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Adsorption of Direct Blue 2 Dye by Dry Biomass of *Bacillus cereus* from Aqueous Solution

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Abstract

One of the significant environmental problems is the pollution of water by dyes;. Biological treatment method was used, which is one of the effective ways to reduce this sort of pollution as it is environment friendly, economic and does not require any expertise. Under controlled conditions, this study estimated the efficacy of dry biomass for *Bacillus cereus* to reduce Direct Blue 2 dye from the aqueous solution. The optimum conditions such as pH values, contact time and concentration of dyes, were used in this research. The end results showed that the adsorption efficiency, when using a weight of bacterial biomass 0.2 g/50mL, reached 69.2% at a concentration of 10 ppm after one hour at 40°C and pH5. While it reached 54.6% for a concentration of 15 ppm with a contact time of three hours and pH 7. At 40ºC and one hour contact time, the adsorption efficiency for a concentration 20 ppm was 46.5% at pH9. The optimum conditions for the highest adsorption efficiency were achieved after one hour and pH5.

Keywords: Adsorption, Direct Blue 2, *Bacillus cereus*.

امتزاز صبغة Direct Blue 2 من المحلول المائي بواسطة الكتلة الحيوية الجافة لعزلة *Bacillus cereus*

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الخلاصة :

تعد مشكلة تلوث المياه بالصبغات من اهم المشاكل البيئية لذلك تم استخدام احد طرق المعالجات الحيوية والتي تعد من الطرق الفعالة في خفض الصبغات لكونها ذات جدوى اقتصادية ، صديقة للبيئة كما انها لاتحتاج الى خبرات .في هذه الدراسة تم اختبار كفاءة بكتريا*Bacillus cereus* على خفض صبغة ال Direct Blue 2من المحلول المائي تحت ظروف مسيطرعليها .استخدمت الظروف المثلى (قيم الأس الهيدروجيني وزمن التلامس بالاضافة الى تركيز الصبغة).اظهرت النتائج ان نسبة الإمتزاز عند استخدام وزن 0.2غرام 50 /مل من الكتلة الحيوية الجافةوصلت الى% 69.2 عند التركيز10 جزء بالمليون بعد ساعة واحدة عند درجة الحرارة40° م ودالة حامضية 5 ، بينما كانت 54.6% للتركيز 15 جزء بالمليون وزمن تماس3 ساعة ودالة حامضية7 ،اما بالنسبة لتركيز 20جزء بالمليون فأن نسبةالإمتزاز بلغت 49.5% عند دالة

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حامضية 9 وزمن تماس 1 ساعة ضمن درجة حرارة 40 م °. توصلت الدراسة الى ان الظروف المثلى التي رافقت اعلى فعالية للإمتزاز قد تحققت بعد 1 ساعة وعند الاس الهيدروجيني 5.

1. Introduction

Colored water originating from wastewater discharge from textile, plastic, food, paper, leather and cosmetics industries causes serious environmental pollution and raises concerns [1, 2]. Textile industries are a significant source of pollution due to dyes as they produce large quantities of highly colored effluents which are generally toxic and resistant to destruction by biological treatment methods. Azo dyes, defined by their distinctive azo group (−N = N−), are one of the most important groups of synthetic dyes, account for 50–70 % of the total 7.0×10^5 tons of dyes produced each year globally [3, 4]. It has been observed that a large proportion of these dyes do not bind to the target fibers during the dying process and are, hence, discharged into the environment along with the effluents [5, 3]. Widespread discharge of these dyes into the water resources causes a change in the variety of physicochemical properties, including light penetration, pH, electrical conductivity (EC), biochemical oxygen demand (BOD5) and chemical oxygen demand (COD) [6, 7]. As well as several of their degradation intermediates have also been linked to mutagenic and carcinogenic [5, 6, 8].

Various biological, chemical and physical methods are used to treat textile dye effluents [1, 9]. Although the chemical and physical processes are technically practical for treating color wastewater, they have disadvantages too such as intensive energy consumption, high operative cost and formation of hazardous byproducts [10, 11]. The biological treatment methods using several aerobic and anaerobic microorganisms have been selected for dye adsorbing, depending on their activities and adaptability to the environment [12]. These organisms have received increasing interest as they are eco-friendly and have high effectiveness with lower sludge production [13]. Several studies have reported that isolating and characterizing bacteria from textile effluent discharging sites is considered to be more efficient than fungi in most studies of azo dyes adsorbing. The benefits of using bacteria as adsorbent agent includes removing color over a wide range of pH in contrast with a narrow pH range of fungi and their ability to adsorb a wide range of azo dyes, utilization of azo compounds as the sole carbon and energy sources and oxidoreductive enzymes production for adsorption of synthetic azo dyes [10, 14, 15]. The aim of the current research was to reduce Direct Blue 2 by using *B. cereus* from aqueous solutions under optimal laboratory conditions.

2. Material and Methods

2.1 Preparing the solutions

The stock solution of Direct Blue 2 dye ($C_{32}H_{21}N_6Na_3O_{11}S_3$), which is used by Iraqi textile industry, was prepared by dissolving 0.1g/1000ml of dye in order to prepare the solution at a concentration of 100 ppm. Concentrations ranging 10, 15 and 20 ppm were prepared from ths diluted solution. Different pH (5, 7 and 9) values were used. The pH was adjusted using 0.1 M solutions of HCL and 0.1 molarity of NaOH. Figure 1 shows the molecular structure of Direct Blue 2 dye.

2.2 Determination of the calibration curve of the dye

To determine the wavelength of the formula by scanning electron spectroscopy, the appropriate focus is UV which uses UV rays to set the dye's UV rays wavelength during the scanning electron spectrogram procedure, the proper concentration of the dye and its comparison with the literature within the range 200-800 nm maximum wavelength. Maximum wavelength for Direct Blue 2 is 570 nm [16].

2.3 Preparing the dry biomass

Bacillus cereus isolate was obtained from the Directorate of Water and Environment, Ministry of Science and Technology, Baghdad, Iraq. This isolate was cultured on brain heart infusion to activate the bacteria after incubation at 37°C for 24 hours. 10 ml from an inoculum of culture was added to 1L of brain heart infusion and then incubated at 37°C for 48 hours under shaking conditions at 120 rpm/40 ± 20°C. It was then centrifuged at (6000 rpm/20 min). The supernatant was drawn to collect biomass and then dried at 30°C [1].

2.4 Dyes adsorption experiment

Adsorption experiment was performed by using 0.2 g of dried biomass for *B. cereus* with 50 mL of dyes in a glass container and then incubated under shaking conditions at 120 rpm/40 ± 20°C for different incubation times (1–3 hr.) and pH (5, 7 and 9) [17]. The samples were then taken after 1, 2 and 3hr. before being centrifuged at 6000 rpm for 20 min); to estimate and analyze the supernatant of dye concentration in the aqueous phase by UV-visible spectrophotometer and to determine changes in absorbance to assess the adsorption percentage of the DB2 dye [18] at 570 nm. Dye removal percentage was calculated by the following equation [19, 20]:

$$\textbf{Percentage of adsorption \%} = \frac{C_i - C_e}{C_i} \times 100$$

Where, Ci is the initial concentration of the sample and C_e is a final concentration at a specific time after the biodegradation process by the microorganism.

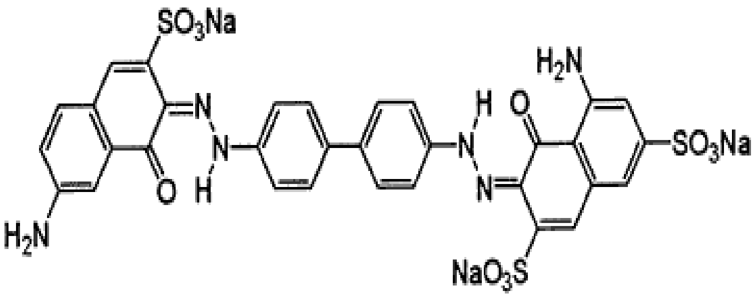

Figure 1: Molecular structure of Direct Blue 2 dye

2.5 Statistical Analysis:

Statistical Analysis System - SAS (2012) program was used to detect the effects of different factors on the study parameters. Least significant difference – LSD test (Analysis of Variation-ANOVA) was used to compare between means [21].

3. Results and discussion

Many factors such as dye concentration, pH values and contact time were optimized to be used to get the maximum capability of *B. cereus* to adsorption DB2 dye (dye concentration, pH values, and contact time). The results showed the adsorption percentage when used 0.2 g from dried *B. cereus* with different concentrations of the DB2 (10, 15 and 20) ppm, pH values (5, 7 and 9), and contact times (1, 2 and 3hr.) at 40°C temperature. The adsorption efficiency is displayed in Table 1. The residual of DB2 concentration after treatment is displayed in Figures 2, ‹3 and 4.

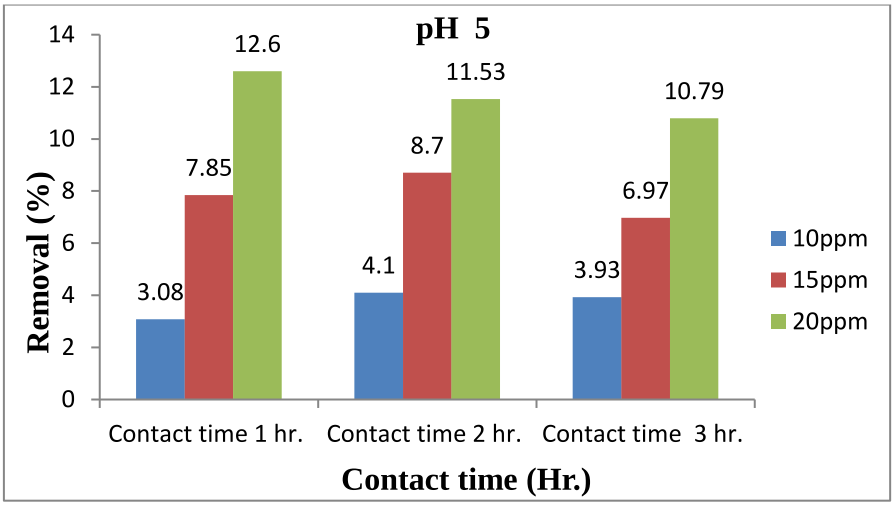

Figure 2: The residual DB2 (ppm) in the solution after adsorption process by *B. cereus* at 3 different concentrations and at pH 5.

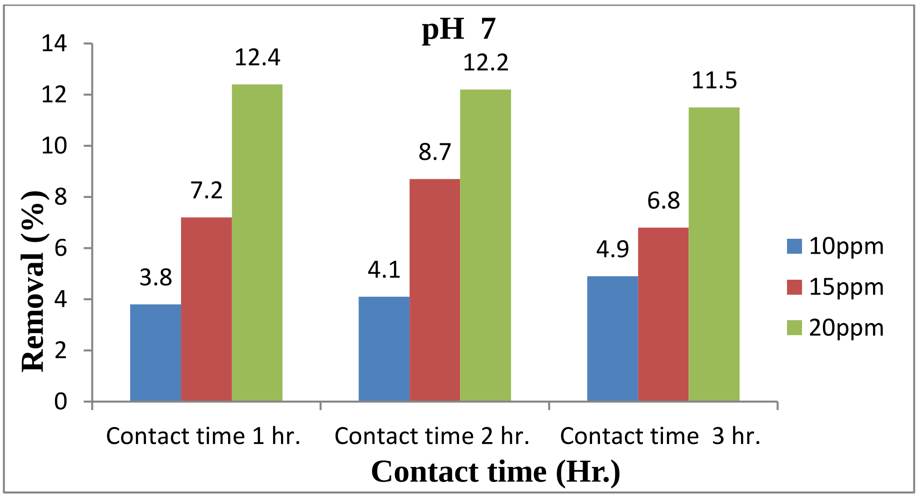

Figure 3: The residual DB2 (ppm) in the solution after adsorption process by *B. cereus* at 3 different concentrations and at pH 7.

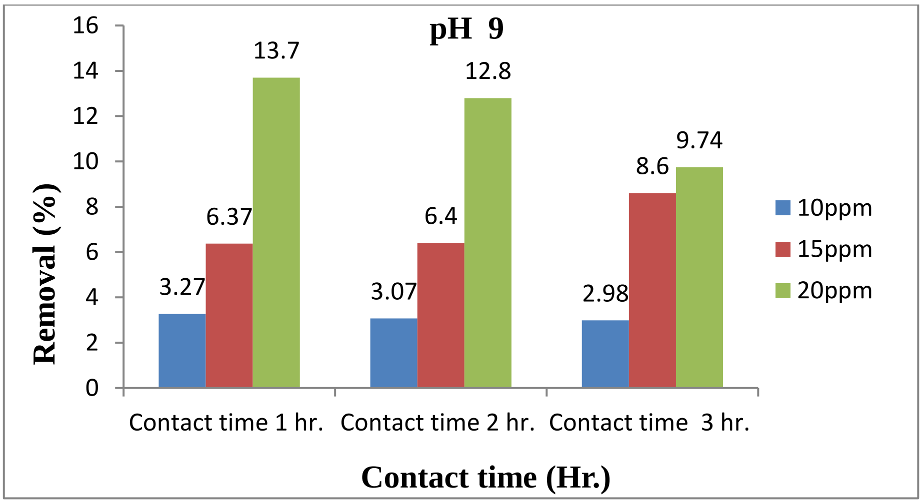

Figure 4: The residual DB2 (ppm) in the solution after the adsorption process by *B. cereus* at 3 different concentrations and at pH 9.

Table 1 shows that after 1hr the highest adsorption percentage for concentration 10 ppm was 69.2% at pH (5). While the lowest adsorption percentage for concentration 10 ppm after 3 hr was 14% at pH9. The highest adsorption percentage for concentration 15 ppm was 54.6% at pH (7), while the lowest percentage for adsorption of 15 ppm concentration after 1hr was 31.5% at pH (9). After 1hr and at pH (9), the highest adsorption percentage for 20 ppm concentration was 49.5%. While after 2hr and at pH (9), the lowest adsorption percentage recorded for 20 ppm concentration was 36%.

The results for adsorption efficiency of DB2 showed significant differences for 10 and 20 ppm concentrations at pH (5), while the 15 ppm concentration had non-significant effects after different contact time. Whereas at pH (7). 10 and 15 ppm concentrations had significant effects., And 20 ppm concentration had non-significant effects. The results show that all concentrations had significant effects at pH (9).

Table 1: Adsorption efficiency of DB2 by *B. cereus* at three different dye concentrations with different contact time at pH (5, 7 and 9)

pH	Initial Concentration (ppm)	Contact Time (hr.)			LSD Value
		1	2	3	
5	10	69.2%	68.1%	60.7%	7.92 *
	15	47.6%	53.5%	53.5%	6.44 NS
	20	37%	42.5%	46.05%	7.69 *
7	10	60.7%	59%	50.9%	7.25 *
	15	52%	41.6%	54.6%	8.05 *
	20	38%	39%	42.5%	6.13 NS
9	10	37%	36%	14%	8.62 *
	15	31.5%	52.4 %	35%	8.06 *
	20	49.5%	36%	40.5%	7.37 *
LSD value		9.38*	8.66*	*8.02**	.....
		* (P≤0.05).			

Previous research found that concentration of dye is one of important parameters that might have an impact on the efficacy of bacterial dye adsorption via a variety of factors, including toxicity imposed by greater dye adsorptions [22]. Adsorptions efficiency of DB2 by *B. cereus* increased at lower concentrations, whereas it decreased at higher concentrations. These observations can be explained that at higher concentration the saturation would happen and then the desorption will affect the removal efficiency and leading to its decrease. Whereas at low concentration there will be complete bindings and adsorption [23]. However, the current investigation demonstrated that greater dye concentration (20 ppm) was not hazardous to *B. cereus*. As result, this culture may have a lot of promise for cleaning industrial effluents with a lot of dye. Indeed, it has been noted that dye adsorptions vary depending on the structure and complexity of the dyes, specifically the kind and location of the substituent in the aromatic rings [13]. Dyes are, by their nature, organic compounds that contain electrons withdrawing and impulse groups that have an effect on the amount of adsorptions. The surface of *B. cereus* used contains negative and positive surface charge, as different physical forces arise from this surface, including the hydrogen bonding that occurs between the effective functional groups in the adsorbed compounds and hydroxyl groups on the surface as well as electrostatic, inductive and dipole forces. The surface of *B. cereus* contains negative surface charge as different physical forces arise from this surface, including the hydrogen bonding that occurs between the functional groups in the adsorbent compounds (dyes) and the hydroxyl groups on the surface as well as the electrostatic forces of attraction and the forces of induction and dipole. Hence, this culture could prove to be very promising for cleaning industrial effluents with dye. This is consistent with some studies conducted and thus leads to an increase in the dye bonding to the surface more than its tendency to bond to its solvent molecule. However, no statistical data was collected to validate this result. At pH> 4.5, the whole responsibility of the bio-sorbent surface is natural and there is a higher electrostatic interaction between the surface groups and species of dye [24]. As a result, biosorption was greater at pH > 4.5. Lower pH can be linked to a rivalry among proton and dye species [1]. Influence of pH on the rates of dye degradation was also studied, but no convincing association was found.

However, they remarked that reducing the pH caused an exponential rise in the adsorption rate, although this connection depended on the dye being tested [24]. The outcomes of this study are similar to the other studies [19] which found that, under anoxic conditions, the best pH for color removal was between 5.5 and 10.0, while Asfaram *et al.* mentioned that the most favorable pH for azo dyes adsorption would be neutral [20]. Data of this study disagrees with the findings of another study that the removal efficiency of concentration 10 ppm of direct blue (DB71) anionic dye on flint clay in aqueous solution was 44.7% at pH (5), while it was 89% at pH (7) and 75% at pH (9)[25]. The study results also conflicted with the findings of a study which found that the maximum adsorption capacity was 90.48 mg DB71 per 1 g of pistachio hull waste at pH 2, dye concentration of 100 ppm, at 50°C and 210 minutes contact time[26]. Another study isolated a potential bacterial strain, capable of degrading acid red 337 (AR 337) dye from a textile wastewater effluent. Using 16S rRNA sequence analysis, the bacterium was identified as *Bacillus megaterium* KY848339.1. The decolorization capability of *B. megaterium* for AR 337 dye was optimized. The bacterium could remove 91% of dye from a concentration of 500 ppm within 24 hr. where the solution pH was 7 and 30°C incubation temperature [27].

Another important factor is the contact time. Generally, the higher efficiency for dye adsorption at 10 ppm concentration was after 1hr. At the same time, after 3hr the lowest efficiency was 14%. While, after 3h and at 15 ppm concentration , the highest efficiency adsorption was 54.6%. And after 1 hour, the lowest efficiency reported was 31.5%, while at 20 ppm concentration and after 1 hour, it was 49.5%. And after 2 hours, the lowest efficiency for adsorption was 36%. This can be attributed to the extent of *B. cereus* growth which is proportional to the consumption of these dyes as an organic carbon source by multiplying cells, resulting in increased surface area and numerousnumber of active sites for the binding and accumulation of dyes [28]. The structure of azo dye can influence the rate of biodegradation. Some dyes biodegrade faster than others, contingent on the amount and the location of the azo linkages. Universally, the slower decreasing rate is the extra azo connection that must be broken. While few studies directly address this topic [29] that two poly-azo dyes had only moderate to variable biodegradation compared to four mono-azo and six di-azo dyes. According to a scientist, poly-azo dyes are less prone to deterioration than mono- or diazo dye types.

4. Conclusions

Dry biomass of *B. cereus* was reduced from Direct Blue 2 from aqueous solution so that the percentage of adsorption was 69.2% in an acidic medium and 40°C. It is possible to benefit from the results of the current study in treating industrial wastewater.

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