to achieve the highest decolorization rate, effect of various factors on decolorization rate for Reactive Orange 122 and Reactive Red 194 by the selected species of microorganisms was studied. Triplicate measurements were done for each experiment.

Azo dye concentration

Three levels (20, 40, and 60 ppm) of Reactive Orange 122 and Reactive Red 194 were applied to define the optimum concentration necessary for maximum decolorization. The above mentioned procedure in the substrate concentration section was repeated.

The effect of pH

The influence of different pH values (5, 7, 9, and 11) of the culture medium on the decolorization of dyes was studied at the concentration 20 ppm of Reactive Orange 122 to *Oscillatoria* sp. mixed with *S. obliquus* and Reactive Red 194 (20 ppm) to *Oscillatoria* sp. mixed with *S. obliquus* under static conditions. The steps, as mentioned above, were repeated, excluded changing the pH of the growth medium. The concentrations were adjusted by using HCl and NaOH solutions, followed by measurements using a pH meter WTWpH91 (Keith and John 2008).

Effect of temperature

Decolorization of Reactive Orange 122 and Reactive Red 194 by the selected organisms was measured at different temperatures, 12, 25, and 60 °C by using the method of Gómez and González (2005).

Effect of different culture conditions

Continuous illumination The flasks were kept at room temperature under continuous light of white fluorescent lamps (40 W), having 33.75 $\mu molm^{-2}\ s^{-1}$ at 25–30 °C.

Light/dark duration The cultures were kept in the laboratory window in natural light (sunlight) among light and dark period at 25–30 °C (Lee and Lee 2001).

Dark condition The flasks were kept in a dark place after covering with cellophane paper at 25–30 °C.

Effect of aeration using an air pump with sterilized media The flasks were kept at room temperature under continuous illumination using the sterilizing air pump at 25–30 °C. Aeration was one of the three flasks using the air pump, which pumps the air at a rate of 150 per minute bubble through the drip set (plastic tubes) with regulator.

Effect of different media

Oscillatoria sp., *S. obliquus*, *C. vulgaris,* and *Chlorococcum* sp. were cultivated in four different nutrient media to find the best culture medium (BG11, Kuhl, Allen, and BBM media) (Sharma et al. 2011).

Effect of nitrogen (N)

Kuhl's medium was prepared and used for the cultivation of the algal species. KNO_3 was added with various concentrations (2.525, 5.05, 10.1, 10.6, and 20.2 g l^{-1}) to the medium (Hamouda et al. 2018). The impact of extra N and P levels on microbial decolorization of dyestuffs was studied.

Effect of phosphorus (P)

Various concentrations of phosphorous as disodium hydrogen phosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$) were added to the medium (0.23, 0.45, 0.89, 1.35 and 1.78 g l^{-1}) and Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$) (0.16, 0.31, 0.62, 1.12, and 1.24 g l^{-1}) (Hamouda and Abou-El-Souod 2018).

Effect of some vitamins

Five various concentrations of ascorbic acid and thiamine were added to the medium (2, 4, 6, 8, and 10%) (Provasoli et al. 1974).

FTIR analysis of decolorized dyes

The decolorized dyes were analyzed by FTIR spectroscopy (Perkin-Elmer, Spectrum one). The obtained data were compared with the control dye. The FTIR analysis was done in the mid-IR region (400–4000 cm^{-1}) with 16 scan speed (Sarwa et al. 2013; Shyamala et al. 2014).

Statistical analysis of data

Standard error

The data presented in the figures and tables are the average of at least three replicates per treatment and means ± SE.

Statistics

Analysis of the data was performed using ANOVA, and significant variation in treatment means was compared according to the least significant difference test (LSD) at $P < 0.05$ (Yusop et al. 2017).

Results and discussion

Dyestuffs, including reactive dyes, are utilized in the weaving process. This leads to effluent streams containing intense color based on the existence of azo dyes. The expulsion of azo dyes from effluents is significant because of their mutagenicity and cancer-causing nature together with their serious coloration. Low molecular weights and simple structures dyes usually have high rates of color removal, whereas dyes with highly substituted and higher molecular weight have less efficiency for color removal (Pearce et al. 2003).

The decolorization efficiency

The ability of algae for biosorption is credited to their relatively high surface district and high binding affinity (Donmez and Aksu 2002). The physical processes that mediate algal biosorption are electrostatic attraction and complication in the cell wall of algae (Satiroglu et al. 2002). El-Sheekh et al. (2009) stated that *Oscillatoria* had the ability to degrade and remove azo dyes as methyl red, orange II and G-Red compared to some species that did not have any ability to degrade those dyes in their culture media. The results were achieved; the elimination of color changes with varying initial dye concentrations. The decolorization efficiency of *Oscillatoria* sp., *S. obliquus*, *C. vulgaris,* and *Chlorococcum* sp. was studied by measuring the optical density after 3, 5, and 7 days of incubation as recorded in Tables 1 and 2. The maximal removal of Reactive Orange 122 was observed with *Oscillatoria* sp. mixed with *S. obliquus* (98.54 ± 0.006%) and (98.40 ± 0.010%) of *Oscillatoria* sp. at 20 ppm in 7^{th}day incubation while the minimum results were recorded (0.00%) and appearance of turbidity (Table 1). The molecular structure of the dye is an essential factor that controlling the increment of decolorization of dyestuffs (Jinqi and Houtian 1992), absorption to the algae (Chen et al. 2003a, b), and fast decomposition of the dye (Daneshvar et al. 2007). As observed from Table 2 in Reactive Red 194, the maximum decolorization recorded (97.58 ± 0.003%) with *Oscillatoria* sp. mixed with *S. obliquus* and (91.53 ± 0.010%) in *Oscillatoria* sp. at 20 ppm in seventh day incubation, whereas the minimum value was recorded (0.00%) and turbidity. A breakdown of algae occurred on the first day or two as a result of this degradation, the algae extracted toxic substances that made the breakdown of the other alga or algae, and this led to the appearance of turbidity and the degradation of azo dye (0.00%). Anaerobic decolorization, followed by aerobic biodegradation, may or may not reduce toxicity as anaerobic decolorization by cleavage of azo bond generates aromatic amines in some cases more toxic than the parent dyes (Da Silva et al. 2012; Montano et al. 2008).

It was observed that the Reactive Orange 122 was more decolorized than Reactive Red 194. Zimmermann et al. (1982) explained these results as follows: the charged groups (sulfonic group) in the proximity to the azo group; hinder the reaction (decolorization). The sulfonic groups to azo bond (–N=N–) in Reactive Orange 122 were a strong electron-withdrawing group through resonance to cause an enhanced color removal to be easily decolorization (McMurry 2004; Hsueh and Chen 2007). Some of the components of

Table 1 Decolorization (%) of different concentrations of Reactive Orange 122 by different algae and consortium

Decolorization (%)												
Dye	Day	*S. obliquus*	*C. vulgaris*	*Chlorococcum* sp.	*S. obliquus* + *C. vulgaris*	*S. obliquus* + *Chlorococcum* sp.	*C. vulgaris* + *Chlorococcum* sp.	*Oscillatoria* sp.	*Oscillatoria* sp. + *Scenedesmus obliquus*	*Oscillatoria* sp. + *C. vulgaris*	*Oscillatoria* sp. + *Chlorococcum* sp.	All organisms
Reactive Orange 122 20 ppm	3	41.26 ± 0.069	8.74 ± 0.012	45.15 ± 0.066	18.45 ± 0.03	37.38 ± 0.05	29.61 ± 0.014	55.83 ± 0.018	73.30 ± 0.018	24.76 ± 0.039	15.53 ± 0.052	29.13 ± 0.045
	5	47.57 ± 0.013	9.71 ± 0.014	59.22 ± 0.01	20.87 ± 0.051	46.12 ± 0.058	33.98 ± 0.043	67.96 ± 0.020	87.38 ± 0.012	0.00 ± 0.090	0.00 ± 0.050	0.00 ± 0.019
	7	72.82 ± 0.039	15.05 ± 0.021	77.18 ± 0.017	46.12 ± 0.057	72.33 ± 0.033	46.60 ± 0.015	98.40 ± 0.010	98.54 ± 0.006	0.00 ± 0.021	0.00 ± 0.034	0.00 ± 0.033
Reactive Orange 122 40 ppm	3	17.74 ± 0.146	5.38 ± 0.122	37.10 ± 0.087	12.63 ± 0.098	38.17 ± 0.057	23.39 ± 0.033	52.96 ± 0.081	54.84 ± 0.017	21.77 ± 0.017	40.59 ± 0.011	23.12 ± 0.067
	5	43.82 ± 0.076	34.14 ± 0.107	55.65 ± 0.042	32.80 ± 0.059	46.24 ± 0.011	37.37 ± 0.04	59.41 ± 0.057	61.02 ± 0.047	27.15 ± 0.070	36.02 ± 0.018	13.98 ± 0.141
	7	82.26 ± 0.041	56.45 ± 0.046	90.32 ± 0.014	45.97 ± 0.069	57.80 ± 0.014	59.41 ± 0.028	62.63 ± 0.037	64.25 ± 0.040	33.33 ± 0.091	19.89 ± 0.036	4.84 ± 0.140
Reactive Orange 122 60 ppm	3	39.70 ± 0.02	37.79 ± 0.106	43.10 ± 0.02	37.79 ± 0.024	40.13 ± 0.01	27.18 ± 0.015	59.66 ± 0.016	59.24 ± 0.013	30.36 ± 0.113	38.43 ± 0.037	34.18 ± 0.022
	5	57.11 ± 0.07	38.85 ± 0.016	52.02 ± 0.012	39.92 ± 0.027	41.83 ± 0.02	27.81 ± 0.063	62.63 ± 0.017	63.27 ± 0.031	23.99 ± 0.107	31.00 ± 0.139	33.33 ± 0.097
	7	76.22 ± 0.044	50.74 ± 0.095	74.95 ± 0.021	58.17 ± 0.057	79.41 ± 0.011	62.00 ± 0.027	66.67 ± 0.029	67.94 ± 0.028	2.97 ± 0.036	5.31 ± 0.010	12.74 ± 0.120
LSD*		0.88	0.115	0.961	0.369	0.901	0.968	0.382	0.015	0.233	0.098	0.322

*The mean difference is significant at the 0.05 level

Table 2 Decolorization (%) of different concentrations of Reactive Red 194 by different algae and its consortium

Decolorization (%)												
Dye	Day	*S. obliquus*	*C. vulgaris*	*Chlorococcum* sp.	*S. obliquus* + *C. vulgaris*	*Scenedesmus. obliquus* + *Chlorococcum* sp.	*C. vulgaris* + *Chlorococcum* sp.	*Oscillatoria* sp.	*Oscillatoria* sp. + *S. obliquus*	*Oscillatoria* sp. + *Chlorella. vulgaris*	*Oscillatoria.* sp. + *Chlorococcum* sp.	All organisms
Reactive Red 194 20 ppm	3	54.48 ± 0.085	47.70 ± 0.08	58.35 ± 0.073	53.03 ± 0.108	54.72 ± 0.104	49.88 ± 0.119	81.84 ± 0.030	85.96 ± 0.029	78.21 ± 0.015	74.58 ± 0.048	80.15 ± 0.022
	5	60.53 ± 0.069	48.67 ± 0.06	60.05 ± 0.034	52.78 ± 0.113	59.81 ± 0.071	50.36 ± 0.091	86.44 ± 0.022	87.89 ± 0.020	53.03 ± 0.044	63.44 ± 0.020	69.25 ± 0.017
	7	61.74 ± 0.049	49.15 ± 0.059	64.41 ± 0.055	52.30 ± 0.111	61.50 ± 0.078	51.09 ± 0.117	91.53 ± 0.010	97.58 ± 0.003	49.15 ± 0.050	38.74 ± 0.100	64.16 ± 0.016
Reactive Red 194 40 ppm	3	26.21 ± 0.172	27.59 ± 0.177	22.53 ± 0.096	29.43 ± 0.173	24.14 ± 0.182	22.99 ± 0.189	49.89 ± 0.058	49.66 ± 0.050	22.76 ± 0.023	32.18 ± 0.077	24.60 ± 0.054
	5	27.59 ± 0.163	13.1 ± 0.118	32.87 ± 0.126	14.02 ± 0.194	16.55 ± 0.193	9.43 ± 0.160	53.10 ± 0.060	54.02 ± 0.036	0.00 ± 0.028	3.22 ± 0.049	20.23 ± 0.159
	7	40.92 ± 0.151	9.66 ± 0.109	51.26 ± 0.109	8.97 ± 0.117	14.48 ± 0.101	5.75 ± 0.122	54.94 ± 0.013	61.15 ± 0.058	0.00 ± 0.016	0.00 ± 0.050	14.71 ± 0.023
Reactive Red 194 60 ppm	3	30.79 ± 0.180	36 ± 0.164	25.30 ± 0.224	24.54 ± 0.235	24.39 ± 0.233	28.96 ± 0.263	42.38 ± 0.094	46.49 ± 0.035	27.74 ± 0.071	32.32 ± 0.019	28.81 ± 0.038
	5	40.24 ± 0.163	25 ± 0.191	34.45 ± 0.252	30.34 ± 0.251	35.37 ± 0.234	19.97 ± 0.216	40.85 ± 0.016	43.60 ± 0.032	1.98 ± 0.061	14.63 ± 0.013	28.05 ± 0.018
	7	41.46 ± 0.240	24.54 ± 0.220	35.67 ± 0.224	32.62 ± 0.209	38.11 ± 0.226	12.96 ± 0.213	40.70 ± 0.150	40.55 ± 0.043	0.30 ± 0.029	14.18 ± 0.032	15.40 ± 0.076
LSD*		0.004	0.003	0.016	0.002	0	0.001	0	0	0.008	0.023	0

*The mean difference is significant at the 0.05 level

sediment increase in contrast with the blank; this might be because of the structure of intermediate compounds through biotransformation or biodegradation operation when blue-green algae were applied (El-Sheekh et al. 2014). From the previous data we observed that the capability of algae to degrade the two dyes depends on the algal species and its capacity to resist the impact of dyes on their growth, while the difference in the concentrations of the dye is not significantly affecting the results, these disagree with Acuner and Dilek (2004) stated that decreasing the algal growth with rising dye concentration. Chen et al. (2003a, b) concluded that the decolorization was due to reduced biological azo pigment and absorption. The dye removal is highly concentration-dependent and is approximately attributed to bioconversion (Aydin and Baysal 2006), and also due to the molecular structure of the dyes (Jinqi et al. 1992).

Effect of pH

The pH affects the dye decolorization efficiency because the difference in pH affects several functional groups such as amino and carboxyl on the surface of algal cell walls, which are responsible for the binding of dye molecules (Aravindhan et al. 2007; Aksu and Karabayir 2008). Removal efficiency of azo dye depends mostly on the pH in the middle, and the ability of endurance azo dye analyzers, and the stability of the pH in the oxidative enzymes. According to the microalgae physiology and dye constituents, any parts of the microalgae cell carry out their vital functions in specific pH. The highest percentage of Reactive Orange 122 uptake was (79.61%) at pH 11, and the lowest was (56.31%) at pH 5 by *Oscillatoria* sp. mixed with *S. obliquus* at 20 ppm in seventh day incubation at (Fig. 2). pH of the medium normally increases with microalgal growth: microalgae remove CO_2 from water, inducing pH to reach high levels (it is a consequence of its activity!). All abiotic parameters will be very variable and change rapidly in space and time. In addition to that, microalgae release some extracellular metabolites, which are acidic in nature and in turn, neutralize the high alkaline pH. This is one of the strategies to protect microalgal cells. Due to the physiology of micro-members of microalgae, any parts of the cell microalgae (thylakoid of chloroplasts) perform vital functions in a certain degree of acidity. Representation of the process of photosynthesis is also affected by the number pH of the media. Since the optimum pH value is between 8.2 and 8.7 for microalgae growth, the alkaline medium is preferred for growing algae. Bitog et al. (2011) found the optimum pH at 8 to remove the maximum dyes (DB71 and DR1). The pH has a significant effect on decolorization efficiency at the optimum pH for color removal which is often between 6.0 and 10.0 for most dyes (Chen et al. 1999).

The pH tolerance of decolorizing bacteria is very significant because dyestuffs link to cotton filaments by expansion or replacement instruments under alkaline conditions and at high temperatures (Aksu and Donmez 2003). Some researchers as Chen et al. (2003a, b), Guo et al. (2007) and Kilic et al. (2007) found the optimal pH for color removal frequently somewhere in the range of 6.0 and 10.0 for the vast majority of the dyes. Reactive azo dyes lose hydrogen ions under alkaline conditions, leading to the ionization of the dye, affect the stability, and facilitate the removal of solutions (Mahmoud et al. 2007). Preferably remove color in alkaline conditions in general commercial applications, when treated with reactive azo dyes, they are made under alkaline conditions (Table 2).

Effect of temperature

Removing of the dye Reactive Orange 122 and Reactive Red 194 was found optimal at temperature 25 °C when treated with *Oscillatoria* sp. mixed with *S. obliquus* was able to degrade the dye by 83.98% and 87.41% after fifth day of incubation, respectively, as in Table 3. The optimum dye

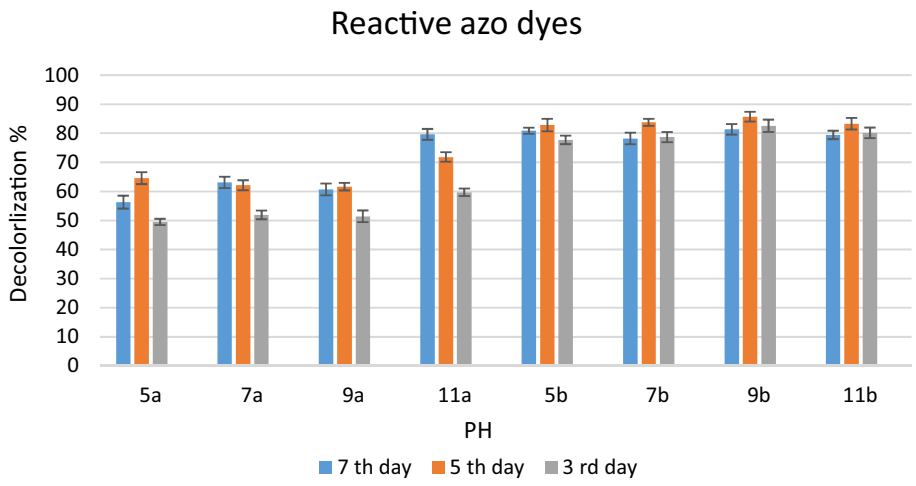

Fig. 2 Effect of pH on decolorization of Reactive azo dyes by *Oscillatoria* sp. mixed with *S. obliquus. a* Reactive Orange 122 and *b* Reactive Red 194

Table 3 Decolorization (%) of reactive azo dyes by *Oscillatoria* sp. mixed with *S. obliquus* at different temperatures and culture conditions

Factors	Reactive Orange 122		Reactive Red 194	
	Third	Fifth	Third	Fifth
Temperature (°C)				
12	66.99 ± 0.021	73.79 ± 0.016	78.69 ± 0.015	85.23 ± 0.013
25	75.73 ± 0.012	83.98 ± 0.007	85.23 ± 0.017	87.41 ± 0.013
60	40.29 ± 0.014	21.36 ± 0.021	63.20 ± 0.018	57.63 ± 0.014
Culture conditions				
Light	83.50 ± 0.005	93.69 ± 0.002	82.57 ± 0.014	87.65 ± 0.014
Light/dark	79.13 ± 0.01	88.35 ± 0.005	80.63 ± 0.01	85.71 ± 0.01
Dark	71.84 ± 0.013	82.52 ± 0.012	80.15 ± 0.01	85.47 ± 0.019
Aeration	81.07 ± 0.01	89.32 ± 0.01	82.57 ± 0.01	86.44 ± 0.014

± Means the standard error of the mean of three replicates

decolorization by the selected algal strain was observed at 25 °C. The high temperature may cause thermal inactivation of the algae enzyme(s) responsible for removing the color of the azo pigments. The removal of azo dyes increases to the optimum temperature, after which there is a marginal decrease in the decolorization activity. This can be attributed to the decline in high temperature to the loss of cell viability of life or denaturation enzyme azoreductase (Chang et al. 2001; Saratale et al. 2009). Cetin et al. (2006) was reported that the decolorization activity was significantly suppressed above 45 °C.

Effect of different culture conditions

Microalgae are everywhere, requiring sunlight and some simple nutrients, although growth rates can be precipitated by the addition of specific nutrients and adequate aeration (Pratoomyot et al. 2005; Renaud et al. 1999). Anaerobic microbial wastewater treatment can be highly effective in decolorizing, mainly through the azo reductive activity that splits the azo bond to produce the corresponding amines, which are often toxic, mutagenic, carcinogenic, and withstand further degradation under anaerobic conditions (Gottlieb et al. 2003; O'Neill et al. 2000; Pinheiro et al. 2004; Van der Zee et al. 2001; Van der Zee and Villaverde 2005). In this operation, azo dye acts as a final electron receiver in anaerobic respiratory oxidation of carbon sources and other electronic donors (Carliell et al. 1995; Ryan et al. 2010). At light condition, both reactive dyes are recorded maximum degradation activity in *Oscillatoria* sp. mixed with *S. obliquus* 93.69% and 87.65% to Reactive Orange 122 and Reactive Red 194, respectively (Table 3). In contrast, the minimum degradation activity for both dyes is recorded in dark conditions for *Oscillatoria* sp. mixed with *S. obliquus* 82.52% and 85.47% for Reactive Orange 122 and Reactive Red 194, respectively.

Effect of different media

Figure 3 shows that BG11 media and Allen media have the maximum percentage of degradation activity after treatment by *Oscillatoria* sp. mixed with *S. obliquus* to both Reactive dyes. In Reactive Orange 122, both media were recorded 90.78%, while BG11 media and Allen media were recorded 94.92% and 93.46%, respectively, for Reactive Red 194 in seventh day. Kuhl's medium recorded 82.04% and 93.22% degradation activity for Reactive Orange 122 and Reactive Red 194, respectively. While the minimum percentage of degradation activity for both Reactive dyes was recorded with BBM media, it recorded 81.07% and 90.80%

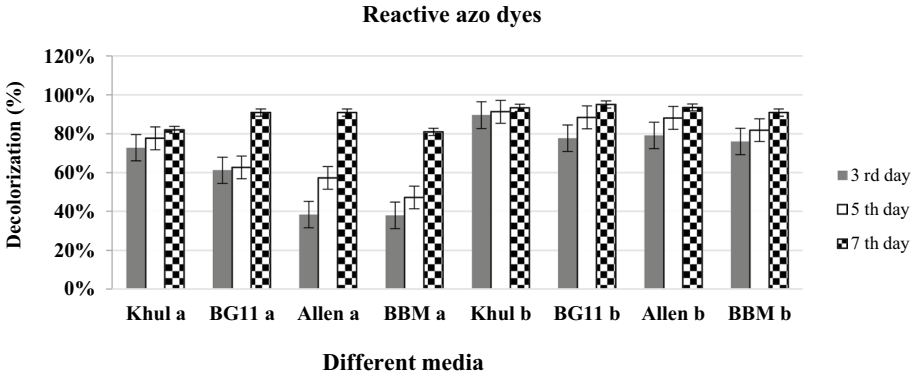

Fig. 3 Influence of different media conditions on decolorization of Reactive azo dyes by *Oscillatoria* sp. mixed with *S. obliquus*. *a* Reactive Orange 122. *b* Reactive Red 194

decolorization with Reactive Orange 122and Reactive Red 194, respectively. BG11 was found to be the optimum medium for algal growth (Dayananda et al. 2007). The rise of nutrients results in higher growth and higher biomass of cyanobacteria. Many studies reported similar observations as stated by Gerald et al. (1950), nutritional data with the difference in the amounts of essential elements in the solution may explain that BG11 will result in a faster rate and more considerable amount of growth of many of the algae. Different algae require different components in the media for their growth. Growth of algae, in general, depends upon the availability of nitrogen and phosphate.

Effect of nitrogen (N)

Nitrogen is an essential component involving about 10% of cyanobacterial cell dry weight. Cyanobacteria can use various inorganic and natural nitrogen (Flores and Herrero 2005; Perez-Garcia et al. 2011). Dyestuffs are weakly in carbon and nitrogen sources, and the biodegradation of dyes without any supplement of these sources is very difficult (Razia Khan et al. 2013). The supplement of nitrogen and phosphorus in the liquid medium activated the biodegradation operation (Kassim 2002; Aslan and Kapdan 2006). The influence of different concentrations of KNO_3 on the removal of Reactive Orange 122 by *Oscillatoria* sp. mixed with *S. obliquus* has been reported in Table 3. The best decolorization activity of dye was at 20.2 g l^{-1} KNO_3 followed by 10.6, 10.1, 5.05, and 2.525 g l^{-1} KNO_3, respectively. While in Reactive Red 194, the highest degradation values were recorded with *Oscillatoria* sp. mixed with *S. obliquus* (10.6 g l^{-1}) KNO_3 concentration followed by10.1, 5.05, 20.2, and 2.525 g l^{-1} KNO_3, respectively (Table 4). The recorded data indicated that the decolorization of dyestuffs increased with nitrogen concentration raise. Different concentrations of nitrogen sources in different proportions may change the growth conditions ranging from pure photoautotrophy to heterotrophy, thus leading to assimilatory variations (Kumar and Bera 2020).

Table 4 Influence of nitrogen, phosphorus and vitamins (thiamine and ascorbic acid) concentrations on decolorization (%) of reactive azo dyes by *Oscillatoria* sp. mixed with *S. obliquus*

Concentrations	Reactive Orange 122		Reactive Red 194	
	Third	Fifth	Third	Fifth
Elements				
Nitrogen (g L^{-1})				
2.525	68.45 ± 0.017	75.73 ± 0.021	86.20 ± 0.017	79.90 ± 0.025
5.05	71.84 ± 0.024	76.70 ± 0.022	86.92 ± 0.017	85.23 ± 0.018
10.1	67.96 ± 0.011	79.13 ± 0.012	87.17 ± 0.022	85.47 ± 0.019
10.6	57.77 ± 0.016	83.50 ± 0.011	85.96 ± 0.021	85.71 ± 0.018
20.2	54.37 ± 0.028	92.72 ± 0.01	86.68 ± 0.016	84.50 ± 0.015
Phosphorus (g L^{-1})				
0.23	84.47 ± 0.011	91.26 ± 0.01	80.87 ± 0.017	79.42 ± 0.021
0.45	83.01 ± 0.01	90.29 ± 0.01	80.63 ± 0.031	79.90 ± 0.035
0.89	82.04 ± 0.018	89.81 ± 0.01	79.66 ± 0.041	79.18 ± 0.026
1.35	78.64 ± 0.01	89.32 ± 0.01	79.90 ± 0.029	78.21 ± 0.027
1.78	77.18 ± 0.018	85.44 ± 0.013	78.69 ± 0.026	76.27 ± 0.031
Vitamins				
Thiamine (%)				
2	83.98 ± 0.01	77.67 ± 0.01	82.81 ± 0.025	83.78 ± 0.018
4	84.47 ± 0.013	72.33 ± 0.017	80.87 ± 0.011	84.02 ± 0.01
6	82.04 ± 0.014	79.13 ± 0.011	79.66 ± 0.031	81.60 ± 0.034
8	83.01 ± 0.01	74.76 ± 0.01	79.42 ± 0.034	85.23 ± 0.026
10	74.76 ± 0.017	70.87 ± 0.014	79.42 ± 0.032	84.75 ± 0.01
Ascorbic acid (%)				
2	64.56 ± 0.018	65.53 ± 0.01	79.90 ± 0.017	81.36 ± 0.01
4	64.56 ± 0.014	75.73 ± 0.011	79.90 ± 0.024	84.26 ± 0.022
6	68.93 ± 0.016	77.18 ± 0.01	79.90 ± 0.021	84.02 ± 0.017
8	65.05 ± 0.014	71.36 ± 0.019	78.93 ± 0.024	84.02 ± 0.01
10	61.65 ± 0.01	66.99 ± 0.01	79.18 ± 0.01	82.57 ± 0.01

± Means the standard error of the mean of three replicates

Effect of phosphorus (P)

Phosphorus is the most nutritional factor that affects metabolism and cell growth. Phosphorus concentrations can affect the biochemical of microalgae. Algae utilization of nitrogen and phosphorous rely upon different factors as the composition of the nutritional medium, light intensity, and nitrogen/phosphorous ratio (Aslan and Kapdan 2006). The highest decolorization activity of Reactive Orange 122 treated with *Oscillatoria* sp. mixed with *S. obliquus* was 84.47% at 0.23 g l^{-1} of $Na_2HPO_4{\cdot}2H_2O$ at third day and increased by 91.26% after fifth day of incubation. At 1.78 g L^{-1} $Na_2HPO_4{\cdot}2H_2O$, the decolorization of Reactive Orange 122 recorded the lowest percentage on third day (77.18%) and increased by 85.44% after fifth day of incubation as shown in Table 4. In Reactive Red 194, the highest decolorization by *Oscillatoria* sp. mixed with *S. obliquus* was 80.63% at 0.45 g l^{-1} $Na_2HPO_4{\cdot}2H_2O$ after third day and increased to 79.90% after fifth day of incubation. In Reactive Orange 122, the lowest percentage of Reactive Red 194 of degradation at 1.78 g l^{-1} $Na_2HPO_4{\cdot}2H_2O$ was recorded 76.27% at fifth day of incubation (Table 4). Concerning phosphorus, when phosphorus is reduced, the organism needs phosphorous in the dye, so it needs degrading of the dye. However, with the increase in days, some algae die and give color in the environment as a result of degradation or form intermediate compounds that give color.

With regard to phosphorous, algae grow under hard conditions of phosphorous deficiency, so they break down the dye, and then, as a result of decolorizing, it produces intermediate compounds that stop the growth, and the algae decompose. For nitrogen, the rate of degradation is increased with the increase in nitrogen concentration. The metabolism of organic nitrogen sources regenerates NADH, which acts as an electron donor for the reduction of azo dyes by the bacterial system. (Lalnunhlimi and Krishnaswamy 2016). Algae utilization of nitrogen and phosphorous rely upon different factors as the composition of the nutritional medium, light intensity, and nitrogen/phosphorous ratio (Rasdi and Qin 2014). Dyes deficient in carbon and nitrogen sources, and the decomposition of biological dyes without any supplements from these sources is extremely difficult. Microbial cultures generally require complex organic sources, such as yeast extract, peptone, or a mixture of complex organic sources and carbohydrates to remove and degrade the dye.

Effect of some vitamins

In biochemical reactions in the plant cells, vitamins act as an activator, as well as, vitamins are required as among organic nutritional factors for continued growth and metabolic activities of living organisms. Ascorbic acid and thiamine vitamins were utilized in the treatment of the adverse effects of stress and heavy metals (Desouky 1995, 2003). Table 4 shows that 6% of both thiamine and ascorbic acid recorded the maximum degradation activity of Reactive Orange 122 when treated *Oscillatoria* sp. mixed with *S. obliquus* (79.13% and 77.18%), respectively. While in Reactive Red 194, the highest degradation activity in treated with *Oscillatoria* sp. mixed with *S. obliquus* was 85.23% at 8% of thiamine and 84.26% of ascorbic acid at 4% (Table 4).

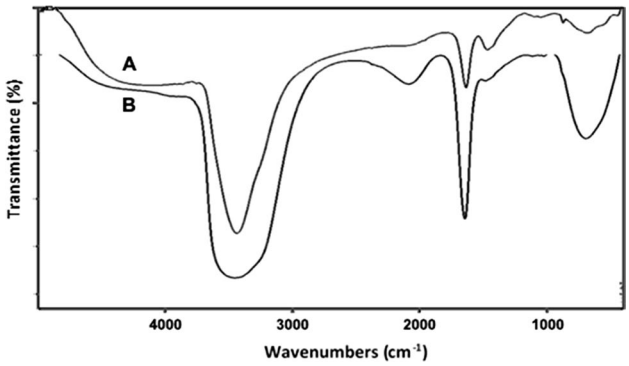

Fig. 4 Infrared of aqueous solution of *Oscillatoria* sp. mixed with *S. obliquus* after treatment by Reactive Orange 122 (*a*) and control of Reactive Orange 122 (*b*)

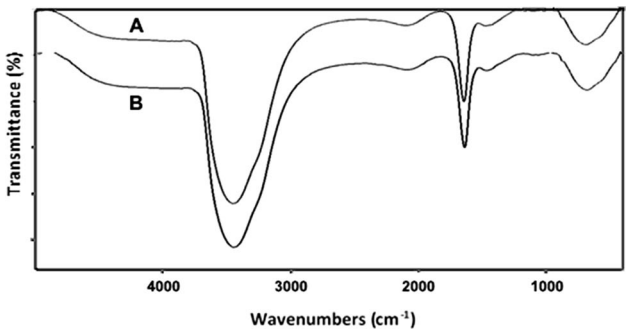

Fig. 5 Infrared of aqueous solution of *Oscillatoria* sp. mixed with *S. obliquus* after treatment by Reactive Red 194 (*a*) and control of Reactive Red 194 (*b*)

FTIR analysis of decolorized samples

The FTIR spectrum of *Oscillatoria* sp. mixed with *S. obliquus* before and after the absorption of the dye participation of different functional groups revealed algae in the absorption of the dye. Figures 4, 5, 6, and 7 shows infrared spectra of Reactive Orange 122 and Reactive Red 194, respectively, to recognize their structural differences before and after algal treatment. Figures 4 and 5 show the IR of the biomass of *Oscillatoria* sp. mixed with *S. obliquus* before and after treatment with Reactive Orange 122 and Reactive Red 194, respectively, and there was a difference in the intensity of peaks, especially in the region from 1646 to

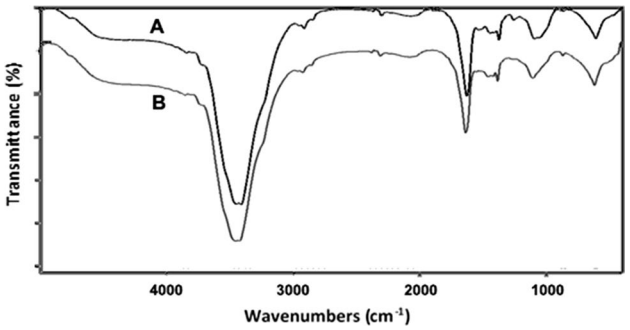

Fig. 6 Infrared of biomass of *Oscillatoria* sp. mixed with *S. obliquus* after treatment by Reactive Orange 122 (*a*) and control of *Oscillatoria* sp. mixed with *S. obliquus* before treatment (*b*)

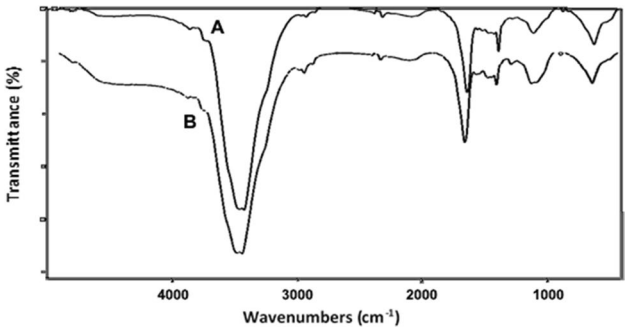

Fig. 7 Infrared of biomass of *Oscillatoria* sp. mixed with *S. obliquus* after treatment by Reactive Red 194 (*a*) and control of *Oscillatoria* sp. mixed with *S. obliquus* before treatment (*b*)

1544 cm^{-1}. Figures 6 and 7 show the FTIR spectrum of algae after dye absorption and represent that some peaks turn or disappear, and some new peaks arise after absorbing dye.

No intermediates of degradation/oxidation/reduction/substituent removal etc., have been explicitly shown. Sabnis (2017) reported that raw materials used as intermediate materials in the production of organic dyes and dyestuffs for manufacture. It is colorless and almost vary in complexity. There are three types of interactions used to produce of intermediates of dyes: (a) electrophilic substitution, (b) nucleophilic substitution, and (c) unit processes.

Conclusion

This study discovers azo dye degrading by the consortium of organisms that can be beneficial for the environment. A new theory on the effect of physicochemical conditions on decolorization and degradation of azo dyes is proposed. It is also explored the effect of the consortium of organisms on the degradation efficiency of azo dyes. This study revealed that the efficiency of azo dye biodegradation depends on the suitable environmental conditions and the type of organism. The study recommends using *Oscillatoria* sp. mixed with *S. obliquus* in the degradation of azo dyes under the environmental conditions referred to in the study to achieve the best result of the decomposition.

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