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EFFECT OF TEMPERATURE VARIATION ON THE EFFICACY OF *CHLORELLA VULGARIS* IN DECOLORIZATION OF CONGO RED FROM AQUEOUS SOLUTIONS

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ABSTRACT : The current study deal with the effect of temperature variation on the efficacy of greenalgae *Chlorella vulgaris* on the removal of the cancerous synthetic dyes of Congo red from their aqueous solutions. An algae isolated from local Iraqi aquatic environments and cultured under controlled laboratory conditions using different concentration (50, 150 and 250 ppm) and different temperatures (15, 25 and 35ºC) in different period times (3, 7, 9, 11 and 13 days). The results showed that the best temperature for decolorization by *Chlorella vulgaris* was (35ºC) for an period of (9 days) and concentration (50) ppm with the complete removal (100%), the efficiency of the *Chlorella vulgaris* in the removal of pollutants fall under the influence of many different environmental factors including temperature. The capacity of *Chlorella vulgaris* to survive and grow in the presence of high concentrations of the congo red dye, show that this strain may possess potential to be used in bioremediation of dyes contaminated environments.

Key words : Temperature, congo red, *Chlorella vulgaris*, water pollution, biotreatment.

INTRODUCTION

Biotreatment involved the use of biological systems for the removal or reduction of pollutant from different environmental media (Luka *et al*, 2018). It has confirmed to be a safe, effective, low-cost and environmentaly friendly alternative for sustainable treatment environmental pollution by hazardous and recalcitrant pollutants (Singh, 2006; Shukla *et al*, 2010). Biotreatment is use to convert organic pollutants into harmless metabolites or to mineralize the pollutants into carbon dioxide and water (Alexander, 1999). Microalgae have many roles in biotreatment and are widely used to remove different aquatic pollutants (Hwang *et al*, 2016). Specifically, greenalgae is used in transforming and degrading congo red and removing these compounds from the environment (Gupte *et al*, 2016). They have been used to degraders recalcitrant pollutants such as *Chlorella vulgaris* (Vimonses *et al*, 2009).

Congo red (CR) is a benzidine-based, direct, anionic diazo dye prepared by coupling tetrazotised benzidine with two molecules of napthionic acid. Congo red is the first synthetic azo dye produced that is capable of dying cotton directly. Congo red containing effluents are generated from a number of industrial activities: textiles, printing and dyeing, paper, rubber, plastics industries (Purkait *et al*, 2007; Mittal *et al*, 2009). Exposure to the dye has been known to cause allergic reactions. The substance is considered as toxic exhibiting acute, algal, bacterial, protozoan, cutaneous, environmental, microbial, yeast toxicity; cytotoxicity; genotoxicity; hematotoxicity; neurotoxicity, as well as carcinogenicity and mutagenicity (Han *et al*, 2008; Sabnis, 2010; Shu *et al*, 2015).

The capability of CR to form carcinogenic amines such as benzidine through cleavage of one or more azo groups is the reason why it falls under the category of banned azo dyes (Raymundo *et al*, 2010). The recalcitrance of CR has been attributed to the presence of aminobiphenyl group and azo bonds, two features generally considered as xenobiotic (Pielesz, 1999; Sponza and Isik, 2005).

MATERIALS AND METHODS

Culture and algal growth

The unicellular green microalga *C. vulgaris* (Aksmann and Tukaj, 2008) was grown on Chu-10 (Chia *et al*, 2013) for 15 days under constant laboratory conditions at 25±2°C and a light system of 16:8 hours light/ dark (Pinheiro *et al*, 2004). This culture transported into 1000 ml of media and incubated for 14 days to increase of algal biomass (Chia *et al*, 2013).

Experimental design

Chlorella vulgaris (100ml) was cultured in 1 liter

from Chu-10 medium and left for at least two weeks before starting experiment under constant laboratory conditions. This alga is exposure in congo red and delivered to the culture medium to prepared final concentrations (50, 150 and 250 mg/l), which is prepared in 250 ml of liquid culture media and using culture media with algae as control without adding congo red under same constant conditions to compared with effect alga by cong red, anther control using culture media and congo red without algae in the same concentration in order to study the effect of light on congo red and the compared to treatment by algae. Three replicates were done for this experiment. DMSO (0.1% v/v) control sample was prepared for each experiment; this chemical had shown no significant effect on *C vulgaris* (Tredici, 2004). We take (3 ml) of the sample. Samples were taken at different intervals under sterile conditions for measurements. The samples were centrifuged at 5000 rpm for 15 min. The supernatant was evaluated via a light absorption method and percentage reduction rates were calculated after being compared with control (culture medium without algae) (Aksmann and Tukaj, 2008).

In the culture medium after 3, 5, 7, 9, 11 and 13 days. Dye decolourization at different temperature (15, 25 and 35°C). In addtion, effect of different concentration of congo red on *C. vulgar*.

Spectroscopic analysis

Culture fluid without algae incubated under the same conditions as the test specimens was used as the control. Some of the suspension was assayed with the (UV–visible – Unvisible spectrum method) Accessory interface U.V vis. Spectrophotometer. (50, 150 and 250 ppm) congo red dye solution in distilled water was scanned spectrophotometrically (Systronics20) to find out maximum absorbance (λmax) for congo red, the wavelengths of congo red dyes were as follows, 50 (498 nm), 150 (502 nm), 250 (523 nm), and measurement the culture fluid with algae to found Congo red residual concentration is measuring by spectrophotometer after period treatment (Zamora *et al*, 2015).

The concentration of residual congo redwas determined by the following formula :

$$\text{Removal Effeciency RE (\%)} = \frac{A-B}{A}\times 100$$

A = Initial absorbance of Congo red

B = congo red absorbance after treatment.

Statistical Analysis of fall treatments were performed by sigma blotprogram (version 21).

RESULTS AND DISCUSSION

Biotreatment of congo red

The results showed that *C. vulgaris* had high ability to removed congo red after 3, 5, 7, 9, 11 and 13 days of treatments because *C. vulgaris* is identified as tolerating and effectively degrading congo red that may be toxic in the environment and converting these compounds into non-toxic form. A survey of the literature suggests that algae are capable of degrading azo dyes through an induced form of anzo reductase) (Patel and Tiwari, 2015). Color removal by algae is duemechanism of assimilative utilization of chromophores for the production of algal biomass, CO_2 and H_2O transformation of colored molecules to non-colored ones and adsorption of chromophores on algal biomass. Several species of *Chlorella* sp. and *Oscillatoria* sp. are capable of degrading azo dyes to their aromatic amines and can further metabolize the aromatic amines to simpler organic compounds or CO_2 (Acuner and Dilek, 2004; Vijayaraghavan and Yun, 2008). The results observed significant differences ($p \leq 0.01$) between treatments and residual concentration) attributes the decolorization to biosorption followed by bioconversion and biocoagulation using algae. It has been reported that more than 30 azo compounds canbe biodegraded and decolorized by *Chlorella pyrenoidosa*, *Chlorella vulgaris* and *Oscillateria tenuis*, with the azo dyes decomposed into simpler aromatic amines (Mohan *et al*, 2002). These results in comparison with other studies (Acuner and Dilek, 2004) on the same alga, showed degradation of congo red, where the removal percentage was 83% (Yan and Pan, 2004).

The results of use by *C. vulgaris* to degradation congo red dye showed significant differences ($p \leq 0.01$) between different treatments and residual concentration after (3, 5, 7, 9, 11, 13) days (Table 1, Fig. 1) shows removed of congo red in temperature 15^0C in compared with control. The higher removal percentage efficiency after 13 days of treatment, in concentration 50ppm the removal percentage was 71%. Minimum removal percentage efficiency showed after 3 day of treatment was 24%.

While in concentration 150 ppm, the higher removal percentage recorded in 13 day of treatment was 66%, while the minimum removal percentage efficiency showed after 3 day was 18%.

The result showed higher removal percentage efficiency was 59% recorded by concentration 250 ppm after 13 day, but lowest removal percentage was 11% after 3 day of treatment.

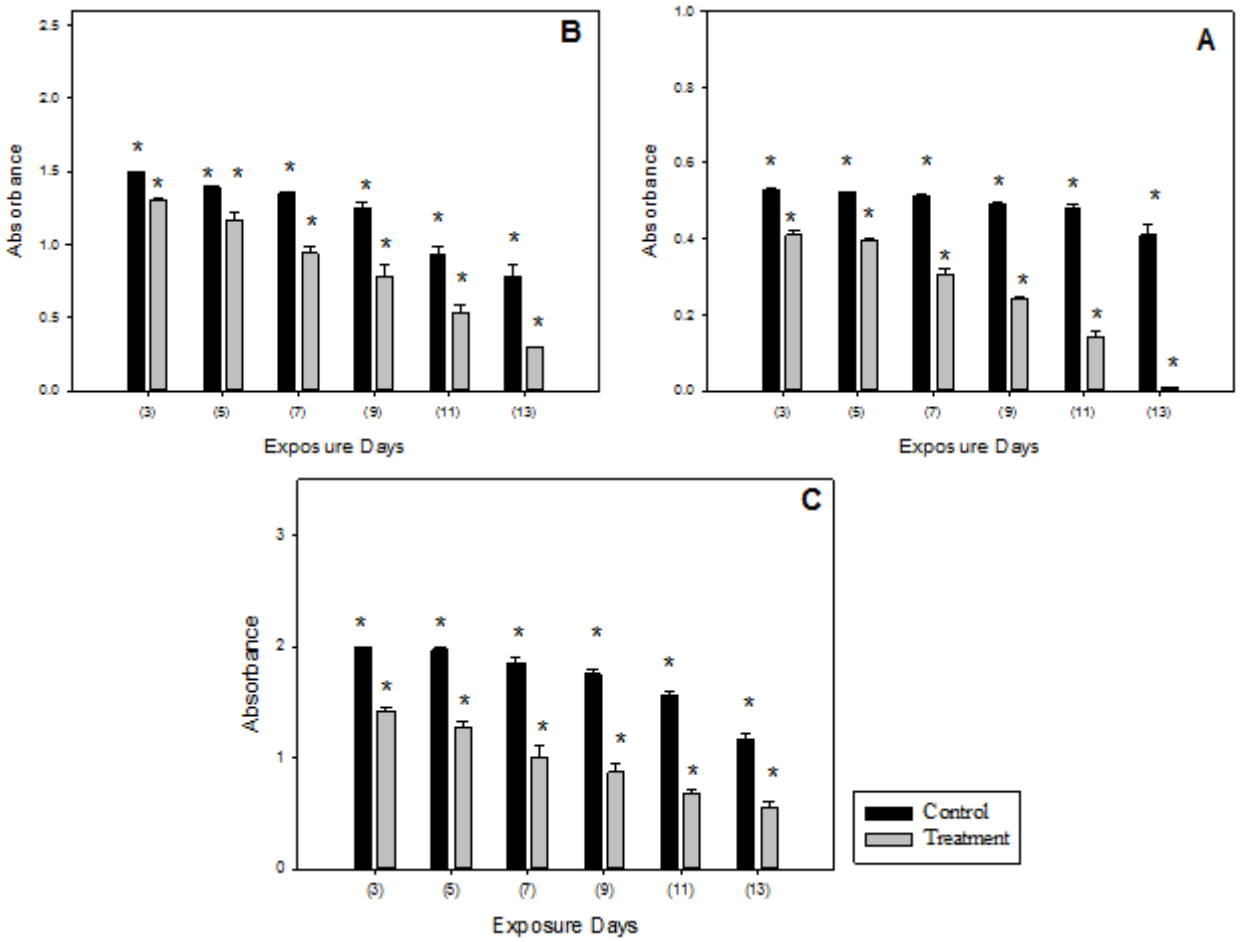

Fig. 1 : Effects of *Chlorella vulgaris* on decolorization of congo red at temperature 15°C and concentration (A = 50,B = 150 and C = 250mg/L). * Significant (≤ 0.01).

Table 1 : Decolorization of concentration of congo red at temperature 15°C by *C. vulgaris.*

Dye concentration (ppm)	Dye removal %					
	3day	5day	7day	9day	11day	13day
50	24	29	36	46	56	71
150	18	25	33	42	50	66
250	11	16	30	39	49	59

Table 2 : Decolorization of concentrationof congo red at temperature 25°C by *C. vulgaris*.

Dye concentration (ppm)	Dye removal %					
	3day	5day	7day	9day	11day	13day
50	26	34	49	57	71	98
150	22	24	43	53	67	79
250	12	19	34	42	66	72

Table 2, Fig. 2 shows removed of congo red in temperature 25^0C the maximum removal percentage efficiency after 13 days in concentration 50 ppm was 98% as maximum value but minimum removal percentage efficiency was 26% recorded after 3 day of treatment.

While in the concentration 150 ppm the result showed maximum removal percentage in 13 day of treatment 79%, while minimum removal percentage efficiency after 3 day of treatment the was 22%.

Also the maximum percentage in concentration 250 ppm was recorded after 13 day to 72%, but the minimum percentage was recorded after 3 day of treatment was 12%.

Table 3 and Fig. 3 shows the result of congo red in temperature 35^0C. In concentration 50 ppm, the maximum removal percentage efficiency recorded after 13 days of treatment 100% but the minimum removal percentage efficiency recorded after 3 day of treatment was 40%.

Table 3 : Decolorization concentration of congo red at temperature 35°C by *C. vulgaris.*

Dye concentration (ppm)	Dye removal %					
	3day	5day	7day	9day	11day	13day
50	40	52	82	96	100	100
150	29	36	49	59	69	86
250	11	18	40	46	59	74

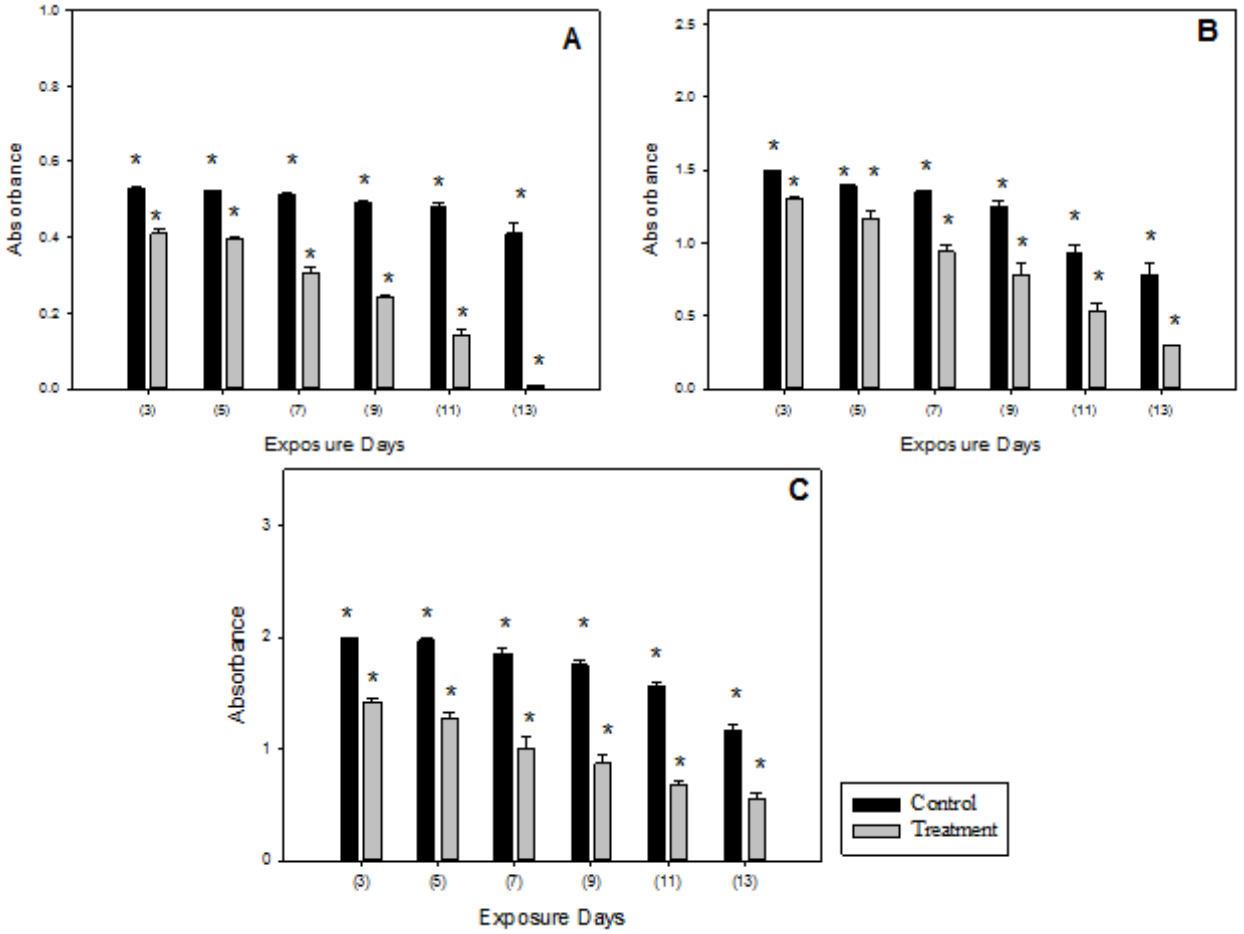

Fig. 2 : Effects of *Chlorella vulgaris* on decolorization of congo red at temperature 25°C and concentration (A = 50, B = 150 and C = 250 mg/L).

While, in concentration 150 ppm, the maximum removal percentage showed in 13 day of treatment was 86%, the minimum removal percentage efficiency after 3 day of treatment the removal percentage was 29%.

Minimum removal percentage efficiency in concentration 250 ppm was 74% after 13 day but removal percentage after 3 day of treatment was 11%.

Tables 1, 2, 3 shows the percentage of dye removal every day. The results showed that *C. vulgaris* appear higher ability to removal congo red after 3, 5, 7, 9, 11 and 13 days of treatments. Where in most results, there is a significant difference between treatments and control in all days of exposure.

The results indicated that the rate of dye removal progressively increased as the agitation time increased. The increase inthe rate of color removal with agitation time may be attributed to the decrease in the diffusion layer thickness surrounding the adsorbent particles. The equilibrium time increases with dye concentration. The highest values of removal percentage were found for the lowest initial dye concentrations of 50mg L^{-1}. After of exposure, the bioremoval percentages were 100%, but these percentages decreased to 66 and 59% with higher concentrations of the dye (150 and 250 mg L^{-1}). These study agreement with Namasivayam and Yamuna (1992) Yan (2004), Zamora *et al* (2015).

However, Hanan (2008) showed that the microalga *Chlorella vulgaris* was able to bioremove the azo dye Tartrazine by 48, 43 and 20% from initial concentrations of 5, 10 and 15 mg L^{-1}, respectively. He also demonstrated that the bioremoval ability of *Chlorella vulgaris* for the Yellow dye Tectilon G decreases when the dye concentration increases. They observed 69, 66 and 63% reductions by bioremoval of the concentrations 50, 200, and 400 mg L^{-1}, respectively. However, Acuner and Dilek (2004) reported that *Chlorella vulgaris* was unable to bioremove Methyl Red dye at the concentration of 20 mg L^{-1}.

Temperature is a factor of paramount importance for all processes associated with microbial vitality, including the remediation of water and soil. Study with the activation energy of microbial decolorization of azo dyes has been undertaken.

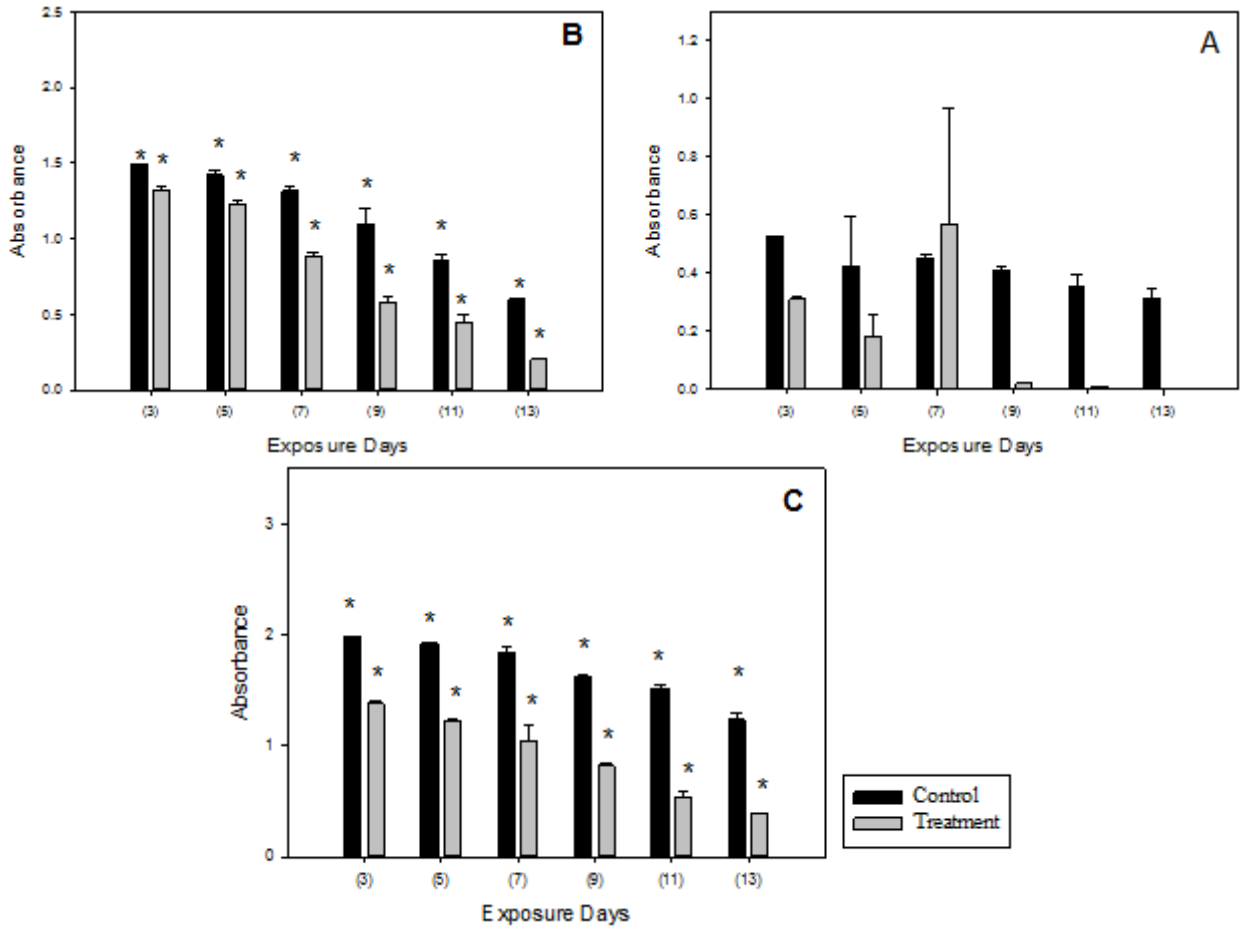

Fig. 3 : Effects of *Chlorella vulgaris* on decolorization of congo red at temperature 35°C and concentration (A = 50, B = 150 and C = 250 mg/L).

An increase in adsorption of the dye with the rise in temperature. Where at 35°C the removal rate is 100% while the temperature is 25°C with a removal rate of 98 but the exposure time is greater while at 15°C was 71% removal. These study agreement with El-Sheekh *et al* (2009), Mittal *et al* (2013).

CONCLUSION

The capacity of *Chlorella vulgaris* to survive and grow in the presence of high concentrations of the congo red dye, show that this strain may possess potential to be ýused in bioremediation of dyes contaminated environments.

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