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Biodegradation of Two Textile Dyes by *Bacillus Subtilis*

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Abstract: This study revealed the efficiency of *Bacillus subtilis* in degrading two textile dyes (disperse red and disperse yellow), the rates of red dye removal when measured after 24, 48, 72 and 96 hours for the concentrations of 50 ppm were 51.67, 67.56, 84.67 and 95.33%, for the concentration 150 ppm were 41.67, 62.67, 80.67 and 89.67%, while for the concentration 300 ppm were 25.67, 42.67, 71.67 and 84.33%. The results of yellow dye removal showed that the concentration of 50 ppm were 49.67, 65.33, 83.33 and 92.67%, for the concentration of 150 ppm were 38.33, 60.33, 77.33 and 87.33%, and for the concentration, 300 ppm were 24, 36.67, 68.33 and 81.67%, when measured after 24, 48, 72 and 96 hours. Results recorded a slight decrease in pH values for all concentrations for each dye during 96 hours. Results also revealed a high reduction of EC and TDS values for both dye concentrations after 96 hours.

Key words: *Bacillus subtilis*, biodegradation, pH, EC, TDS, textile dyes.

Introduction

The development of industry led to add large quantities of chemicals to the environment, and textile industries contribute to pollution and toxicity of water because of dye residual in the effluent (Yagub et al., 2014). Untreated discharged waters (about 40–65 L/kg) contain a lot of chemicals such as salts, azo dyes and heavy metals in addition to a large of difficult-to-treated compounds (Manu and Chaudhari, 2002; Saratale, 2012). These dyes are mostly mutagenic, carcinogenic and toxic, because they are made of carcinogenic chemicals like aromatic hydrocarbons for example benzidine (Khan and Husain, 2007), or end up being bio-accumulated (Baughman and Perenich, 1988). Srivastava and Prakash (1991) reported that heavy metals accumulated in high concentrations in higher plants and algae which were exposed to wastewater. The need for safe and clean water has been elevated to face the shortage of safe water, therefore, the effluents must be treated (Yagub et al., 2014).

To decolourise wastewater some properties must be studied (Imran et al., 2015). pH value is very important because the effluent could be acidic, neutral or alkaline according to the type of dyes, the degradation rate could be changed by pH which means the bacteria which is used to treat wastewater must be able to tolerate all pH values (Aslam et al., 2004; Hussain et al., 2013).

The term electrical conductivity, a parameter, measures the electrical conductivity that is affected by concentrations, temperature, presence and mobility of ions in a material (Pavithra and Kousar, 2016). It is used as a wastewater salinity measurement, the density of water is affected by high solids concentrations which influences water gases' solubility (O_2) and also affects freshwater organisms (Ahmed et al., 2012). If the EC

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value is high, it will affect water organisms lives such as the dehydration process through the skin because of the presence of salts (Morsy, 1999).

Total Dissolved Solids (TDS) measures the total inorganic and organic chemicals found in the water (Chockalingam et al., 2019). TDS results from the chemical substances that are used in addition to soluble substances in the units of processing (Patel and Vashi, 2015). They involve many harmful and hazardous substances (mostly heavy metals, e.g, copper, zinc, chromium, cadmium, nickle), and they are highly toxic for human and aquatic organisms (Wang et al., 2019).

Many treatment methods were used, such as physical, chemical and biological (Yeap et al., 2014). Chemical and physical treatment methods are less used to treat textile discharge, these processes include electrocoagulation, adsorption, ion exchange, flocculation, membrane filtration, reverse osmosis and ozonation (Mukherjee et al., 2018), the treatment using these methods is expensive and produce sludge which must be removed immediately (Lalnunhlimi and Veenagayathri, 2016). Therefore, a safe green treatment must be used to avoid these problems, the biological process includes dye-adsorption on the biomass of bacteria and fungi (Crini et al., 2006), and this method is considered good substitution to other methods because of its low cost and less sludge productivity; so, it is considered an eco-friendly method (Vikrant, 2018). Several microorganisms have been reported as efficient dye degraders such as yeast, fungi, bacteria, actinomycetes and algae (Solis et al., 2012).

In 1970s, bacterial pure isolates were used to treat azo-dyes, when researchers isolated *Aeromonas hydrophila*, *Bacillus cereus* and *Bacillus subtilis* (Singh, 2014). The aim of this study is the use of *Bacillus subtilis* bacteria to treat polluted water, as a a part of green technology.

Methods

Dyes concentrations Preparation

Dyes (Disperse Red and Disperse Yellow) were taken from the Al-Hilla textile factory, and three concentrations (50 ppm, 150 ppm, 300 ppm) were prepared from each dye with three replicates for each concentration and control group.

Preparation of Inoculation

Bacillus subtilis culture (nutrient broth) was incubated for 24 hours (37°C) (Nwadinigwe and Onyeidu, 2012), and then added to the concentrations solutions.

Measurement of Absorbance

We measured absorbance with a spectrophotometer at 487 nm. We measured absorbance for four days daily.

$$\frac{Dye\,(i) - Dye\,(I)}{Dye\,(i)} \times 100$$

where:

D% = removal ratio
i = first absorbance
I = last absorbance

pH, EC and TDS Measurements

The measurements were taken using a portable electronic multi-measurement meter (HANNA).

Results

The obtained results of this study recorded that the rates of red dye removal when measured after 24, 48, 72 and 96 hours for the concentration 50 ppm were 51.67, 67.56, 84.67 and 95.33%, and the concentration 150 ppm were 41.67, 62.67, 80.67 and 89.67%, while for the concentration 300 ppm were 25.67, 42.67, 71.67 and 84.33)%, as shown in Figure 1. The results of yellow dye removal showed that for the concentration of 50 ppm were 49.67, 65.33, 83.33 and 92.67%, while the concentration of 150 ppm was 38.33, 60.33, 77.33 and 87.33)%, and for the concentration of 300 ppm were 24, 36.67, 68.33 and 81.67% when measured after 24, 48, 72 and 96 hours as shown in Figure 2.

Results recorded a slight decrease in pH values of all concentrations for each dye during 96 hours as shown in Figures 3 and 4.

Results also revealed a high reduction of EC and TDS values for all dye concentrations after 96 hours of degradation, as shown in Figures 5- 8.

Discussion

The results recorded a decreased rate of dye decolourisation with increased concentrations because the degradation rate has an inverse relationship with textile dye concentration. After all, the molecular weight increased with increased concentrations related to the complexity of dye structure (Junnarkar et al., 2006). The adsorbent activity to adsorb dye is determined by the initial adsorption of different dye concentrations, in other words when the dye concentration increases the bacterial efficiency decreases, therefore high concentrations need more time for degradation (Mohan et al., 2012). This results from the inhibition of bacteria

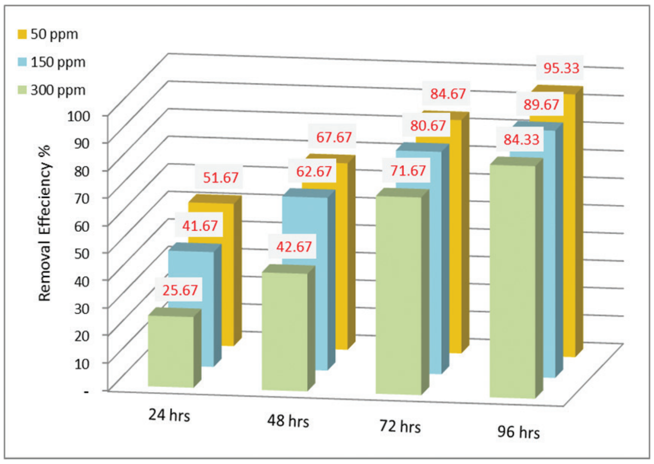

Figure 1: Efficiency % of *Bacillus subtilis* for the red dye removal.

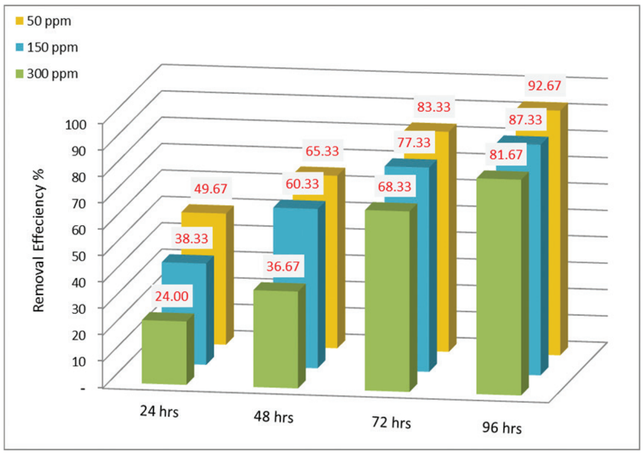

Figure 2: Efficiency % of *Bacillus subtilis* for the yellow dye removal.

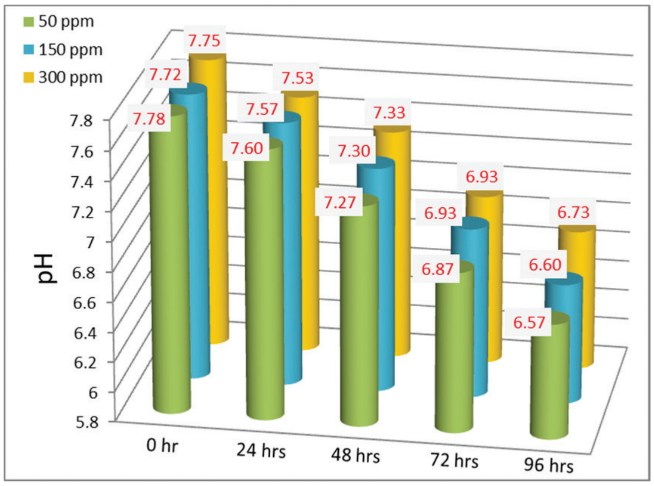

Figure 3: The pH values during 96 hours for the red dye concentrations.

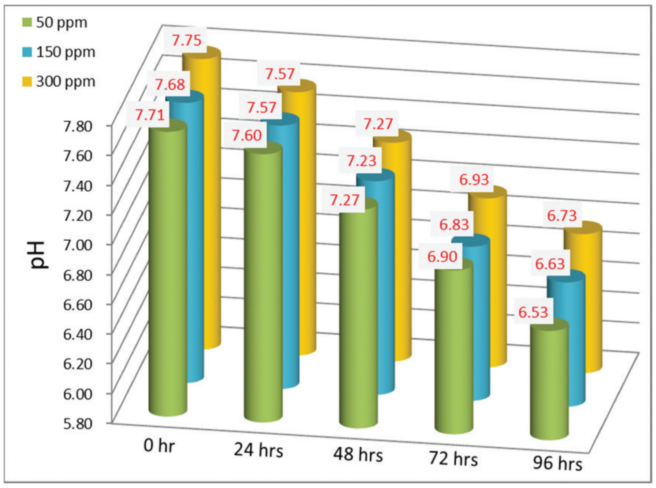

Figure 4: The pH values during 96 hours for the yellow dye concentrations.

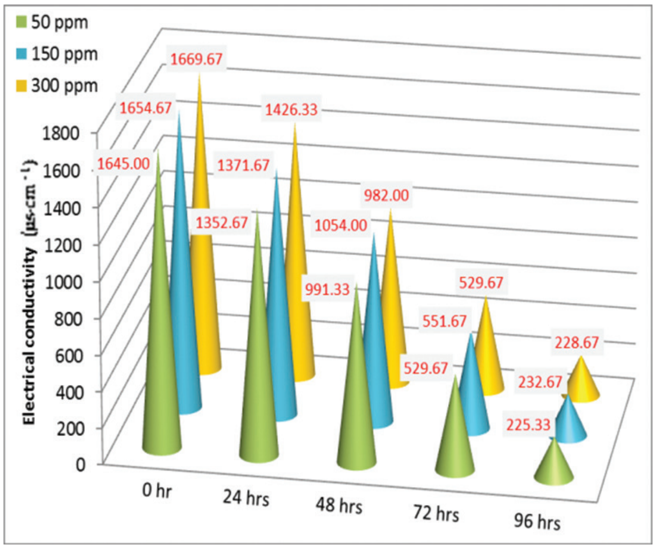

Figure 5: The EC values for the red dye concentrations during 96 hours.

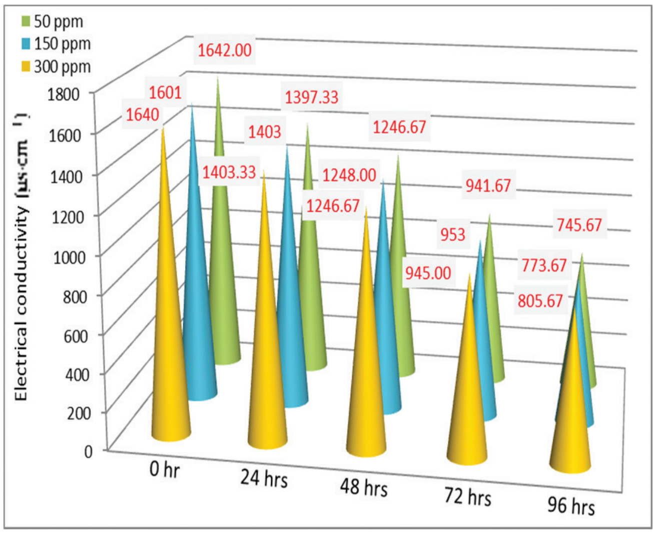

Figure 6: The EC values for the yellow dye concentrations during 96 hours.

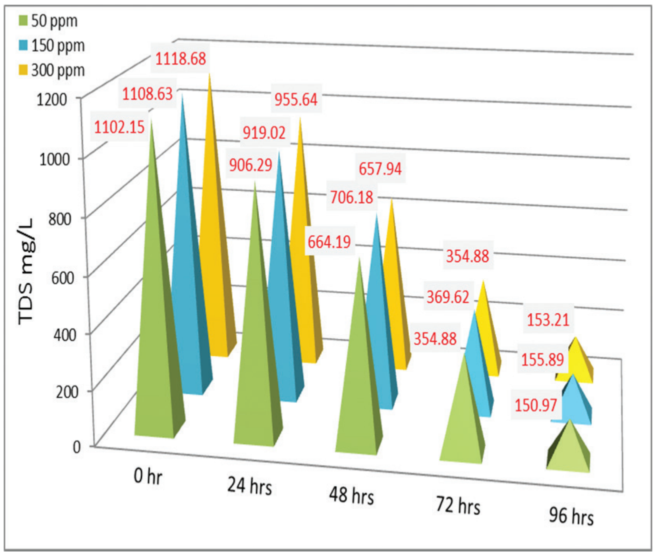

Figure 7: The TDS values for the red dye concentrations during 96 hours.

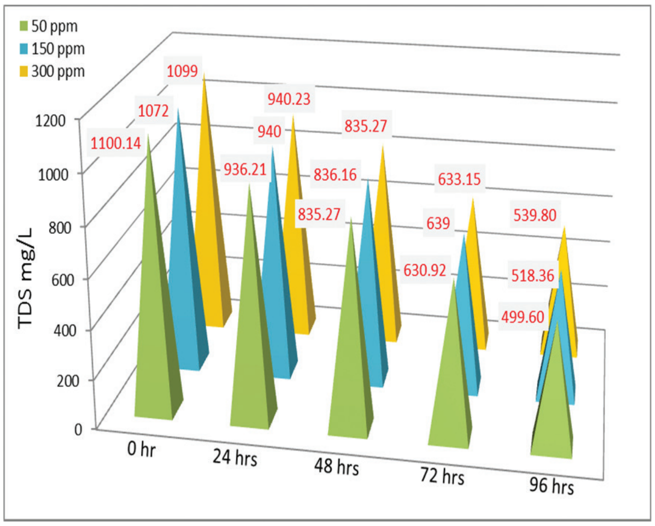

Figure 8: The TDS values for the yellow dye concentrations during 96 hours.

by dye higher concentration (Anjaneyulu et al., 2005), and could be as a result of dye toxicity to bacteria or azo-reductase active site blockage (Tony et al., 2009).

The pH, EC and TDS initial values (before treatment) agree with the results of Almamoori et al. (2023). The measurement of water pH value considered is an important parameter for its role in controlling nutrients' bio-availability and solubility such as heavy metals (e.g., iron) (Rostern, 2017). pH also controls all bio-chemical reactions and bacterial growth, it also affects dye molecule movement across the plasma membrane of the cell, this movement is considered a limiting process for dye degradation (the limiting step for degradation rate) (Kodamet al., 2005). pH value determines the charge of the bio-sorbent surface and the speciation degree in addition to the ionisation of the dye solution (Verma et al., 2019).

Reduction in the pH and EC values explains the main bacterial role as a result of organic acid release and the uptake of nutrients by bacteria (Rehman et al., 2018). Also, the reduction of EC and TDS values could be related to the physico-chemical and biological binding of chemicals (Gunatilake, 2015; Rehman et al., 2019). The suspended particles of the solution will be trapped by bacterial biofilm and may be degraded by the biofilm or adsorbed or precipitated at the bottom (Borne et al., 2013). Therefore, the reduction of pH, EC and TDS values revealed that the toxic compounds of both dyes solutions were reduced significantly (Mohanty and Kumar, 2021).

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