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RESEARCH ARTICLE

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Biodegradation of some dyes by the cyanobacteria species *Pseudoanabaena* sp. and *Microcystis aeruginosa* Kützing

ABSTRACT:

The processes of biodegradation have widely used to remove hazardous material from aquatic and soil system is which an environmental friendly tool. The present study was conducted to investigate the decolorization and biodegradation of some dyes using some microalgal species isolated from polluted industrial regions. Two microalgal species (cyanobacteria) were isolated and identified as *Pseudoanabaena* sp. and *Microcystis aeruginosa.* The ability of the two algae to remove and decolorize Disp.orange 2RL, Reactive yellow 3RN, Reactive Black NN and Tracid Red BS was investigated. The results revealed that the removal of these dyes depends on the algal species, its growth rate and the structure of dyes. The maximum decolorization was observed in Reactive Black NN and Disp.orange 2RL by *M. aeruginosa* (55.12% and 65.07%, respectively) after 7 days of incubation. Also, *Pseudoanabaena* sp. decolorized Reactive yellow 3RN and Tracid red BS by (58.47% and 78.44%, respectively). Azo reductase enzyme in algae which is the responsible for degradation of azo dyes into aromatic amine by cleaving the azo linkage was estimated. The results showed that treatment of *M. aeruginosa* with Disp.orange 2RL induced the azo reductase enzyme by 68.04% and *Pseudoanabaena* sp. with Reactive yellow 3RN by 55.64% after 7 days of incubation. The degradation product after decolorization was identified and confirmed by spectroscopic analysis and Fourier transformed infrared spectroscopy analysis.

KEY WORDS:

Biodegradation, dyes, microalgae, wastewater.

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INTRODUCTION:

Nowadays, the public become more interested and sensitive towards the protection of the environment and general awareness has now increased about the potential adverse effects of industrial effluents contaminated with various pollutants, including dyes, on the environment (Sarita *et al*., 2012; Karthik *et al*., 2014). It was reported that effluent from the dyeing industry is one of the most difficult waste waters to be treated, not only because of its high chemical and biological oxygen demand, suspended solid and content of toxic, carcinogenic, or mutagenic compounds, but also its color, which is visually the most noticeable contaminant (Aksu, 2005; Khataee *et al.,* 2009). So, the removal of dyes from aqueous effluent has received considerable attention within environmental research (Khataee, 2009).

Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds (Alexander, 1994). Indeed, it is the operation by which organic substances are broken down into smaller compounds by living microbial organisms (Bhatnagar *et al.,* 2013; Joutey *et al.,* 2013). When biodegradation is complete, the operation is called "mineralization". However, in most cases the term biodegradation is generally used to characterize almost any

biologically mediated change in a substrate (Bennet *et al.*, 2002).

Dyes are generally “chemicals” which binds to one of the material to imports color due to presence of chromophore group. Industrially, the azo dyes are commonly used, including Acid dye, Basic dye, Direct dye, Disperse dye, Mordant dye, Reactive dye, and Solvent dyes (Sudha *et al.,* 2014). Azo dyes are the most important group of synthetic dyes that are extensively used in the pharmaceutical, textile and printing industries (Syed *et al*., 2009).

Azo dyes contain groups such as anthraquinone, polycyclic and triphenylmethane which make these dyes toxic to environment. They are toxic to aquatic organisms (fish, bacteria, etc.), as well as to animals and thus it affects ecosystem function. Azo dyes cause serious contamination to rivers and groundwater around dyeing industry outfalls. Dissolved materials in industrial effluents change the chemical and biological properties of the soil and water, which may have negative effects on growth and productivity of plants (Puvaneswari *et al*., 2006).

Azo dyes, one of the greatest groups of synthetic dyes, have one or more azo bounds (N=N-) and because of their solubility, low expense, stability and color variety, are widely used in many applications (Lucas and Peres, 2007; Entezari *et al*., 2008). Removal of azo dyes from colored effluents due to their complex composition, toxicity, poor degradability and high solubility, have attracted great interest in the last decade (Qu *et al*., 2008).

Algae are photosynthetic organisms, which are distributed in nearly all parts of the world and in all kinds of habitats. Also, algae can degrade many dyes, presuming that the reduction appears to be related to the molecular structure of the dyes and the species of algae used. Algae species, being very versatile, have developed enzyme systems for the decolorization and mineralization of organic dyes under certain environmental conditions. Many algae can degrade and decolorize of azo dyes in wastewater effluents (Ertugrul *et al*., 2008; Wang *et al*., 2009).

Marungrueng and Pavasant (2007) reported that many functional groups (such as carboxyl, carbonyl, hydroxyl, phosphoryl and amide) in the algal cell wall plays the important role in dye removal. Several other factors may have roles in dye bioremediation, such as pH, dye concentrations, and amount of biomass (Wafaa *et al.,* 2008). Therefore, the present study was carried out to investigate the ability of *Pseudoanabaena* sp. and *Microcystis aeruginosa* to remove and decolorize some of the most widespread dyes and azo dyes.

MATERIAL AND METHODS:

Algae and growth conditions:

Microcystis aeruginosa and *Pseudoanabaena* sp. were isolated from polluted locations in the industrial region in Quisna, and Sadat city, Menoufia Governorate, Egypt and purified in axenic cultures (bacterial free) as described by Bolch and Blackburn (1996) and identified according to Pontin (1978), Prescott (1984), Yamagishi (1992), and Vymazal (1995). The two cyanobacteria were cultured in Allen medium (Allen, 1968) in sterilized Erlenmeyer conical flasks and the pH was adjusted to 6.8. After inoculation, they were kept in a culture room at 28 ±1^{o}C under continuous illumination (120 ± 4 µE/m^{2}s).

Dyes and azo dyes used:

The chemical structures of the dyes and azo dyes used are given in figure 1. (Reactive yellow 3RN, Disp.orange 2RL, Reactive Black NN and Disp. Red BS (Tracid Red BS) were used for decolorization and biodegradation study. They were obtained from Dyeing Factory at the industrial region district in Quisna, Menoufia Governorate, Egypt.

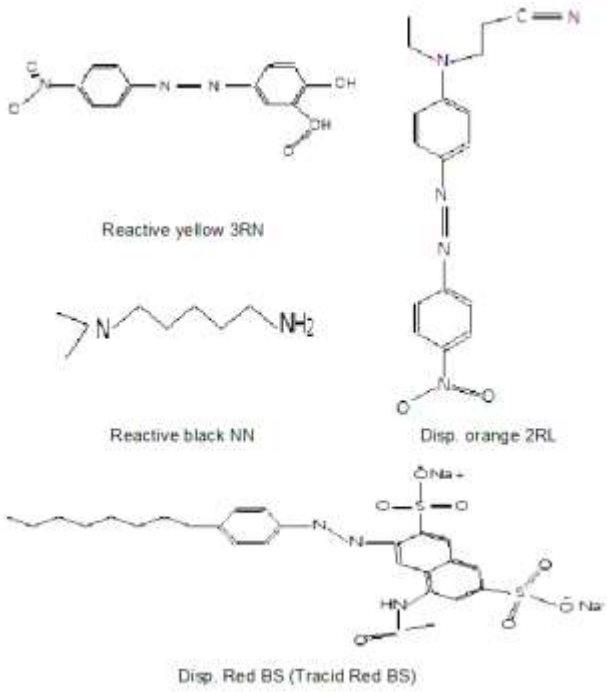

Fig. 1. Chemical structure of the dyes and azo dyes used.

Decolorization study and spectroscopic analysis:

The experiments were conducted in sterilized 250 ml Erlenmeyer flask containing 120 ml of the sterile medium with 30 ml of algal culture and the azo dye at concentration 20 ppm. The culture was incubated at 25^{o}C for 7 days. The degradation ratio was determined after 3, 5, and 7 days of incubation by measuring the absorbance of the cell free supernatant of the sample at the

maximal absorption wave length 415, 429, 597, and 515 nm for reactive yellow 3RN, Disp.orange 2RL, Reactive Black NN and Tracid red BS, respectively. A sterile cell-free medium was used as control. The percentage of decolorization was calculated by using the equation according to Telke *et al*. (2010).

Decolorization (%) = (Initial absorbance – Final absorbance/100) × Initial absorbance

FT-IR Analysis of Decolorized Samples:

After 7 days of incubation, the biodegraded dye samples were characterized before and after treatment using FTIR spectroscopy (Tensor 27 Bruker, Spectrum one). The analyses of the biodegraded dyes were compared with the control dyes. The FTIR analysis was done in the mid IR region (400 - 5000 cm^{-1}) with 16 scan speed (Sarwa and Verma, 2013; Shyamala *et al*., 2014).

Physiological studies:

Dry weight estimation:

Aliquots of 5 ml were filtered through a pre-dried (24h at 80^{o}C) and pre-weighed Whatman GF-52 filter (47 mm diameter). After filtration, the cells were washed with deionized water and dried in an oven at 80^{o}C for 24 h. After cooling to room temperature in desiccators, the filters with algal cells were weighed again and the dry weight was calculated and expressed as mg/l (Leganes *et al*., 1987).

Estimation of protein by Lowry assay:

The amount of proteins in the sample was estimated via reading the absorbance (at 750 nm) of the end product of the Folin reaction against a standard curve of a selected standard protein solution (Bovine serum albumin BSA) as recommended by Lowry *et al*. (1951).

Azo reductase activity:

The azo reductase preparation was made according to the procedure described by Idaka *et al*. (1987 a&b). The reductase activity of tested microalgae was studied after 3, 5, and 7 days of incubation with 20 ppm azo dye concentration.

RESULTS AND DISCUSSION:

It is known that biomass adsorption is effective when conditions are not always favorable for the growth protection of the microbial population, because the use of biomass has its advantages, especially if the dye containing effluent is very toxic. Treatment of dye effluent presents many problems mainly due to the toxicity of dyestuffs. Discharge of dye effluent into the natural streams may be toxic to the aquatic lives. Color affects the nature of water and inhibits the sunlight penetration into the stream and reduces photosynthetic activity (Mahalakshmi *et al.,* 2015). The present results agree with the previous findings where the presence of dyes imparts an intense color to effluents, which lead to environmental problem and algae undoubtedly have the potential to rapidly, efficiently and effectively remove dyes to low concentrations and less toxic compounds.

Decolorization experiments:

The present results show the potential of *M. aeruginosa* and *Pseudoanabaena* sp. to biodegrade a variety of dyes and azo dyes depending on the type of the dye and the algal species. The degree of decolorization of Reactive yellow 3RN, Disp. orange 2RL, Reactive Black NN and Disp. Red BS by cyanobacterial species were studied at 20 ppm dyes concentration for 3, 5, and 7 days incubation. Table 1 shows that generally there was an increase in the decolorization rate with increasing the incubation time. Table 1 also shows that *M. aeruginosa* has the maximum percentage of degradation after treatment with Disp. orange 2RL of 52.23%, 60.59%, and 65.07% after 3, 5, and 7 days of incubation, respectively.

Decolorization of Disp. Red BS by *Pseudoanabaena* sp. is also affected by the algal growth and incubation period, where 60.34% was degraded after 3 days that increased to 70.68% after 5 days and to 78.44% after 7 days of incubation as shown in table 1. On the other hand, 20 ppm of the same dye was decolorized and degraded by *M. aeruginosa*, the percentages of decolorization were 41.37%, 47.41%, and 50.01% after 3, 5, and 7 days of incubation, respectively. Also *M. aeruginosa* removed 51.16%, 52.31%, and 55.12% of Reactive Black NN after 3, 5, and 7 days of incubation, respectively, while the decolorization of the same dye by *Pseudoanabaena* sp. was 30.21% after 3 days which increased to 32.15% and 36.24% after 5 and 7 days, respectively, as shown in table 1.

The decolorization of Reactive yellow 3RN by *Pseudo anabaena* sp. was 55.71% at 3 days and increased by 57.81% and 58.47% after 5 and 7 days of incubation, respectively. The decolorization of the same dye by *M. aeruginosa* was 47.83 % after 3 days and this value increased to 49.21% and 52.47% respectively, after 5 and 7 days as shown in table 1.

Many cyanobacterial strains possess to their outer membrane, additional surface substances mainly of polysaccharide nature (Bertocchi *et al*., 1990). De philippis *et al*. (2001) surveyed the ability of cyanobacteria polysaccharide to remove pollutants from aqueous solutions and this explain why *Pseudoanabaena* sp. had a decolorization ability higher than *M. aeruginosa*.

In the degradative pathway of azo dyes by algae there are three limiting factors:

(1) Degradability of dyes, this is mainly dependent on the structural features of dyes.

(2) Ability of algae to utilize dyes, this was determined by algal physiological characteristics; and

(3) effects of environmental conditions (Jinqi and Houtian, 1992). In the current study, the isolated algae have the ability to degrade the industrial dyes and azo dyes pollutants, and this is determined by algal physiological characteristics and dependent on the structure of dyes.

The obtained results agree with that obtained by Chen *et al*. (2003) who reported that decolorization of dyes by microorganisms may be due to adsorption to biomass of the algae or biodegradation action by the algae. The amount of color removal varies with varying initial dye concentrations. The removal of dyes by algae and microorganisms was concentration dependent and approximately attributed to the bioconversion (Aydin and Baysal, 2006). Bafana *et al*. (2008) concluded in this study that the decolorization of azo dyes may be attributed to biological azo dye reduction and adsorption of the dye on the surface of algal biomass.

Govidan (1979) reported that bacteria are the main consumers of organic compounds in algae- bacteria degradation system in ponds stabilization. The author also concluded that the algal component primarily contribute to photosynthesis and serve as oxygen source for the aerobic action of bacteria. The results of this study, however, suggest that algae also have a direct effect on the degradation of dyes.

From table 1 we can record that;

- *Pseudoanabaena* sp. can degrade efficiently Disp. Red BS and Reactive yellow 3RN (Azo dye)

- While *M. aeruginosa* can well degrade Disp. Orange 2RL (Azo dye) and Reactive Black NN.

Table 1. Percentage of biodegradation of different dyes and azo dyes by *Pseudo anabaena* sp. and *Microcystis aeruginosa* (± means the standard error of the mean of three replicates.)

Degradation percentage (%)			
Dyes name	Day	*Pseudo anabaena* sp	*Microcystis aeruginosa*
Disp.orange 2RL (Azo dye)	3	10.55 ± 4.410	52.23 ± 3.93
	5	33.73 ± 6.943	60.59 ± 3.292
	7	42.98 ± 4.630	65.07 ± 2.56
Tracid red Bs	3	60.34 ± 2.073	41.37 ± 1.520
	5	70.68 ± 2.791	47.41 ± 0.499
	7	78.44 ± 2.485	50.01 ± 0.288
Reactive Black NN	3	30.21 ± 0.256	51.16 ± 2.285
	5	32.15 ± 0.604	52.31 ± 2.499
	7	36.24 ± 0.508	55.12 ± 1.529
Reactive Yellow 2RN (Azo dye)	3	55.71 ± 0.232	47.83 ± 1.180
	5	57.81 ± 0.538	49.21 ± 0.942
	7	58.47 ± 0.127	52.47 ± 1.221

Dry weight estimation:

Table 2 shows that different dyes significantly decreased the dry weight of *Pseudoanabaena* sp. as compared with that of control. However, there is a gradual slight increase in growth by increasing incubation time and this is concomitant with increase in the degradation ratio.

Table 2. Effect of different dyes on growth of *Pseudo anabaena* sp. measured as biomass dry weight (gm/l)

	Pseudo anabaena sp.				
Day	control	Disp.orange2RL	Reactive yellow 3RN	Disp.Red BS	Reactive Black NN
3	1.30 ± 0.283	0.82 ± 0.600	1.18 ± 0.440	1.14 ± 0.183	0.81 ± 0.245
5	1.47 ± 0.176	0.93 ± 0.066	1.27 ± 0.275	1.21 ± 0.516	0.97 ± 0.098
7	1.87 ± 0.133	1.27 ± 0.466	1.41 ± 0.127	1.40 ± 0.450	1.12 ± 0.144
L.S.D*	0.340	0.067	0.026	0.206	0.500

Each value is mean ± SE (n=3), (SE means the standard error of the mean of three replicates.)

* The mean difference is significant at the 0.05 level

The results also show that the high inhibition of dry weight (0.81 gm/l, 0.97 gm/l, and 1.12 gm/l) of *Pseudoanabaena* sp. grown in the presence of Reactive Black NN as compared with the control (1.30 gm/l, 1.47 gm/l, and 1.87 gm/l) after 3, 5, and 7 days of incubation, respectively. The growth of the same alga with Disp.orange2RL was decreased to (0.82 gm/l, 0.93 gm/l, and 1.27 gm/l) after 3, 5, and 7 days of incubation, respectively compared with the control. The results also show that there was reduction in the growth of *Pseudoanabaena* sp. on Disp.Red BS by (1.14 gm/l, 1.21 gm/l, and 1.40 gm/l) after 3, 5, and 7 days of incubation, respectively as compared with that of control. The results also illustrated that the lowest inhibition in dry weight of *Pseudoanabaena* sp. was attained with Reactive yellow 3RN (1.18 gm/l, 1.27 gm/l, and 1.41 gm/l) after 3, 5 and 7 days of incubation, respectively as compared with the control (Table 2).

Different dyes significantly decreased the dry weight of *M. aeruginosa* as compared with that of control. However, there is a gradual increase in growth by increasing incubation time and this is concomitant with increase in the degradation ratio as shown in Table (3). The present results recorded that

the dry weight of *M. aeruginosa* on Disp.Red BS was inhibited by (0.22 gm/l, 0.35 gm/l, and 0.59 gm/l) compared with control (0.60 gm/l, 0.80 gm/l and 1.00 gm/l) after 3, 5 and 7 days of incubation, respectively. The growth of *M. aeruginosa* after treatment with Reactive yellow 3RN was decreased to (0.36 gm/l, 0.58 gm/l, and 0.73 gm/l) after 3, 5 and 7 days of incubation, respectively, as compared with that of control. It is also clear that the growth of *M. aeruginosa* on Reactive Black NN was decreased to (0.27 gm/l, 0.47 gm/l, and 0.61 gm/l) after 3, 5, and 7 days of incubation, respectively as compared with the control. The results also illustrated that the lowest inhibition in dry weight of *M. aeruginosa* was attained with Disp.orange2RL (0.40 gm/l, 0.67 gm/l, and 0.86 gm/l) after 3, 5 and 7 days of incubation, respectively as compared with the control (Table 3).

Table 3. Effect of different dyes on growth of *Microcystis aeruginosa* measured as biomass dry weight (gm/l)

Microcystis aeruginosa					
Day	control	Disp.orange2RL	Reactive yellow 3RN	Disp.Red BS	Reactive Black NN
3	0.60 ± 0.200	0.40 ± 0.001	0.36 ± 0.061	0.22 ± 0.011	0.27 ± 0.066
5	0.80 ± 0.305	0.67 ± 0.066	0.58 ± 0.064	0.35 ± 0.043	0.47 ± 0.066
7	1.00 ± 0.416	0.86 ± 0.066	0.73 ± 0.066	0.59 ± 0.070	0.61 ± 0.197
L.S.D*	0.092	0.003	0.017	0.004	0.240

Each value is mean ± SE (n=3), (SE means the standard error of the mean of three replicates.)

* The mean difference is significant at the 0.05 level

The present results are in agreement with that obtained by El-Sheekh *et al*. (2009) who reported that different concentrations of the industrial pollutants such as dyes significantly decreased the dry weight production of different algae as compared with control by increasing incubation time. Also, Chia *et al*. (2013 a &b) reported that the indigo dye exhibit negative effects on the growth and biomass production of the freshwater microalga *Chlorella vulgaris* at environmentally relevant concentrations. Dyes were capable of interacting in an additive, synergistic or even antagonistic way to affect the growth, biomass production and biochemical composition of microalgae (Chia *et al.*, 2013a).

Protein content:

The obtained results demonstrate that the protein content was increased with respect to incubation period. As shown in table 4, different dyes influence protein content in *Pseudoanabaena* sp. The effect of reactive yellow 3RN on the protein content of *Pseudoanabaena* sp. was 31.74 mg/ml after 3 days of incubation and increased to (43.03 mg/ml and 48.62 mg/ml after 5 and 7 days of incubation, respectively), compared to those of control (45.94 mg/ml, 57.27 mg/ml, and 65.07 mg/ml) after 3, 5 and 7 days respectively. In case of Disp.orange 2RL the protein content was (21.32 mg/ml, 23.27 mg/ml, and 25.47 mg/ml) after 3, 5, and 7 days of incubation respectively, compared to the control. Then the protein content of *Pseudoanabaena* sp. treated by Reactive Black NN was (20.55 mg/ml, 21.73 mg/ml, and 23.78 mg/ml) after 3, 5 and 7 days of incubation respectively, compared to the control. Finally, the protein content of *Pseudoanabaena* sp. treated by Disp.Red BS was (43.32 mg/ml, 50.61 mg/ml, and 56.87mg/ml) after 3, 5, and 7 days of incubation, respectively, compared to the control.

Table 4. The effect of different dyes on the protein content of *Pseudo anabaena* sp.

Protein content (mg/ml)				
Dyes \ Days	3	5	7	L.S.D*
control	45.94 ± 2.93	57.27 ± 5.19	65.07 ± 3.79	0.118
Reactive yellow 3RN	31.74 ± 1.45	43.03 ± 4.28	48.62 ± 1.58	0.021
Disp.orange2RL	21.32 ± 0.512	23.27 ± 0.369	25.47 ± 0.438	0.002
Reactive Black NN	20.55 ± 0.678	21.73 ± 0.894	23.78 ± 0.971	0.037
Disp.Red BS	43.32 ± 2.223	50.61 ± 3.84	56.87 ± 2.29	0.028

Each value is mean ± SE (n=3), (SE means the standard error of the mean of three replicates.)

* The mean difference is significant at the 0.05 level

The protein content of *Pseudoanabaena* sp. showed the highest content after 7 days with Disp.Red BS, then Reactive yellow 3RN, and Disp.orange2RL and the lowest protein content was with Reactive Black NN.

Table 5 indicated that different dyes influence protein content in *M. aeruginosa.* The Reactive yellow 3RN induce a decrease in the protein content of *M. aeruginosa* was 38.31 mg/ml after 3 days of incubation and increased to 44.46 mg/ml and 47.53 mg/ml

after 5 and 7 days, respectively, compared to those of control (43.08 mg/ml, 47.54 mg/ml, and 50.61 mg/ml) after 3, 5, and 7 days, respectively. In case of Disp.orange2RL the protein content was 30.46 mg/ml, 33.69 mg/ml, and 40.15 mg/ml after 3, 5, and 7 days of incubation, respectively, compared to the control. Then the protein content of *M. aeruginosa* treated by Reactive Black NN was 23.43 mg/ml, 24.81 mg/ml and 27.74 mg/ml after 3, 5, and 7 days of incubation, respectively, compared to the control. Finally, the protein content of *M. aeruginosa* treated by Disp.Red BS was 23.78 mg/ml, 24.66 mg/ml, and 26.24 mg/ml after 3, 5, and 7 days of incubation, respectively, compared to the control. The protein content of *M. aeruginosa* showed the highest content after 7 days with Disp.orange2RL, then Reactive Black NN, and Disp.Red BS and the lowest protein content was with Reactive yellow 3RN.

Table 5. The effect of different dyes on the protein content of *Microcystis aeruginosa*

	Protein content (mg/ml)			
Dyes \ Days	3	5	7	L.S.D*
control	43.08 ± 0.778	47.54 ± 0.694	50.61 ± 0.935	0.001
Reactive yellow 3RN	38.31 ± 1.62	44.46 ± 1.05	47.53 ± 1.27	0.000
Disp.orange2RL	30.46 ± 0.47	33.69 ± 0.223	40.15 ± 0.901	0.001
Reactive Black NN	23.43 ± 2.78	24.81 ± 0.894	27.74 ± 0.941	0.289
Disp.Red BS	23.78 ± 0.623	24.66 ± 1.02	26.24 ± 0.712	0.171

Each value is mean ± SE (n=3), (SE means the standard error of the mean of three replicates.)

* The mean difference is significant at the 0.05 level

These results illustrated in table 4 and table 5 are in agreement with that obtained by Sathyaprabha *et al*. (2011) who reported that protein concentration, carbohydrate concentration and chlorophyll – a content were lower as compared to the control. Also, Srashti (2013) reported that *Spirulina platensis* proved to be efficient in degrading the dyes like Congo red, mordant green and metanil yellow which show cytotoxicity at higher concentration as well as lower biochemical activity was low (i.e. carbohydrate content, chlorophyll content, protein content).

Azo reductase enzyme estimation:

Figure 2 shows that the azo reductase enzyme of *Pseudoanabaena* sp. after treatment with Reactive yellow 2RN. It is obvious that the addition of Reactive yellow 2RN induced azo reductase enzyme activity after 3, 5, and 7 days of incubation, by about 50.26%, 53.07%, and 55.64%, respectively, as compared with the control.

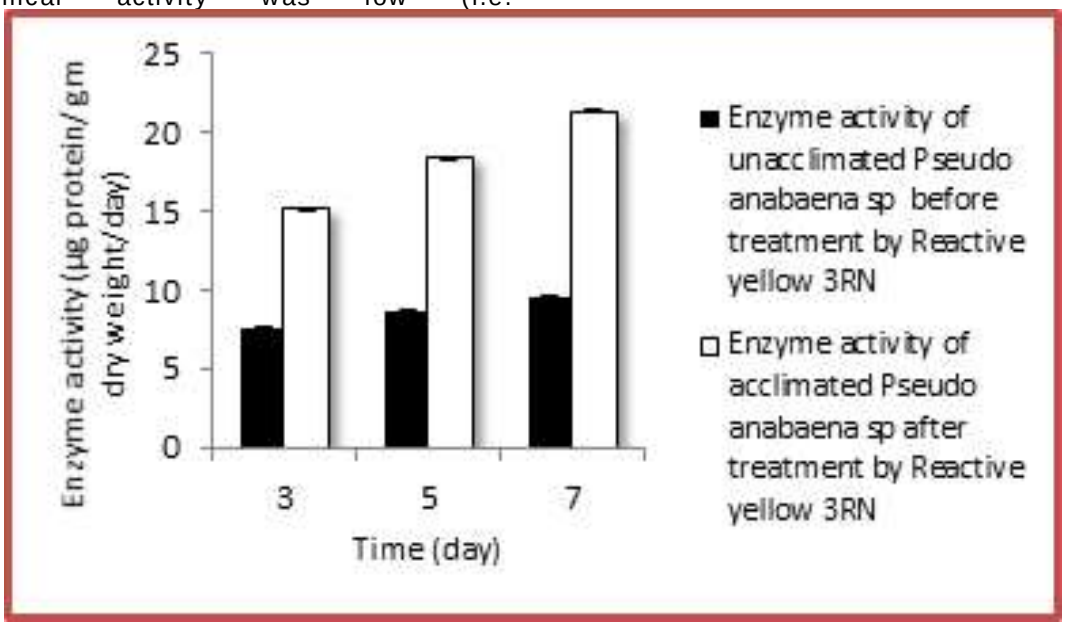

Fig. 2. The effect of azo reductase enzyme activity of *Pseudo anabaena* sp. before and after treatment by azo dye (Reactive yellow 3RN)

Also, the addition of Disp.orange 2RL induce the Azo reductase enzyme activity in *M. aeruginosa* by about (66.29%, 67.96%, and 68.04%, respectively), after 3, 5, and 7 days of incubation as compared with the control as shown in figure 3.

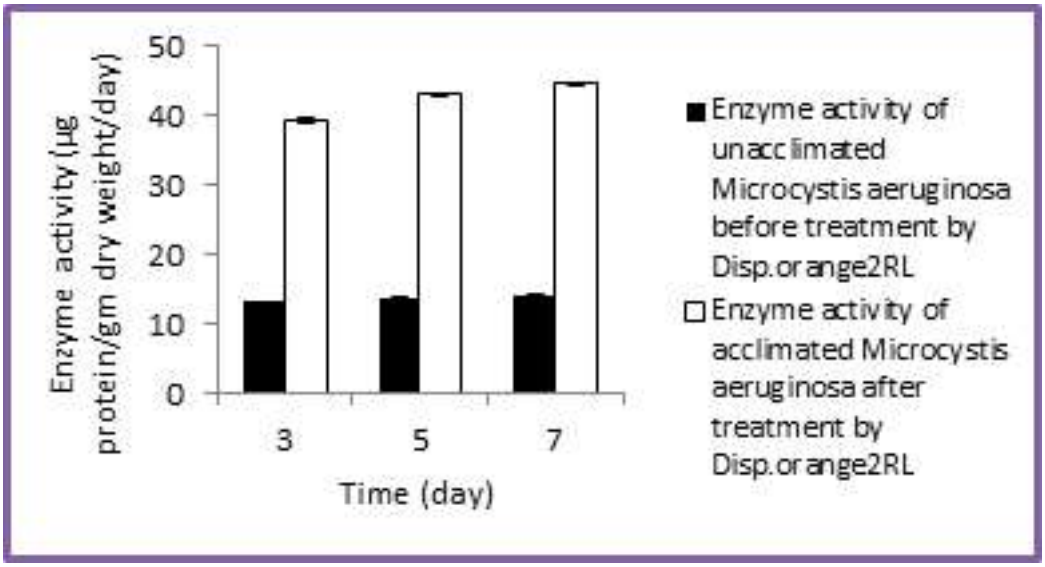

Fig. 3. The effect of azo reductase enzyme activity of *Microcystis aeruginosa* before and after treatment by azo dye (Disp.orange 2RL)

These results are agreement with that obtained by Urushigawa and Yonezawa (1977) who reported that algae can induce azo dye reductase activity under azo dye stress. Also, Pandey *et al*. (2007) stated that azo reductase can act in breaking down of the azo band as follows:

$$Ar - N{=}N - Ar` \xrightarrow{\text{azo reductase}} Ar - NH_2 + Ar` - NH_2$$

Moreover, these results also showed that activity of azo reductase in *Pseudo anabaena* sp. and *M. aeruginosa* was increased by addition of P-amino azo benzene. This indicates the probable induction of this enzyme by the dyes. Puvaneswari *et al.* (2006) stated that azo reductase of different microorganisms is useful for the development of biodegradation systems as they catalyze reductive cleavage of azo groups (–N=N–) under mild conditions.

FT-IR Analysis of Decolorized Samples:

Infrared analysis was used to determine the functional groups on the carbon surface and to identify the structure variation of the compounds before and after algal treatment. The absorption frequencies were shifted to higher wave numbers with the absorption of all the dyes after degradation (Kishore *et al*., 2015). The results recorded in figure 4 illustrated that there was difference in the IR peak of the biomass of *M. aeruginosa* before and after treatment with Reactive yellow 3RN dye and it shows reduction in azo bond within the range 1650 – 1546 cm^{-1} as indicated by shaded part.

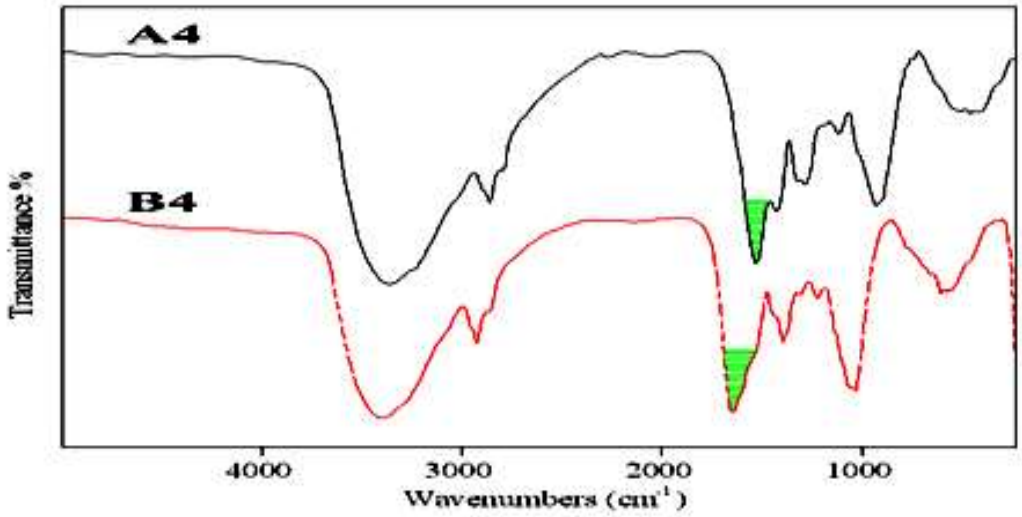

Fig. 4. Infrared of biomass of *Microcystis aeruginosa* (A4) control *Microcystis aeruginosa,* (B4) *Microcystis aeruginosa* after treatment by Reactive yellow 3RN.

Figure 5 shows the IR of biomass of *M. aeruginosa* with Disp.orange2RL before and after dye absorption and shows that some peaks are shifted or disappeared and some new peaks emerged after dye absorption. These results suggest that Reactive yellow 3RN dye degradation was the lowest percentage by (52.47%) than that of Disp.orange2RL (65.07%) after 7 days during incubation time.

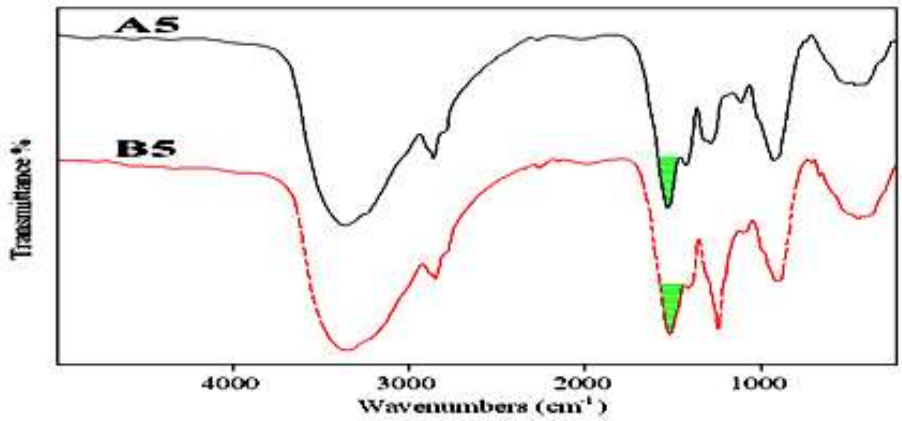

Fig. 5. Infrared of biomass of *Microcystis aeruginosa* (A4) control *Microcystis aeruginosa,* (B5) *Microcystis aeruginosa* after treatment by Disp.orange2RL

Figure 6 shows the IR of biomass of *Pseudo anabaena* sp. before and after treatment with Tracid red Bs and there was difference in the intensity of peaks especially in region from 1646 -1544 cm^{-1}. So, the degradation of this dye shows high percentage reached 78.44 %. These results may be due to the ability of algae to induce azo reductase enzyme under azo dye stress which is in agreement with that reported by Urushigawa and Yonezawa (1977).

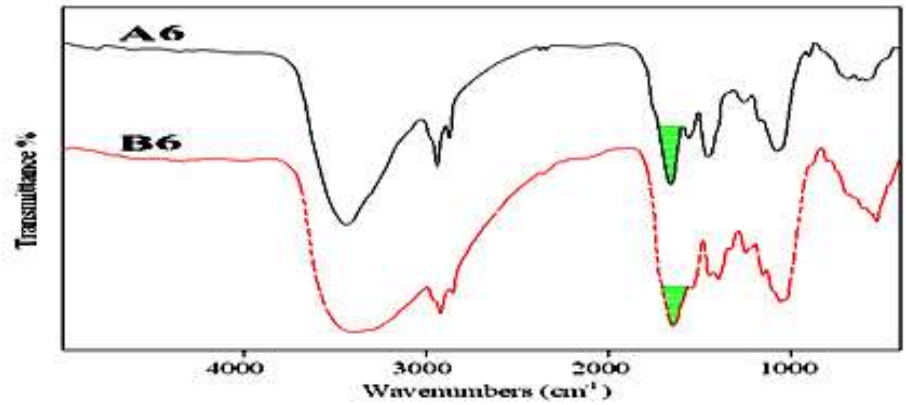

Fig. 6. Infrared of biomass of *Pseudo anabaena* sp. (A6) control *Pseudo anabaena* sp. (B6) *Pseudo anabaena* sp. after treatment by Tracid red Bs.

The infrared spectrum of Disp.orange2RL before and after *M. aeruginosa* action showed that there are shifting or disappearance of some peaks at 1516 cm^{-1} and 1600cm^{-1} for -N=N- stretching vibrations, (2977 cm^{-1}) for aromatic C-H stretching vibrations and 1342 cm^{-1} for NO_2 group, due to the degradation of Disp.orange2RL by *M. aeruginosa* and the percentage of degradation was 65.07% after 7 days incubation time. Therefore, the concentration of Disp.orange2RL was decreased after treatment by *M. aeruginosa* (Fig. 7). These results are agreement with that obtained by Kishore *et al*. (2015).

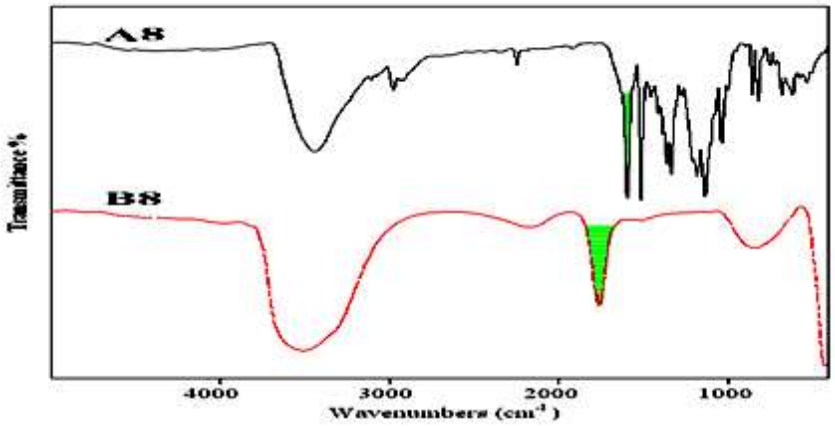

Fig. 7. Infrared spectrum of Disp.orange2RL before *Microcystis aeruginosa* action (A7) after *Microcystis aeruginosa* action (B8).

As shown in figure 8 there are difference in the infrared spectrum of Tracid red Bs before and after *Pseudoanabaena* sp action, it is evident that there were stretching vibration of sulphonic acid group (SO_3^-) diminished within the range 1251 – 1110cm^{-1} and stretching vibration of Tracid red Bs at 1598 cm^{-1} and FTIR absorption band at 2971 cm^{-1} assigned to a symmetric CH_2 bending vibration, as indicated by shaded part in the figure. These results confirm that the concentration of Tracid red Bs was decreased after treatment by *Pseudoanabaena* sp.

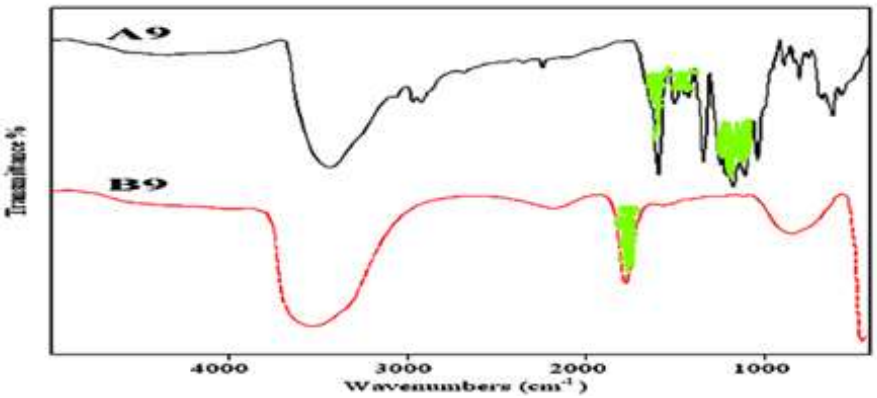

Fig. 8. Infrared spectrum of Tracid red Bs before *Pseudo anabaena* sp. action (A9), after *Pseudo anabaena* sp. action (B9)

CONCLUSION:

In this work, both algae have sufficient biodegradation potential for removing some dyes and azo dyes toxicants from its aqueous solution. The results revealed that the removal of these dyes depend on the algal species, its growth rate and the structure of dyes. It has been also found that *Pseudoanabaena* sp. has more potential for biodegradation than *M. aeruginosa*. Depending on the final results in this study, we may conclude that both species of algae can be used for removing these dyes. Knowledge from present work may be employed on large scale at actual contamination sites. Our future study aims to find out the mechanism of biodegradation of these dyes by *Pseudoanabaena* sp. and *M. aeruginosa*.

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التحلل الحيوي لبعض الصبغات بواسطة انواع الطحالب الدقيقة (السيانوبكتيريا) *Pseudo anabaena* sp. و *Microcystis aeruginosa*

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أصبحت مشكلة التلوث البيئي الصناعي خطرا يهدد الجنس البشرى وكل الكائنات الحية والنباتات، وقد ازداد الوعي العام الآن حول الآثار السلبية المحتملة للنفايات السائلة الصناعية الملوثة بمختلف الملوثات، بما في ذلك الأصباغ على البيئة. وتعتبر النفايات السائلة الناتجة عن صناعة الصباغة هي واحدة من أكثر مياه النفايات تعقيدا التي ينبغي معالجتها وذلك بسبب احتوائها على المركبات السامة أو المسببة للسرطان أو الطفرات للإنسان وجميع الكائنات الحية. وقد وجد العديد من الطرق المختلفة لإزالة هذه الصبغات من النفايات الصناعية منها المعالجة الفيزيائية والكيمائية وايضا المعالجة البيولوجية والتي تم التركيز عليها في هذه الدراسة وذلك باستخدام بعض أنواع الطحالب الدقيقة. وقد أجريت هذه الدراسة للبحث في كيفية ازالة الصبغات السامة وايضا التحلل الحيوي لبعض الاصباغ باستخدام نوعين من الطحالب الدقيقة (السيانوبكتيريا) المعزولة من المناطق الصناعية الملوثة وهم بزيدوانابينا و ميكروسيتسس اريوجينوزا وتم دراسة قدرة هذه الانواع على التحلل الحيوي لأربعة أنواع من الصبغات المسببة للتلوث الصناعي وهم Disp.orange 2RL و reactive yellow 3RN و Reactive black NN وصبغة Tracid red BS. وأظهرت النتائج أن ازالة هذه الأصباغ تعتمد على نوع الطحالب ومعدل نموه وايضا التركيب الكيميائي للصبغات المستخدمة. وقد سجل طلحب ميكروسيستس اريوجينوزا أعلى نسبة تحلل لصبغة Reactive black NN و Disp.orange 2RL بنسبة 55.12 % و 65.07 % على التوالى بعد 7 أيام. بينما سجل طحلب بزيدوانابينا أعلى نسبة تحلل لصبغة Reactive yellow 3RN وTracid red BS بنسبة 58.47% و 78.44% على التوالي بعد 7 أيام. أيضا تم دراسة تأثير انزيم azo reductase enzyme الموجود في الطحالب والمسؤول عن تكسير مركبات الازو وتحويلها الى مركبات امينية عن طريق كسر رابطة الازو الموجودة في الصبغات (-N=N-). واوضحت النتائج بالنسبة لطحلب ميكروسيستس اريوجينوزا بعد المعاملة بصبغة Disp.orange 2RL تحفيز انزيم azo reductase enzyme بنسبة 68.04% بعد 7 أيام من التحضين ومع طحلب بزيدوانابينا بعد المعاملة بصبغة Reactive yellow 3RN كانت النسبة 55.64 % بعد 7 ايام . وايضا تم دراسة نواتج التحلل الحيوي عن طريق التحليل الطيفي واستخدام الاشعة تحت الحمراء.